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Ovitek: Barva in oblika semen različnih akcesij čičerke (*Cicer arietinum* L.) (Foto: Paolo Casini, 1–12)
*Cover: Seed color and shape of chickpea (Cicer arietinum L.) accessions (Photo: Paolo Casini, 1–12)*

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doi:10.14720/aas.2022.118.1.2262 Original research article / izvirni znanstveni članek

# Isolation of phosphate solubilizing bacteria from root rhizosphere to supplement biofertilizer

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**Isolation of phosphate solubilizing bacteria from root rhizosphere to supplement biofertilizer**

**Abstract:** In soil, a large amount of supplemented phosphorus (P) are immediately transferred into insoluble forms and only 0.1 % of them is available for plant uptake. Therefore, exploring naturally occurring phosphate-solubilizing microorganisms is an essential activity to exploit them in reducing mineral phosphorus added to agricultural soils. In this study, we screened and isolated 7 bacteria that solubilized phosphate at different phosphate solubilization indexes, ranging from 4.2 to 226.1. Of them, the most efficient isolate is PSB31, which solubilized tri calcium phosphate ($Ca_3(PO_4)_2$ at a rate of 962 mg $l^{-1}$ and molecularly identified as *Bacillus* sp. (in: Bacteria) strain IMAU61039. This bacterial strain generated the low supernatant pH and the phosphatase, which are involved in the phosphorus solubilization mechanism. Furthermore, greenhouse experiments showed that tomato seedlings grown in PSB31-inoculated soil contained higher P amount and had much higher biomass than those plants grown in soil without PSB31 addition. These results suggest that the PSB31 strain has potential use as a biofertilizer.

**Key words:** phosphates-solubilizing bacteria; plant growth promoting bacteria; biofertilizer; tomato; phosphatase

**Izolacija bakterij, ki sproščajo fosfat iz rizosfere kot nadomestek biognojilom**

**Izvleček:** V tleh se velike količine dodanega fosforja hitro spremenijo v netopne oblike tako, da ostane rastlinam raspoložljivega le okrog 0,1 %. Zaradi tega je izkoriščanje v naravi prisotnih fosfat sproščajočih mikroorganizmov nepogrešljiva aktivnost, ki omogoča njihovo uporabo in zmanjšujej dodajanje mineralnega fosforja v kmetijska tla. V raziskavi smo preverili in izolirali 7 bakterij, ki sproščajo fosfat z različnimi indeksi sproščanja od 4,2 do 226,1. Med njimi je bil najučikovitejši izolat PSB31, ki je sproščal tri kalcijev fosfat ($Ca_3(PO_4)_2$ v velikosti 962 mg $l^{-1}$, na osnovi molekularnih testov določen kot IMAU61039 soj bakterije iz rodu *Bacillus.* Ta soj bakterije je generiral nizek pH v raztopini in fosfataze, ki so vključene v mehanizem sproščanja fosforja. Nadalje je poskus v rastlinjaku pokazal, da so vsebovale sejanke paradižnika, ki so rastle v tleh inokuliranih z izolatom PSB31 večjo vsebnost fosforja in mnogo večjo biomaso kot tiste, ki so rastle v tleh brez dodatka PSB31. Izsledki nakazujejo, da bi se izolat PSB31 lahko uporabljal kot biognojilo.

**Ključne besede:** fosfor sproščajoče bakterije; rast rastlin vzpodbujajoče bakterije; biognojila; paradižnik; fosfataze

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## 1 INTRODUCTION

Phosphorus is an essential macronutrient that together with others such as C, N, plays an important role in the normal growth of a plant. In agriculture, chemical fertilizer was used to supplement the phosphorus source to promote plant development and increase crop yield. However, it is reported that only a small proportion of phosphorus in provided fertilizer is available for plant uptake, and 95 %–99 % of it were insoluble, immobilized, or precipitated under effect of environmental factors such as soil pH (Khan et al., 2009). Subsequently, the proportion of insoluble phosphates deposited in the soil are increased (Morales et al., 2011). Therefore, the best strategy in crop management is limit the addition of phosphorus into soil under chemical fertilizer form; increase the agents converting P reserved in the soil; and reclaim the chemically-bound P from insoluble form (Cordell et al., 2009). Although there are many solutions to balance the input/output ratio, identification of the least risky alternatives to traditional practices is carrying out. Among those, using phosphate-solubilizing bacteria (PSB) that can solubilize the insoluble phosphate is an emerging solution.

Phosphate-solubilizing bacteria (PSB) living in the root rhizosphere present the ability in reversing the insoluble phosphate into a soluble form easily used by plants. Chen et al. (2006) demonstrated the low molecular mass organic acids produced by PSB played role in dissolving the phosphate complexed minerals. In another study, Vyas and Gulati (2009) showed that PSB-produced low molecular mass organic acids also chelated the cations that formed complexes with P ions ($PO_4^{3-}$) to release P directly into the rhizosphere soil. These results demonstrated soil PSB could release P from the insoluble forms to increase soil fertility. In addition, some PSB genera (such as *Arthrobacter*, *Burkholderia*, *Beijerinckia, Erwinia*, *Bacillus*, *Rhizobium*, *Pseudomonas*, and *Mesorhizobium*) have been exploited as soil inoculants to promote plant growth and subsequently increase the yield (Kumar et al., 2017).

Hence, using PSB as a biofertilizer was determined as an alternative for expensive and environmentally damaging fertilizers in the future. Despite the role in solubilizing insoluble P and promoting plant development, the application of PSB as a bio-fertilizer is still needed further studies due to the composition or variation in soils and bacterial community (Barea, 2015).

Thai Binh province play important role in providing agricultural products for market in Vietnam. However, the overuse of chemical fertilizer is one of major factors which has caused soil pollution in Thai Binh. Therefore, development of biofertilizer plays a key role for the sustainable agriculture in this province. Hence, we aimed to isolate and characterize high phosphorus-solubilizing bacteria from agricultural soil collected in Thai Binh, and also investigated their potential in developing these strains as biofertilizer.

## 2 MATERIAL AND METHODS

### 2.1 ISOLATION OF BACTERIA WITH PHOSPHORUS-SOLUBILIZING ABILITY

The soil samples were collected from the field grown maize, rice, and tomato in Thai Binh (Vietnam) on December 14, 2020. Different locations in Thai Binh province were located including Tien Hai (20°24'27.7"N 106°31'00.1"E) and Kien Xuong (20°22'54.0"N 106°23'43.2"E), which provided more than 80% agricultural products (rice, maize and tomato) for the market in Vietnam.

Sampling procedure is carried out according to TCVN 4046-1985 (TCVN 4046 – 85, 1985) as follows: soil samples were taken according to the diagonal or zigzag rule depending on the topography of the land. Each site took from 15 to 20 samples, each sample was about 0.5 kg and the samples were mixed to be represented by the diagonal rule of about 0.5 kg.

PSB was isolated from soil samples by serially diluting up to $10^{-10}$ by sterilized water and inoculating in Pikovskaya's agar (PVK) agar medium by pour plate method (Cao et al., 2018). For control, only sterilized water was used. The incubation of all plates was done at 30 °C for 7 days. After 7 day of incubation, the bacteria generated a clear zone around colonies were identified as strains having the phosphorus-solubilizing ability. Then, the isolated single colonies presenting a clearing zone around were transferred onto new PVK plates. The strains generated the highest clearing zones are considered as potential PSB and were selected for the next experiments.

### 2.2 DETERMINE PHOSPHATE SOLUBILIZING ACTIVITY OF BACTERIA ON AGAR MEDIUM

After 7 days of incubation, the clearing zones around single colonies on the reinoculated plates were measured and validated (Sharon et al., 2016):

Phosphate solubilizing index (PSI) = [(colony diameter + clearing zone)/ colony diameter] x 100

### 2.3 DETERMINE PHOSPHATE SOLUBILIZING ACTIVITY OF BACTERIA IN PVK LIQUID MEDIUM

The P solubilizing efficiency of the isolates was investigated by growing in the PVK liquid medium. 200 µl of selected isolate was cultured in 9.8 ml of PVK medium with 0.5 % $Ca_3(PO_4)_2$ (w/v) on the shaker at 30 °C. The culture was collected and centrifuged to obtain the supernatant using to determine the solubilized P by vanadomolybdate method (Pearson, 1976). Briefly, 1 ml of supernatant (distilled water for the blank) was transferred into a clean cuvette. Then, added 0.25 ml of vanadate-molybdate reagent and mixed well by pipetting up and down several times. After 10 minutes, placed the cuvette with sample into the UV/VIS spectrophotometers (METTLER TOLEDO, USA) and measured.

The efficiency of the bacteria in solubilizing the insoluble phosphorus compound was identified as the percent of the total phosphorus presenting in the medium. The culture pH was also measured by using a benchtop pH meter (Mettler Toledo, USA). All experiments were performed in triplicates.

### 2.4 DETERMINE PHOSPHATE SOLUBILIZING ACTIVITY OF BACTERIA IN A POTTING SAND MATRIX

The P solubilization efficacy of the isolates was also investigated in a less-nutrient environment like acid-washed and sterilized sand. The experiment was prepared in triplicates as follows: 9 g of treated sand were added into a 15 ml tube containing a 5 ml reaction. After mixing well the reaction mixture, added 1 ml of PVK media (5 % $Ca_3(PO_4)_2$) inoculating with or without PSB strain (2 x $10^6$ cfu $ml^{-1}$) into the reaction. Then, the inoculated samples were kept in the incubator at 30 °C for 24 h. After incubation, adding the distilled water into the sample to make a final volume of 10 ml was done before shaking it at 200 rpm for 1 h, and followed by centrifugation at 3,500 rpm. After that, the supernatant was collected by filtrating the culture media through a 0.45 µm filter. Then, the amount of released P was identified by vanadomolybdate method (Pearson, 1976).

### 2.5 PHOSPHATASE ENZYMATIC ASSAY

Phosphatase was explored by using the method described by Tabatabai and Bremner (1969). Briefly, the selected strain was grown in a 250 ml conical flash containing 100 ml broth PVK medium for 80 h. In every 5 h, the culture was taken and removed the bacterial cells by centrifuging at 10,000 rpm for 10 min at 4 °C. After that, 1ml of cell-free supernatant was mixed with 4 ml of modified universal buffer (pH 6.5). Then added 1 ml of 0.025 mM disodium p-nitrophenyl phosphate (tetrahydrate) into this mixture. The solution was mixed well and incubated at 37 °C for 1 h. After 1 hour incubation, 4 ml of 0.5 M NaOH and 1 ml of 0.5 M $CaCl_2$ was added to stop the reaction. The solution was then filtered through Whatman No. 42 filter paper. The filtered solution was used to measure the concentration of p-nitrophenol by using the UV/VIS spectrophotometers (Mettler Toledo, USA) at 420 nm. The values were identified on the standard curve. Each measurement was done in triplicate.

The standard curve was obtained by serially diluting the standard p-nitrophenol solution. The control was also prepared as above procedure but the additions of 0.5M $CaCl_2$ and 0.5M NaOH was applied before the addition of 1 ml of 0.025 mM disodium p-nitrophenyl. The amount of enzyme that used to release 1 µmol of *p*-nitrophenol $ml^{-1}$ $min^{-1}$ from di-Na *p*-nitrophenyl phosphate (tetrahydrate) under the assay condition was defined as one unit (U) of phosphatase activity.

### 2.6 MOLECULAR IDENTIFICATION OF PSB31 STRAIN

The total DNA of strain PSB31 was extracted using a Rapid Bacteria Genomic DNA Isolation Kit (Biobasic, Canada) as per the kit instructions. The PCR amplification of 16S rDNA was done with the extracted DNA by using the universal primers 27 F (5′-AGA GTT TGA TCC TGG CTC AG-3′), and 1492 R (5′-TAC GGT TAC CTT GTT ACG ACT T-3′) (Mohamed et al., 2018). The amplification was done in a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, CA, USA) using the following program: 95 ˚C for 5 min; 30 cycles at 95 ˚C for 30 s, 55˚C for 30 s, and 72˚C for 90 s; and 72 ˚C for 7 min. The fragment of 16S rDNA sequences (1.5 kb) was obtained by running the PCR product on the 1 % agarose gel in an electrophoresis tank. Then the expected band was cut and purified by using the QIAquick PCR Purification Kit (Qiagen, USA). The purified 16S rDNA fragment was sequenced by Fisrt Base Company (Singapore). The obtained sequence was blasted on NCBI to identify the species. The sequences with high similarity (more than 99 %) were used for multiple cluster alignment and phylogenetic analysis on MEGA software (v.7.2).

The nucleotide sequence data reported in this paper deposited on GenBank with the accession numbers is OL753109.

### 2.7 GREENHOUSE TESTING

The ability of selected PSB strains in promoting plant growth was investigated by pot experiments under greenhouse condition (with a temperature of 30°C and constant humidity of 85-95%) at the VNU Central Institute for Natural Resources and Environmental Studies, Ha Noi, Vietnam.

The sand matrix was pretreated by washing with 0.1 M hydrochloric acid (HCl). Then, the acid-washed sand was submerged in the 0.1 M HCl for another 24 h. After that, the submerged sand was drained and washed three times with DI water. The pH of the sand was adjusted to 7-7.5. Finally, this sand was autoclave at 121 ˚C for 15 min. The sterilized sand was used as the potting medium. In this pot experiment, the insoluble form of P used is $Ca_3(PO_4)_2$, which was added into the potting matrix as a P source.

Tomato seeds (*Lycopersicon esculentum* 'Thuan Dien') were used as an indicator and were surface-sterilized by using alcohol and Javen solution as described by Li et al. (2017). After that, the sterilized seeds were germinated on agar plates, which were covered by the aluminum foil for 3 days at room temperature. Homogenous seedlings were chosen for further experiments, some of which were covered with selected bacterial strain by dipping their roots for 30 min in bacterial culture (OD = 1). Then, four bacterized seedlings were planted in each plastic pot, which was kept in a greenhouse with long-day condition. Each treatment was repeated in triplicate.

The experimental treatments were: (T1) tomato seedlings inoculated with selected bacteria; (T2) non-inoculated tomato seedlings were considered as a negative control; and (T3) the positive control is the seedlings that were not bacterized but were regularly watered with the solution added 0.25 mM $KH_2PO_4$. All pots were provided the macro and micronutrients by watering them with 30 ml of the nutrient solution only or added $KH_2PO_4$, where applicable.

The irrigated nutrient solution was referenced from Li et al. (2017) and consists of 0.65 mM $MgSO_4$, 2 mM $NH_4NO_3$, 2 mM $CaCl_2$, 0.75 mM $K_2SO_4$, 0.1 mM KCl, 0.25 mM $KH_2PO_4$, 0.2 mM Fe-EDTA, $1 \times 10^{-3}$mM MnSO4, $1 \times 10^{-3}$mM ZnSO4, $1 \times 10^{-4}$mM CuSO4, and $5 \times 10^{-6}$ mM $(NH4)_6Mo_7O_{24}$, $1 \times 10^{-3}$mM $H_3BO_3$.

The seedlings were grown in three weeks until harvested. The root samples were removed from the adhering soil by washing with sterile water. The measurement of shoot and root length was carried out for three plants. After that, the plant samples were dried in the oven for 30 min at 105 °C to inactivate the enzyme, then reduced the temperature to 65 °C and kept at that temperature until the plant weight is constant. The weight of the dried plant was recorded and analyzed.

Finally, the oven-dried samples were powdered and then digested by an $H_2SO_4$-$H_2O_2$ mixture at 370 °C. The vanadomolybdate method (Hanson, 1950) was applied to identify the P amount in the solution.

### 2.8 STATISTICAL ANALYSIS

All experiments were repeated three times, the results were presented as mean values with ±SD. Tukey's honestly significant difference (HSD) method in SPSS (version 17) was applied to compare the means in all experiments.

## 3 RESULTS AND DISCUSSION

### 3.1 ISOLATION OF PHOSPHATE-SOLUBILIZING BACTERIA

The overuse of chemical fertilizer caused the increase of insoluble P in the soil leading to many problems for humans and other living creatures. Hence, soil-isolated microorganisms having the ability in solubilizing phosphorus is emerging as an alternative to chemical fertilizer because of their environment-friendly nature.

The results showed that a total of 7 colonies grown and generated a circular clearing zone on PVK medium was obtained. Among obtained colonies, five of them (PSB11 to PSB51) were from soil grown maize while only one colony was observed for soil grown rice (PSB61) and tomato (PSB71). The results were presented in Table 1 and illustrated in Figure 1.

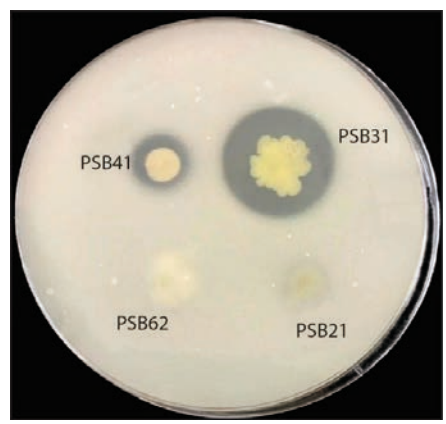


**Figure 1**: Insoluble phosphate solubilization studies on Pikovskaya's agar (PVK): PSB31 show efficient phosphate-solubilizing isolate (large haloes), whereas three others show weak (PSB41 and PSB21) or no activity (PSB62, no visible halo)

**Table 1:** Biochemical properties of isolated PSB strains

| PSB isolates | Phosphate solubilizing index (Agar)[a] | Final pH of PVK Liq. Med. | Color of colonies | Soluble P (mg $l^{-1}$)[b] |
|---|---|---|---|---|
| PVK medium only | 0 | 6.5 | N/A | 11.12 ± 4.5[a] |
| PSB11 | 4.2 | 5.5 | Yellow | 53 ± 7.5[b] |
| PSB21 | 13.8 | 5 | Yellow | 63 ± 9.2[c] |
| PSB31 | 226.1 | 4.5 | Yellow | 962 ± 11.3[f] |
| PSB41 | 78.3 | 4.5 | Yellow | 303 ± 6.3[d] |
| PSB51 | 67.5 | 4.5 | White | 313 ± 7.2[e] |
| PSB61 | 73.4 | 4.5 | White | 301 ± 8.2[d] |
| PSB71 | 91.1 | 4.5 | White | 310 ± 9.1[e] |

[a] All solubilization rates were measured from cultures grown for 24 h in a liquid medium
[b] Data are means ± SE of three independent biological replicates. Bearing different letters in the same row are significantly different from each other according to the least significant difference (LSD) test ($p < 0.05$)

As can be seen from Table 1, the PSB31 strain produced the largest clearing zone, with a PSI of 226.1. On the other side, the lowest index value was 4.2 produced by PSB11 isolate. The results also present the un-correlation between the PSI and the insoluble phosphate solubilization ability of the other isolates including PSB51, PSB61, and PSB71, those were isolated from soil grown maize, rice, and tomato, respectively. These results were demonstrated by a study of Sharon et al. (2016), who reported the solubilization of $Ca_3(PO_4)_2$ of microbial communities living in the rhizosphere of potato roots is higher than the one produced by microbes in the rhizosphere of tomato roots. In addition, the results showed that four of the seven isolates (PSB11, PSB21, PSB31, PSB41) produced colonies that were opaque and bright yellow while the colonies formed by the other three isolates (PSB51, PSB61, PSB71) were opaque and white (Table 1). These results suggest the variation in phosphate solubilization of the isolates could be due to the differences of microbial communities that were strongly affected by soil properties and plant species (Sharon et al., 2016). The explain was strengthened by the discovery of Grayston et al. (1998), in which the microbial diversity in the rhizosphere was highly affected by metabolites exuded by different plant species into the rhizosphere such as amino acids, carbohydrates, and carboxylic acids.

## 3.2 IDENTIFICATION OF BACTERIAL STRAIN PSB31

The 16S rRNA gene sequences of the PSB31 strain was blasted against the one of the other microorganisms on the NCBI (Figure 2). As can be seen from Figure 2, the PSB31 strain closed to the *Bacillus* sp. strain IMAU61039 (Accession number: MF803700.1). The *Bacillus* sp. have been reported as phosphorus solubilizers (Kumar et al., 2017; Mohamed et al., 2018).

## 3.3 FACTORS AFFECTED TO PHOSPHATE SOLUBILIZATION OF PSB31

### 3.3.1 pH

The results also showed that the highest amount

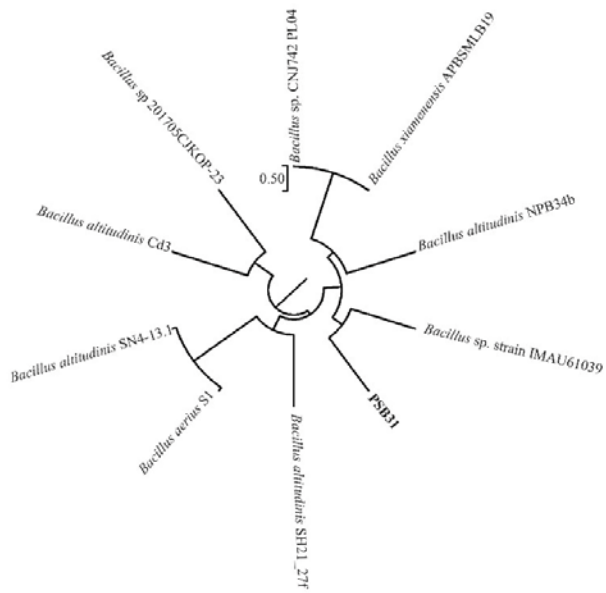


**Figure 2:** A neighbor-joining tree shows the phylogenetic relationships among 16S rDNA sequences of PSB31 and their closely related sequences from NCBI. The scale bar indicates evolutionary distance

of soluble P (962 ± 11.3 mg $L^{-1}$) was calculated for the PSB31 culture, while the lowest one (53 ± 7.5 mg $l^{-1}$) was observed in PSB11 culture (Table 1). Furthermore, the results also indicated that the decrease of pH of final filtrate from isolates compared to the control (Table 1). This phenomenon was reported by some previous studies which demonstrated that the organic acid production of PSB reduced the medium pH facilitating phosphate solubilization (Sharon et al., 2016; Mohamed et al., 2018). Moreover, our results also showed the supernatant pH was uncorrelated with soluble P presented in the culture of PSB31. This could be due to the calcium ion freed from the linkage with $PO_4^{3-}$ by PSB31 strain neutralized the produced acids during the experiment (Nelofer et al., 2015). These results suggest the mechanism of P solubilization by PSB was not only produced low molecular acids but also generated others factors such as hydrolytic enzymes.

### 3.3.2 Phosphatase activity

The result of the phosphatase experiment showed that the insoluble $Ca_3(PO_4)_2$ was completely solubilized in the solution containing 1 ml of supernatant from PSB31 strain after 48 h of incubation was indicated by the color change of culture from milky to transparent. The result suggested that the PSB31 strain produced phosphatase to degrade the $Ca_3(PO_4)_2$ in the medium (Figure 3). The enzymatic activities rapidly increased from 0 to 18 UI after about 25 hours of incubation. After that, the enzymatic activities were slightly increased and reached 20.2 UI after 50 hours of incubation before dropped out to 17.3 UI after 55 hours. Then the enzymatic activities were slightly decreased to 16.1 UI at the end of the experiment.

The current study also showed the presence of phosphatase in the supernatant of PSB31 with amounts that were higher than the results of previous studies (Mendoza-Arroyo et al., 2020). All in all, the result suggested the PSB31 strain is a very promising agent that could be used to solubilize the phosphorus compound in the soil to increase the P availability for crops.

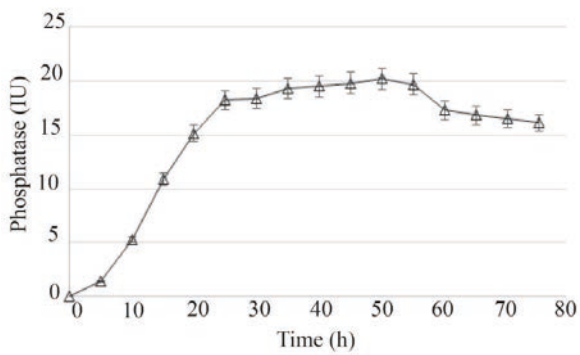


**Figure 3:** Phosphatase profile of phosphorus-solubilizing bacteria PSB31 for 72 h of incubation and 5 g $l^{-1}$ $Ca_3(PO_4)_2$ in the medium

### 3.4 PSB31 ENHANCED THE GROWTH OF TOMATO SEEDLINGS

The results of greenhouse experiments clearly showed that PSB31 was able to promote tomato growth under the stress of nutrient conditions. The results were illustrated in Figure 4A and presented in Figure 4B. As can be seen from Figure 4B, tomato seedlings supplemented with both PSB31 and $Ca_3(PO_4)_2$ (T2) had a greater increase of root and shoot length than those with no bacteria + $Ca_3(PO4)_2$ + $KH_2PO_4$ (T3) and with no bacteria + $Ca_3(PO_4)_2$ (T1).

The result also presented a maximum quantity of shoot length for the seedlings that were not bacterized but were regularly watered with the P solution (positive control). Furthermore, the results also showed the tomato seedlings received both insoluble $Ca_3(PO_4)_2$ and PSB31 strain had the fresh and dry mass moderately higher than the negative control but slightly lower than the positive control (Table 2). It was consistent with reports in which PSB when applied into soil could enhance significantly the development and phosphate uptake in many crop species (Kumar et al., 2017; Mendoza-Arroyo et al., 2020).

Additionally, the results of the experiment for potting medium indicated that PSB31 could solubilize $Ca_3(PO_4)_2$ in sand increasing the soluble P in the sand matrix to 106.7 ± 3.5 mg $l^{-1}$. This could be PSB31 strain generated phosphatase or low molecular organic acids to converted the phosphate from insoluble to soluble form that provided a P balance for plant development resulting in plant growth promotion (Mendoza-Arroyo et al., 2020). These results suggest the potential application of PSB31 as a biofertilizer for sustainable agriculture.

As can be seen from Table 2, tomato seedling inoculated with PSB31 strain had the fresh and dry shoot weights 2 times higher than the one that grew in sand mixed with the $Ca_3(PO_4)_2$ but lower than the one supported with soluble P. These results were similar to the one of Lee et al. (2020), in which the *Arabidopsis thaliana* (L.) Heynh. seedlings bacterized with *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 strain L1 via the roots had a considerable increase in plant mass. These results suggested a possible role of PSB31 strain in the process of assimilation by pho-

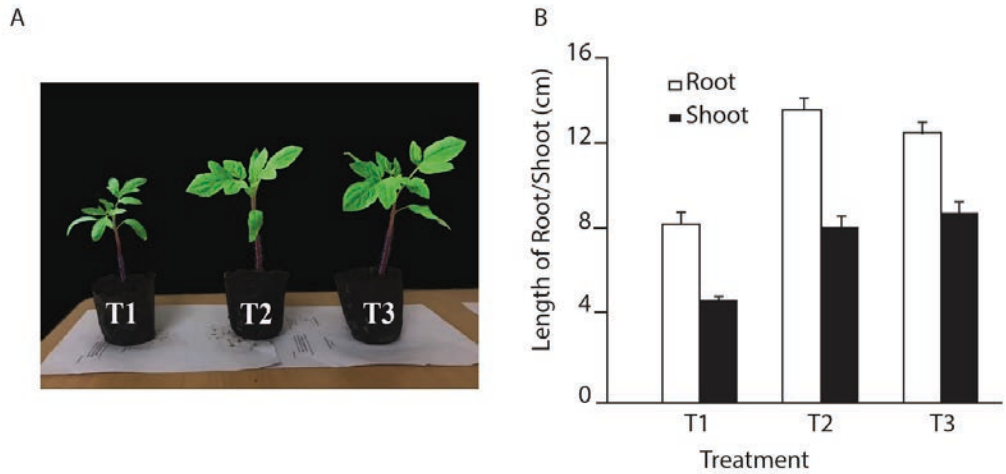


**Figure 4:** Plant growth promotion activity by phosphate-solubilizing bacteria. A. Illustration for plant growth. B. Length of root and shoot of plant under different conditions. T1: no bacteria + $Ca_3(PO4)_2$, T2: with PSB31+ $Ca_3(PO4)_2$, T3: no bacteria + $Ca_3(PO4)_2$ + $KH_2PO_4$

**Table 2:** Effect bacterial isolates on plant biomass production

| Treatment | Root mass (g) | | Shoot mass (g) | | Total P (%) |
|---|---|---|---|---|---|
| | Fresh | Dry | Fresh | Dry | |
| No bacteria + $Ca_3(PO_4)_2$ | 0.153 ± 0.057[a] | 0.022 ± 0.002[a] | 0.378 ± 0.006[a] | 0.044 ± 0.003[a] | 0.15 |
| PSB31 + $Ca_3(PO_4)_2$ | 0.425 ± 0.223[a] | 0.025 ± 0.007[a] | 0.608 ± 0.027[b] | 0.089 ± 0.013[b] | 0.31 |
| No bacteria + $Ca_3(PO_4)_2$ + $KH_2PO_4$ | 0.492 ± 0.257[a] | 0.26 ± 0.004[a] | 0.697 ± 0.034[b] | 0.091 ± 0.032[b] | 0.35 |

Data are means ± SE of three independent biological replicates. Bearing different letters in the same row are significantly different from each other according to the least significant difference (LSD) test ($p < 0.05$)

tosynthesis, subsequently, the plant mass improvement (Wu et al., 2019).

Moreover, the higher P amount measured in bacterized seedlings (0.31 %) compared to the one grown in the sand with only $Ca_3(PO_4)_2$ (0.15 %) indicated that the PSB31 strain functioned in releasing the soluble P from $Ca_3(PO_4)_2$ enhancing P uptake of seedlings. The results also presented the P amount in seedling grown in pot watered with nutrient containing P was the highest among treatments with 0.35 % (Fig. 4B). These results suggested strain PSB31 could enhance P uptake in the bacterized seedlings led to the high amount of water in plant contributing to biomass formation. These results endorsed that the optimistic result of PSB31 on crop yield due to the increase of nutrients uptake (predominantly phosphorus).

## 4 CONCLUSIONS

The soil microorganisms capable of converting insoluble P to soluble P are being explored as an environmentally friendly agent to promote plant development and subsequently increasing yields. The results showed that among 7 isolates, strain PSB31 has good phosphate solubilization activity and promoted the growth of tomato seedlings under phosphate limiting conditions. This *PSB31* strain had been identified belonging to *Bacillus* sp. (in: Bacteria) strain IMAU61039. All of these results suggested the PSB31 strain could be potentially used as a microbial biofertilizer candidate for commercial applications in the future.

## 5 ACKNOWLEDGEMENT

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# Frost hardiness of flower buds of three Hungarian almond cultivars during dormancy

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**Frost hardiness of flower buds of three Hungarian almond cultivars during dormancy**

**Abstract:** Frost hardiness of flower buds of three Hungarian almond cultivars ('Tétényi Bőtermő', 'Tétényi Kedvenc', 'Tétényi Keményhéjú') was investigated by artificial freezing tests during ten dormancy periods. $LT_{50}$ values were calculated after artificial freezing treatments on different temperatures. Based on the results of regular observations, the frost hardiness profile of three cultivars has been described in each dormancy period. Frost tolerance was significantly affected by year and genotype. The potential frost tolerance of cultivars in our geographical location, in the middle of Hungary, has been characterised by $LT_{50}$ values in January 2017, as the best values of them. Flower buds of 'Tétényi Keményhéjú' were the most frost hardy, its $LT_{50}$ in this sampling date was -20.5 °C, 'Tétényi Bőtermő' was the most sensitive ($LT_{50}$: -17.6 °C), while 'Tétényi Kedvenc' showed intermediate frost hardy ($LT_{50}$: -19.1 °C). Nevertheless, in mild winters the cultivars were unable to reach their genetically potential maximum frost hardiness. Hungary is situated at the northern part of almond growing area, so frost tolerance of flower buds is one of the most important traits of cultivars. Based on the results of artificial freezing tests the best cultivars can be selected from the aspect of crop safety.

**Key words:** *Prunus dulcis*; frost tolerance; generative organs; $LT_{50}$ values; artificial freezing tests

**Odpornost na mraz treh madžarskih sort mandljevca v obdobju mirovanja**

**Izvleček:** V raziskavi je bila z umetnim zmrzovanjem preučevana odpornost na mraz cvetnih brstov treh madžarskih sort mandljevca ('Tétényi Bőtermő', 'Tétényi Kedvenc', 'Tétényi Keményhéjú') v obdobju mirovanja. Vrednosti $LT_{50}$ so bile izračunane po obravnavanjih z umetnim zmrzovanjem pri različnih temperaturah. Na osnovi rezultatov rednih opazovanj je bil opisan profil odpornosti na mraz treh sort za vsako obdobje mirovanja. Na toleranco na mraz sta značilno vplivala leto poskusa in genotip. Potencial tolerance na mraz vseh treh sort v geografskem območju osrednje Madžarske je bil najboljše opredeljen z vrednostmi $LT_{50}$ pridobljenimi januarja 2017. Cvetni brsti sorte 'Tétényi Keményhéjú' so bili najbolj odporni na mraz, njihova $LT_{50}$ je bila na ta vzorčni termin, -20,5 °C. Sorta 'Tétényi Bőtermő' je bila najbolj občutljiva ($LT_{50}$: -17,6 °C) med tem, ko je sorta 'Tétényi Kedvenc' izkazala srednjo odpornost na mraz ($LT_{50}$: -19,1 °C). V milih zimah preikušene sorte niso mogle doseči največjega genetskega potenciala največje odpornosti na mraz. Madžarska se nahaja na severnem robu uspevanja mandljevca, zato je odpornost cvetnih brstov na mraz ena od najpomembnejših sortnih lastnosti. Na osnovi rezultatov poskusov umetnega zmrzovanja bi lahko izbrali najboljše sorte z vidika varnosti pridelkov.

**Ključne besede:** *Prunus dulcis*; toleranca na mraz; generativni organi; $LT_{50}$ vrednosti; preiskusi z umetnim zmrzovanjem

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## 1 INTRODUCTION

Frost sensitivity of cultivars is the most important limitation factor of almond production in Hungary (Kállayné, 2003, 2014). Flower buds are the most frost-sensitive overwintering organs of almond trees. They can suffer frost damages during winter and early spring because of low temperatures. Frost tolerance can be tested by various indirect and direct methods. Indirect methods, such as ion leakage observation (Werner et al., 1993; Afshari et al., 2011; Dumanoglu et al., 2019), water, starch and sugar content measurement (Zayan, 1981; Burak & Eris, 1992; Bolat, 1995), or differential thermal analysis /DTA/ (Quamme, 1974, 1978; Proebsting & Sakai, 1979; Faust, 1989; Kang et al., 1998; Kaya et al., 2018, 2019, 2020) are suitable just for estimation of frost hardiness. The direct methods are those used to examine the actual frost damage of plant parts after they have been exposed to low temperatures. This can be a natural frost damage survey or an evaluation of the results of artificial freezing experiments (Tromp, 2005).

There are data of frost hardiness of flower buds of related species of almond based on field studies – such as peach (Szabó & Nyéki, 1988, 1991; Nyéki & Szabó, 1989; Szabó et al., 1998; Szalay, 2001; Szabó, 2002; Miranda et al., 2005; Szalay et al., 2010) and apricot (Szabó & Nyéki, 1991; Szalay, 2001; Miranda et al., 2005). Results of artificial frost treatments of peach (Proebsting, 1970; Proebsting & Mills, 1978; Szalay, 2001), and apricot (Pedryc et al., 1999; Szalay, 2001; Szalay et al., 2006), are available as well. However, only a few studies have addressed the frost resistance of almonds, and the information published is primarily about the spring frost resistance of flowers. Viti et al. (1994) examined frost sensitivity of almond flowers at different phenological stages during blooming time. Based on their experiences, cultivars with late flowering time had higher frost resistance, even if their flowers were in advanced phenological stages. A similar study was published by Snyder and Conell (1996) on the frost tolerance of flowers and fruitlets of Californian almond cultivars. Pink flower buds of the varieties Sonora and Price were less sensitive, they suffered only 30 % frost damage at -5° C, while another seven varieties had higher frost damage. In the case of these two varieties, the open flowers were also more frost tolerant: while 100 % flowers damaged at -3 °C frost of other varieties, critical T was -4.5 and -5.5 °C in a case of Sonora and Price cultivars. Likewise, the differences between several varieties and between various flowering-phenological stages were investigated by Sepahvand et al. (2014). Frost tolerance of almond cultivars and hybrids were tested in different phenological stages during blooming time by field observation and laboratory methods in Iran. There were big differences between genotypes and sampling dates from the aspect of frost hardiness (Imani et al., 2012). In Spain 12 commercial almond cultivars were observed, and the tolerance of flowers to frosts was evaluated by chlorophyll fluorescence after artificial freezing (Kodad et al., 2010). Miranda et al. (2005) examined cultivars 'Marcona' and 'Ferragnes' by artificial freezing during the ecodormancy period. The critical temperature for frost tolerance of flower buds was -16.3 °C. These studies do not track the frost resistance of flower buds during the whole dormant period, but they give only a snapshot of frost tolerance.

Late flowering and frost hardiness are important breeding aims, because almond may be affected by frost due to its early flowering even in subtropical climates (Daneshvar & Sardabi, 2006; Dicenta et al., 2011; García-Gusano et al., 2011; Imani & Mahamadkhani, 2011; Imani et al., 2011, 2012; Moheb et al., 2018). The results of physiological and genetic research can be of great help in this work. Karimi et al. (2016) identified small RNAs that play a role in frost tolerance of reproductive organs in almond. Hosseinpour et al. (2017) identified a cold-shock protein in a frost tolerant genotype which plays role in frost resistance.

In the present study, frost hardiness of the flower buds of three Hungarian almond cultivars were investigated for 10 years (selected between 2004 and 2019) by regular artificial freezing tests during dormancy periods with the aim of determination of frost hardiness profile of them. In our article, the results of this study are presented.

## 2 MATERIALS AND METHODS

Samples were taken from the cultivar collection of the Department of Pomology, Institute of Horticultural Science, HUALS, Budapest. Hungarian cultivars, 'Tétényi Bőtermő', 'Tétényi Kedvenc', and 'Tétényi Keményhéjú' were examined. Six trees of each cultivar were included into examinations. Almond trees, grafted on almond seedling rootstocks were planted in the experimental orchard in 1992, at a row and tree spacing of 6 x 4 meters. The growing system is free vase. Integrated cultivation technology is taking place in the plantation without irrigation.

Investigations were carried out in the dormancy period of the following years: 2004/05, 2005/06, 2006/07, 2007/08, 2010/11, 2013/14, 2014/15, 2015/16, 2016/17, 2018/19. Experimental work began in early September each year and continued until next spring. 7-9 low temperature-treatments were performed each winter. The last test has been done just before flowering. The experiments were performed in a Rumed 3301 (Rubarth

Apparate GmbH, Laatzen, Germany) climate chamber, using a method previously developed by the department (Szalay et al., 2010, 2016, 2017). Each time, 3 or 4 freezing temperatures were applied with a difference of 2 or 3 °C. In order to determine the $LT_{50}$ values (the temperature at which 50 % of the flower buds were damaged) the treatment temperatures were chosen that all cultivars should get frost damage below as well as above 50 %. In the chamber initial room temperature was reduced by 2 °C/h and the samples were kept at the desired freezing temperature for 4 h, after which the temperature was raised by 2 °C/h. After 12 hours at room temperature, the percentage of frost damage was determined by cutting the flower buds in half lengthwise and observing the discoloration of the inner tissues. Five twigs from each cultivar per treatments were put into the climate chamber, where one twig with 30-40 flower buds was considered as a replication for the statistical analysis. Based on the experimental results, the $LT_{50}$ values of each cultivar were determined by linear regression, assuming a linear relationship between the treatment temperature and the percentage of frost damage in the range of 20 % and 80 % (Gu, 1999). The mean and standard deviation of five replications were calculated. Based on the calculated values, the flower bud frost hardiness profile of each cultivar was outlined between 1st of September and 1st of April for each year, characterized by $LT_{50}$ values. Frost hardiness profile of the observed cultivars in averaged of 10 years was determined as well. Due to different sampling times in different years, $LT_{50}$ values were calculated by interpolation from adjacent data in the middle of the months. During the experiments daily minimum and maximum temperatures in the almond orchard were recorded by a local automatic meteorological station. The statistical analysis was performed with Microsoft software, Excel 365 programme. Normality of the error term was proven subsequently by Komogorov Simrnov or Shapiro-Wilks`test ($p > 0.05$). Pair wise comparisons were run by Tukey's post hoc test. For determining year and genotype effects the ANOVA method was applied.

## 3 RESULTS

The frost hardiness profiles which show changes in frost resistance of the studied cultivars throughout the dormancy period were determined based on the $LT_{50}$ values of the flower buds. Data for three highlighted winters and ten-years average are shown in Figures 1-4. The profiles can be divided into two parts. The first part is the hardening, when the frost hardiness of flower buds gradually increased, lasted until December or January, depending on the year. The second part is the dehardening, when the flower buds have gradually lost their frost tolerance. There were significant differences between the years in the change of frost hardiness due to different weather conditions.

Data for the dormancy period 2006/2007 are shown in Figure 1, which was the mildest winter during the studied period. There were nine sampling times during

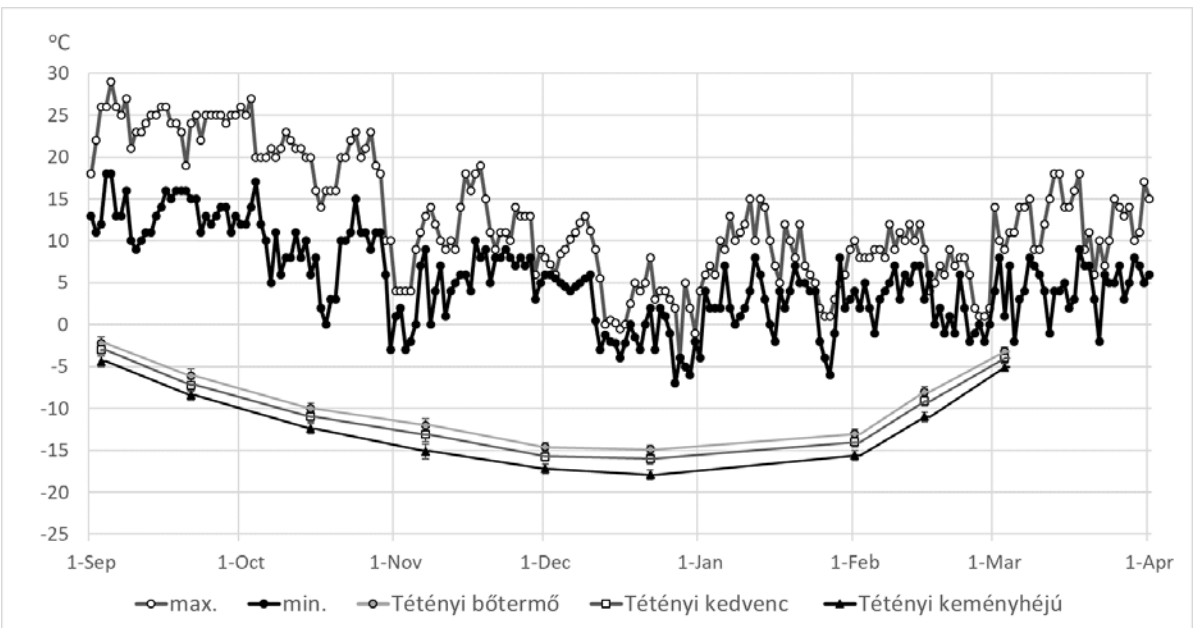


**Figure 1**: Daily maximum and minimum ambient temperatures, and $LT_{50}$ values of flower buds of three almond cultivars observed based on artificial freezing tests in 2006/07 winter

this season. At the beginning of September, the $LT_{50}$ values of the flower buds of the examined cultivars were between -2.2 °C and -4.4 °C. Then, until the second half of December, the frost tolerance of the flower buds has been increased. The highest one was measured on 22 December, when the $LT_{50}$ value of 'Tétényi Bőtermő' was -14.9 °C, while -16.0 °C for 'Tétényi Kedvenc', and -17.9 °C for 'Tétényi Keményhéjú' were detected. In the second half of winter, the frost resistance of flower buds decreased rapidly because of high temperatures. Flowering was very early this year, in early March. The final sampling date was just before blooming time, when the $LT_{50}$ values were between -3.2 °C and -5.1 °C. During this winter there was no natural frost in the orchard, but during the flowering period, low temperatures caused minor damages.

Figure 2 shows the results of the 2013/14 winter. During this season, when the weather was moderate, 8 sampling dates were applied. In early September, the frost tolerance of flower buds was similar to the year presented earlier. Later, frost tolerance increased until mid-December and the differences between cultivars were more pronounced at this time. At the sampling date of 15 December, the $LT_{50}$ of flower buds of the examined cultivars were between -16 °C and -18.6 °C. The most frost hardy was 'Tétényi Keményhéjú', while 'Tétényi Bőtermő' was the most sensitive. In the second half of winter, the frost resistance of flower buds decreased gradually. Cold weather at the end of January and early February caused natural frost damages, however not all flowers were damaged, so we could continue our studies. The flowering time in 2014 started on 10 March. Based on the results of the climate chamber tests at this time, the $LT_{50}$ values were between -2 °C and -3 °C.

The third dormancy period, the results of which are presented in detail, was in 2018/19. It was the coldest of the winters studied. Eight sampling dates were applied (Figure 3). At the first sampling date, in early September, the frost resistance values of flower buds were similar to the other two years introduced earlier, the $LT_{50}$ values ranged between -2.9 °C and -4.8 °C. Then, frost resistance values as well as differences between the cultivars were increased. This winter, the highest cold hardiness values were measured in mid-January, followed by a slow gradual decline in frost tolerance. On 15 January, the $LT_{50}$ value of the flower buds was -17.6 °C for 'Tétényi Bőtermő', -19.1 °C for 'Tétényi Kedvenc', and -20.5 °C for 'Tétényi Keményhéjú'. At the beginning of January, due to the low temperature, there were a natural frost damages in our experimental orchard, but it did not endanger our investigations. In the second half of winter, due to the persistently low temperatures, the decrease in frost tolerance of flower buds was slower than in the other two years, and the flowering started late, after 20 March. Just before flowering, according to the results of the climate chamber studies, the $LT_{50}$ values were between -2 °C and -3.2 °C.

Based on the results of ten years, the average flower bud cold hardiness profile, calculated as a 10-year $LT_{50}$ average, was determined for the studied cultivars. These values show the expected frost resistance of these cul-

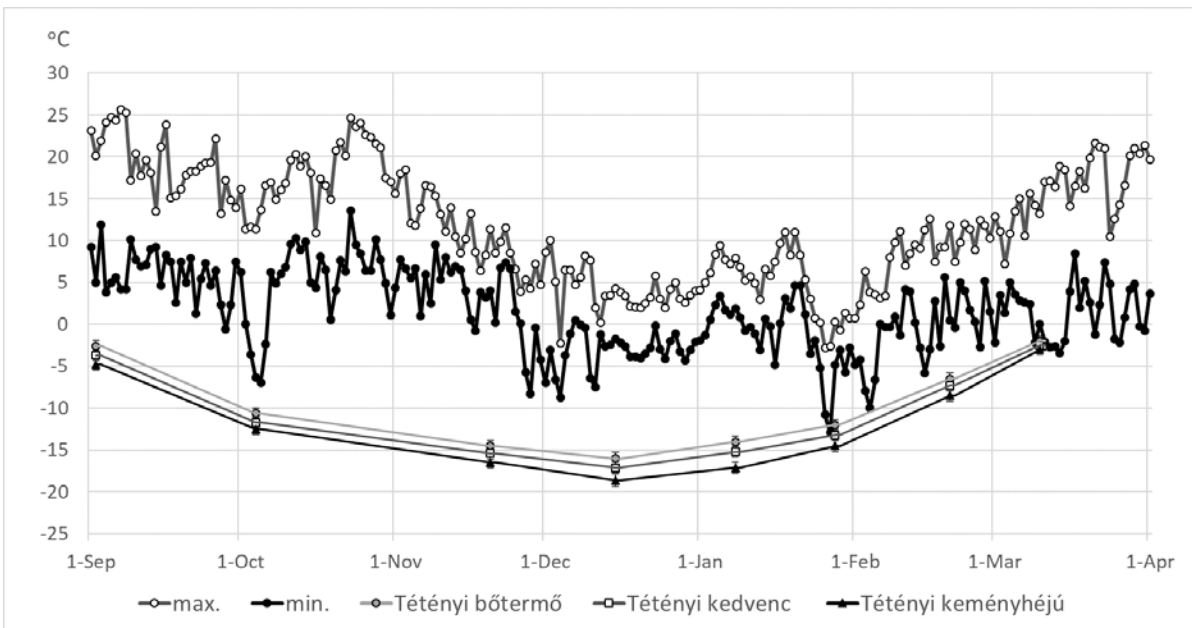


**Figure 2**: Daily maximum and minimum ambient temperatures, and $LT_{50}$ values of flower buds of three almond cultivars observed based on artificial freezing tests in 2013/14 winter

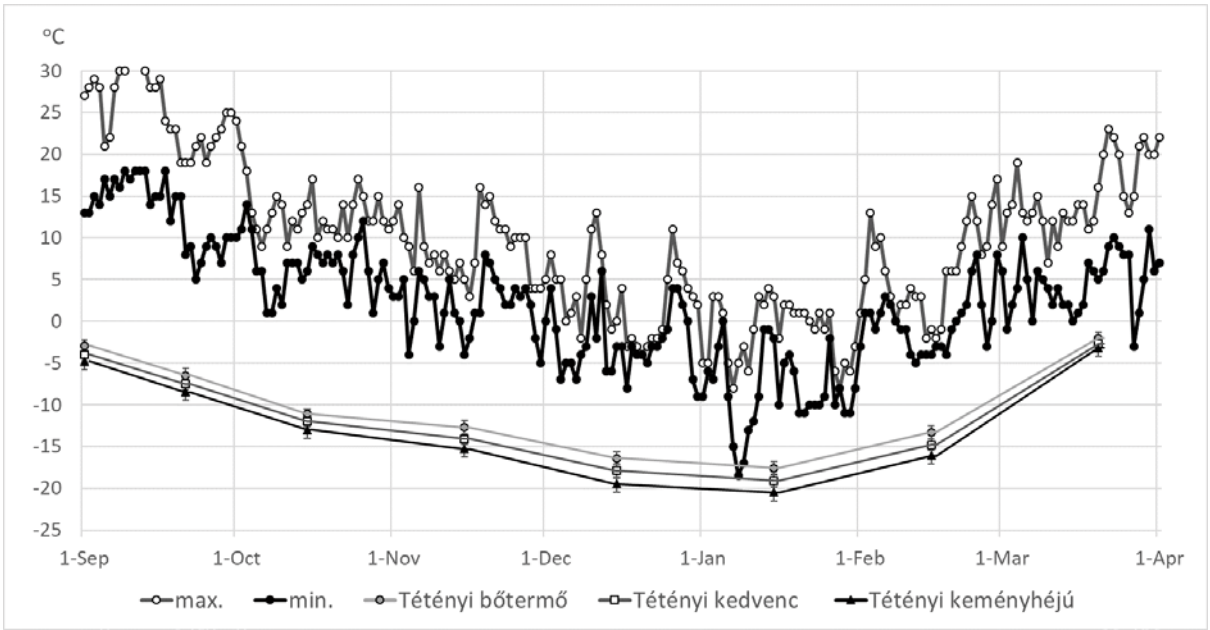


**Figure 3**: Daily maximum and minimum ambient temperature, and $LT_{50}$ values of flower buds of three almond cultivars observed based on artificial freezing tests in 2016/17 winter

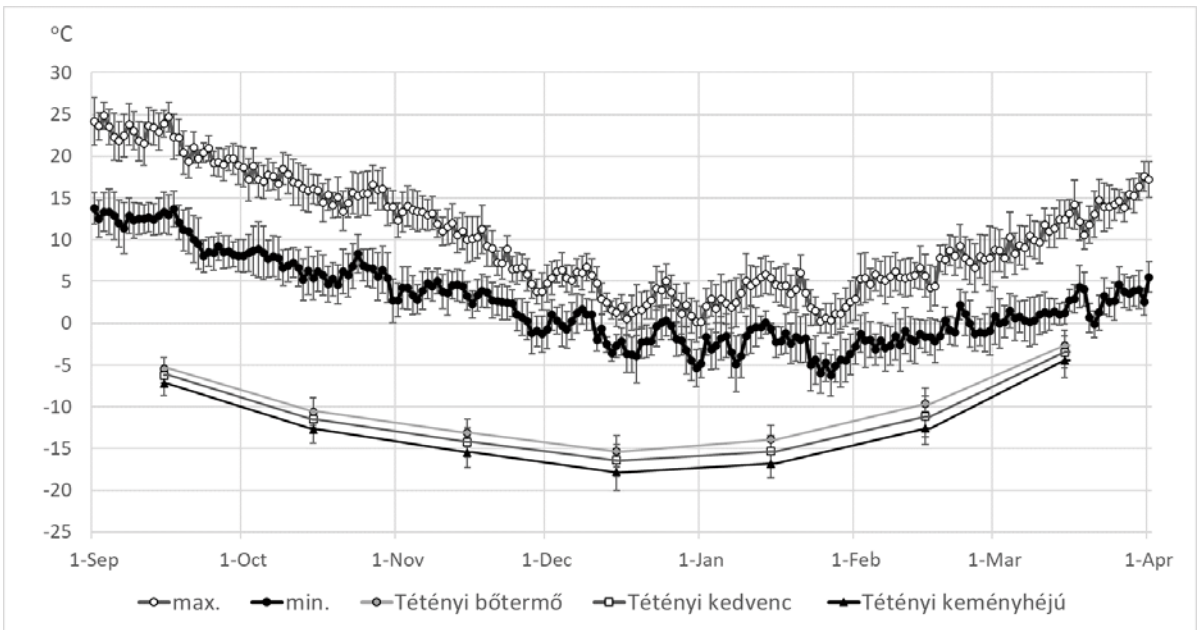


**Figure 4:** Averages of maximum and minimum daily temperature, and averages of $LT_{50}$ values of flower buds of three almond cultivars observed during ten-years winter dormancy

tivars in our geographical location in a common year. Because of technical reason the sampling dates were not the same days in different years, the characteristic points of frost hardiness profiles (in the middle of each month) were calculated by interpolation (Figure 4). In the middle of September average $LT_{50}$ values varied between -5.4 °C and -7.3 °C and parallel with decreasing temperatures frost tolerance of flower buds were increased, first faster, then slower. In the middle of December $LT_{50}$ values were -15.3 °C (± 1.83 °C) for 'Tétényi Bőtermő', -16.4 °C (± 1.91 °C) for 'Tétényi Kedvenc', and -17.8 °C (± 2.18 °C) for 'Tétényi Keményhéjú' in average of the years. It means

significant difference between 'Tétényi Bőtermő' and 'Tétényi Keményhéjú' ($p > 0.05$), but no significant difference between 'Tétényi Bőtermő' and 'Tétényi Kedvenc', and no significant difference between 'Tétényi Kedvenc' and 'Tétényi Keményhéjú'.

By the middle of March average $LT_{50}$ values have decreased to -2.7 °C, -3.5 °C, and -4.5 °C respectively.

The frost hardiness profile of the examined cultivars, which was characterized by the $LT_{50}$ values of flower buds, was different each year. This was due to differences in environmental factors, especially temperature. In all ten years the daily maximum and minimum temperatures showed great daily fluctuations in our experimental station, and the differences between years were also remarkable. The flower buds of the observed cultivars did not reach the genetically programmed maximum frost tolerance each year. The best frost tolerance was expressed in the coldest winter (2016/17), when the daily minimum temperatures dropped below zero after 1 November, and except for a few milder periods, it remained there until the end of February (Figure 3). The daily minimum temperatures stayed below -5 °C for long periods, and even temperatures below -10 °C frequently occurred. In that winter the best frost hardiness was measured in January. The situation was quite similar in the winter of 2015/16 and 2018/19, but in all of other winters the hardening period lasted earlier, in December, and during January the decreasing of frost tolerance was detected. The genetically potential maximum frost hardiness of flower buds of studied cultivars in our geographical location, and the expected values under different weather conditions were calculated based on the best $LT_{50}$ values of certain years (Figure 5). The statistical analysis shows significant differences between years from this aspect. If the autumn temperatures are decreasing gradually, and sub-zero temperatures are lasting, then slow increasing of temperature is detected, and there are no great fluctuations, $LT_{50}$ of flower buds can be -17.5 °C for 'Tétényi Bőtermő', -19 °C for 'Tétényi Kedvenc', and -20.5 °C for 'Tétényi Keményhéjú' in the middle of winter. But in extremely mild winters, with temperature fluctuations, just $LT_{50}$ values between -14.5 °C and -16 °C can be expected in these cultivars under our geographical location.

## 4 DISCUSSION

Almond production is limited by ecological conditions in Hungary. Winter and spring frosts mean the biggest risks. Unfortunately, the Hungarian variety descriptions do not address the issue of frost resistance (Brózik, 1998; Brózik et al., 2003; Apostol, 2013). There is little data on the frost tolerance of almond cultivars in the international literature as well. Some research works on almond have been dealing with frost resistance of flowers in different phenological stages or fruitlets during spring (Viti et al., 1994; Snyder & Conell, 1996; Kodad et al., 2010; Sepahvand et al., 2014), others have observed the

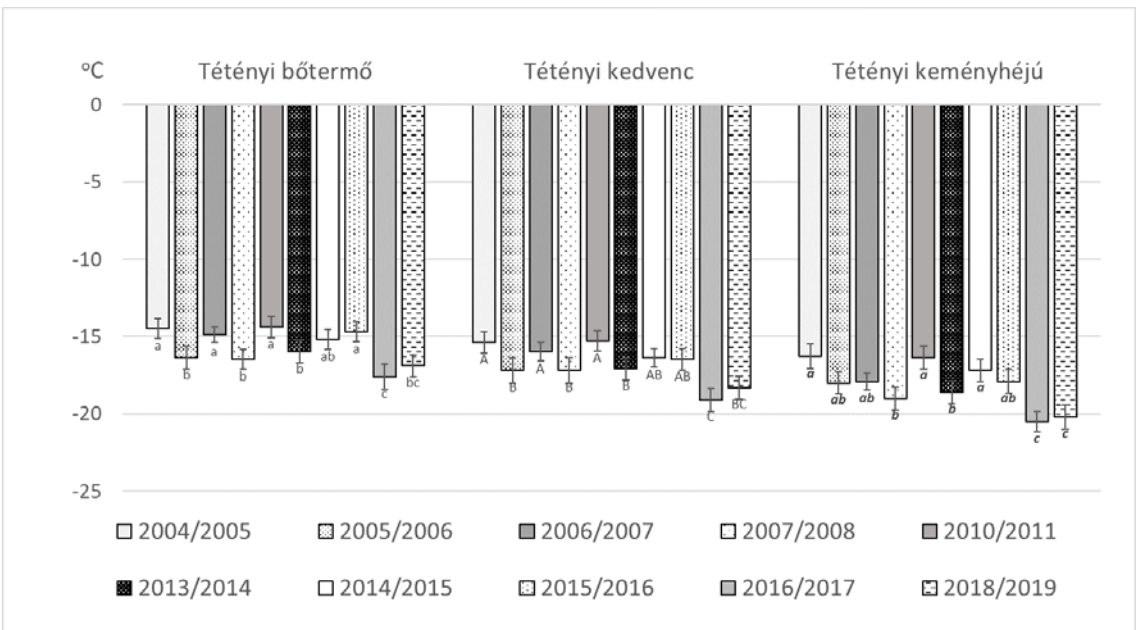


**Figure 5**: $LT_{50}$ values of flower buds of the studied almond cultivars in the middle of winter of different years (2004-2019); The columns show the mean values, the lines the standard deviation, and the letters the homogeneous groups, the different letters indicate statistically significant ($p \leq 0.05$) different values

frost hardiness of overwintering organs during dormancy (Szalay & Fonai, 2002; Miranda et al., 2005). The experimental results are difficult to compare because other cultivars, and different years were studied in different production sites. As general conclusion, however, it was shown that there are big differences between genotypes, and the ecological conditions have significant effect on the frost resistance.

The present paper is the first report about changes in frost hardiness of flower buds of Hungarian almond cultivars during the whole dormancy period. Summarising the results of ten years, frost tolerance of cultivars has varied over the years. In all years studied 'Tétényi Bőtermő' proved to be the most sensitive and 'Tétényi Keményhéjú' was the most tolerant, value of 'Tétényi Kedvenc' could be positioned in between them. In the first half of winter cold hardiness of overwintering organs developed progressively and reached their maximum in December, or, in some cases in January. Then their frost tolerance decreased until spring. Frost hardiness profile of the cultivars has been characterised by the $LT_{50}$ values of flower buds, calculated based on artificial freezing tests. Differences in frost resistance of cultivars were less representative in September and around flowering, however, the most considerable differences were detected in December and January, by the time the maximum frost tolerance developed.

In each year, fluctuation of winter temperatures were observed. Hardening and dehardening processes of almond flower buds were largely affected by weather conditions, especially temperature. Due to differences among years, we can conclude that the more years are studied, the most accurate results can be achieved. On the basis of a ten-year experiment, we made similar conclusions like in the case of apricot and peach, where Szalay (2001) and Szalay et al. (2010, 2016) found out that a gradual decrease in temperature at the first part of winter and later permanent cold is required for developing frost hardiness of flower buds. If any of these factors are missing, the genetic potential of frost resistance of overwintering organs cannot be realised. Among the interpreted years in this paper, the mildest winter resulted the worst frost hardiness of almond, whereas in the coldest season the best frost tolerance profile could be achieved. For describing correlation between changing in temperature and frost tolerance application of statistical analyses are limited. It could be that plant physiological processes are controlled by the inner temperature of plants that is always different from outside temperatures. The other reason is that not only the temperature, but other abiotic factors (light conditions, precipitation, photoperiod, etc.) have effect on plant physiology, therefore on cold hardiness of overwintering organs. Nevertheless, climate change results often mild and fluctuating winter temperatures, which are not conducive to hardening processes. The expected average frost hardiness of a cultivar can be determined as an average of $LT_{50}$ values of different years. In our case, based on 10-years observation, it is -15.3 °C for 'Tétényi Bőtermő', -16.4 °C for 'Tétényi Kedvenc', and -17.8 °C for 'Tétényi Keményhéjú'. The highest genetic potential of frost resistance has been determined, but due to the increasingly mild climate resulting from global warming, this will be less and less achieved by the cultivars. Therefore, it is very important to consider cold hardiness of the selected cultivars and the climate conditions of the growing site when designing an almond orchard.

## 5 CONCLUSIONS

Based on our results it is not recommended to establish an almond orchard in growing sites where winter temperatures regularly drop below -18 °C. From practical point of view it is important to have adequate information on the frost hardiness of almond cultivars that should be included into cultivar descriptions, our work hopefully could contribute to this aim. We can conclude that the growing site and the cultivar must be chosen very carefully when we want to establish an economically functioning plantation from almonds. Such a recommendation is an agreement with several publications dealing with different fruit species (Mohácsy & Porpáczy, 1951; Pejovics, 1976; Brózik et al., 2003; Kállayné, 2003, 2014; Di Lena et al., 2017).

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# Odločanje kmetov z območja Haloz o vključevanju v kmetijsko-okoljske ukrepe za ohranjanje ekstenzivne rabe travinja

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**Farmers' decision to participate in agri-environmental measures for the conservation of extensive grasslands in the Haloze region**

**Abstract:** Understanding the factors that influence farmers' decisions to participate in agri-environmental measures (AEMs) is crucial to meeting the environmental goals of agricultural policy. We investigated the factors of farmers' involvement in AEM aimed at maintaining extensive grassland use by analysing data from a survey of 258 farms and 40 interviews with farmers in the Haloze region. Results show that, in addition to adequate payment, farmers' attitudes towards grassland conservation and their self-identity are also important factors in increasing their willingness to join AEMs. A production-oriented view of farming prevailed among the interviewed farmers, with grassland conservation being important to them primarily in terms of maintaining a tidy landscape and achieving production and economic goals, while biodiversity conservation was often of negligible importance. The results indicate the educational and extension need in terms of farmers' understanding of the relationship between agriculture and nature conservation. The latter can be encouraged by strengthening advisory support and the use of locally and practically oriented knowledge transfer approaches, as well as by introducing result-based AEMs.

**Key words:** agri-environmental schemes; farmers' participation; decision-making process; social capital; self-identity; biodiversity; grassland conservation, Slovenia

**Odločanje kmetov z območja Haloz o vključevanju v kmetijsko-okoljske ukrepe za ohranjanje ekstenzivne rabe travinja**

**Izvleček:** Razumevanje dejavnikov, ki vplivajo na odločitev kmetov za vključitev v kmetijsko-okoljske ukrepe (KOU), je ključnega pomena za doseganje zastavljenih okoljskih ciljev kmetijske politike. Z analizo podatkov, ki smo jih pridobili z anketo na 258 kmetijah in z 40 intervjuji s kmeti na območju Haloz, smo raziskali dejavnike vključevanja kmetov v KOU, ki so namenjeni ohranjanju ekstenzivne rabe travinja. Največji vpliv na pripravljenost kmetov za vključevanje v KOU so imeli višina plačila in s tem vpliv ukrepa na dohodek kmetije ter odnos kmetov do kmetijske dejavnosti in ohranjanja narave. Med anketirani kmeti je prevladovala osredotočenost na proizvodnjo, medtem ko so pomen ohranjanja travinja povezovali predvsem z zagotavljanjem urejene krajine ter doseganja proizvodnih in ekonomskih ciljev. Ohranjanje travinja iz vidika biotske pestrosti je imelo med večino anketirancev manjši, pogosto zanemarljiv pomen. Rezultati kažejo na potrebo po okrepitvi izobraževanja z namenom izboljšanja razumevanja povezav med kmetijstvom in naravo. Slednje je mogoče spodbuditi s krepitvijo svetovalne podpore ter uporabo lokalno in praktično naravnanih pristopov prenosa znanja, pa tudi z uvedbo rezultatsko zasnovanih KOU.

**Ključne besede:** kmetijsko-okoljski ukrepi; vključanje kmetov; odločitveni proces; socialni kapital; samoindentiteta; biotska pestrost; ohranjanje travinja; Slovenija

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## 1 UVOD

Kmetijsko-okoljski ukrepi (KOU) Skupne kmetijske politike (SKP) so pomembno orodje zmanjšanjevanja negativnih vplivov kmetijstva na okolje in naravo ter ohranjanja pozitivnih učinkov kmetijstva v Evropski uniji (EU) (ECA, 2011). V okviru KOU kmetje prejmejo plačilo v zameno za prostovoljno izvajanje nadstandardnih kmetijski praks, ki so usmerjene k ohranjanju biotske pestrosti, varstvu voda in tal ter blaženju in prilagajanju na podnebne spremembe (Uthes in Matzdorf, 2013).

Raziskave o učinkih KOU ugotavljajo nekatere pozitivne vplive (Poláková in sod., 2011; Batáry in sod., 2015), vendar so ti pogosto nezadostni (Kleijn in sod., 2006; Kaligarič in sod., 2019), zato je treba raziskati možnosti za izboljšanje in povečanje uspešnosti KOU (ECA, 2011). Pomanjkljivosti se lahko kažejo predvsem v šibki intervencijski logiki pri načrtovanju ukrepov, neustreznem pristopu k izračunu višin podpor in premajhni ciljnosti ukrepov glede specifičnih okoljskih potreb (ECA, 2011; Batáry in sod., 2015). Podobne pomanjkljivosti kmetijsko-okoljskih plačil se kažejo tudi v Sloveniji (Erjavec in sod., 2018), kjer se kmetijsko-okoljski ukrepi izvajajo od leta 1999 (Travnikar in Volk, 2016). Šibkost ukrepov se dodatno odraža v razmeroma majhnem interesu kmetov za vključitev vanje (Žvikart, 2010; Žgavec in sod., 2013; Kaligarič in sod., 2019).

Zadostno zanimanje kmetov za sodelovanje v KOU lahko poveča verjetnost za uresničitev ciljev politike (Wilson & Hart, 2000), zato je za oblikovalce ukrepov koristen vplogled v dejavnike, ki vplivajo na odločanje kmetov (Falconer, 2000). Dejavniki odločanja kmetov za sodelovanje v KOU so bili v tujini predmet številnih raziskav (npr. Brotherton, 1989; Wilson in Hart, 2000; Ruto in Garrod, 2009; Defrancesco in sod., 2007), nekaj pa jih je bilo opravljenih tudi v Sloveniji. Slednje ugotavljajo, da so med ključnimi razlogi za majhno zanimanje kmetov za sodelovanje v KOU pogoji ukrepov, ki zahtevajo večje prilagoditve tehnologij pridelave na kmetiji (Pust Vučajnik in Udovč, 2008; Žgavec in sod., 2013), nestimulativna finančna nadomestila (Živkart, 2010; Žgavec in sod., 2013) in premajhna informiranost kmetov o KOU (Pust Vučajnik in Udovč, 2008; Žgavec in sod., 2013; Špur in sod., 2018).

Vključevanje kmetov v kmetijsko-okoljske ukrepe lahko pomembno vpliva na ohranjanje kmetijskih območij z visoko naravno vrednostjo (angl. *High Nature Value areas)* (Signorotti in sod., 2013), ki obsegajo predele Evrope, kjer kmetijstvo predstavlja prevladujočo rabo zemljišč in podpira ugodno ohranitveno stanje naravovarstveno pomembnih vrst in habitatov (Paracchini in sod., 2008). Območja z visoko naravno vrednostjo so precej ogrožena zaradi procesov intenziviranja kmetijstva na eni strani in opuščanja kmetovanja na drugi (Cunder, 2008; O'Rourke in Kramm, 2012).

V Sloveniji med območja z visoko naravno vrednostjo uvrščamo tudi gričevnate Haloze v spodnjem Podravju (Ivanjšič idr., 2020), kjer so se zaradi posebnih naravnih in socio-ekonomskih dejavnikov ohranili ekstenzivni načini kmetovanja (Korošec, 2019), ki so sooblikovali suhe traviščne habitatne tipe (Lipovšek, 2015). Med njimi so tudi polnaravna suha travišča in grmišče faze na karbonatnih tleh (*Festuco-Brometalia*) (* pomembna rastišča kukavičevk) (6210*), ki so varovana v okviru Direktive EU o habitatih (Direktiva 92/43/EGS) in so zaradi svoje ogroženosti predmet posebnega akcijskega načrta Evropske komisije za njihovo za ohranitev in obnovo (EC, 2019).

Eden izmed glavnih varstvenih instrumentov, ki ga je za območje Haloz predvideval Program upravljanja območij Natura 2000 v obdobju 2015–2020 (Vlada RS, 2015), je bil ciljni kmetijsko-okoljski ukrep Posebni traviščni habitati (ukrep HAB), ki naj bi spodbujal ohranjanje ekstenzivnih načinov rabe trajnega travinja. Kmetje so se z vključitvijo zavezali k pozni košnji oziroma paši in gnojenju travnikov zgolj z organskimi gnojili (MKGP, 2020). Vendar pa je bilo med kmeti na območju Haloz zanimanje za vključitev v ukrep HAB precej majhno. V letu 2019 je bilo na primer vključenih zgolj 23 kmetij s 76,6 ha travinja (Brdnik, 2019), kar je bistveno manj od varstvenega cilja, ki je predvideval, da bo v obdobju 2015–2020 v ukrep HAB na tem območju vključenih 1.097 ha travinja (Vlada RS, 2015). Zadostna količina površin, vpisanih v kmetijsko-okoljske ukrepe, pa je prvi pogoj za doseganje želenih okoljskih rezultatov (Kus Veenvliet, 2012).

V raziskavi smo želeli s pomočjo mešanih metod raziskovanja (Tashakkori in Teddie, 1998) proučiti zakaj se kmetje na območju Haloz le v redkih primerih odločajo za vstop v kmetijsko-okoljske ukrepe, namenjene ohranjanju ekstenzivne rabe travinja. S tem smo želeli pridobiti nova znanja, ki lahko prispevajo k preoblikovanju ukrepov na način, da bodo bolj zanimivi za kmete in posledično bolj učinkoviti pri varovanju traviščnih habitatov. S tem namenom smo želeli:

- preučiti odnos kmetov do kmetijsko-okoljskih ukrepov, ki so namenjeni ohranjanju ekstenzivne rabe travinja, in
- raziskati povezave med posameznimi dejavniki odločanja na vključevanje kmetov v kmetijsko-okoljske ukrepe.

### 1.1 DEJAVNIKI ODLOČANJA KMETOV GLEDE KOU

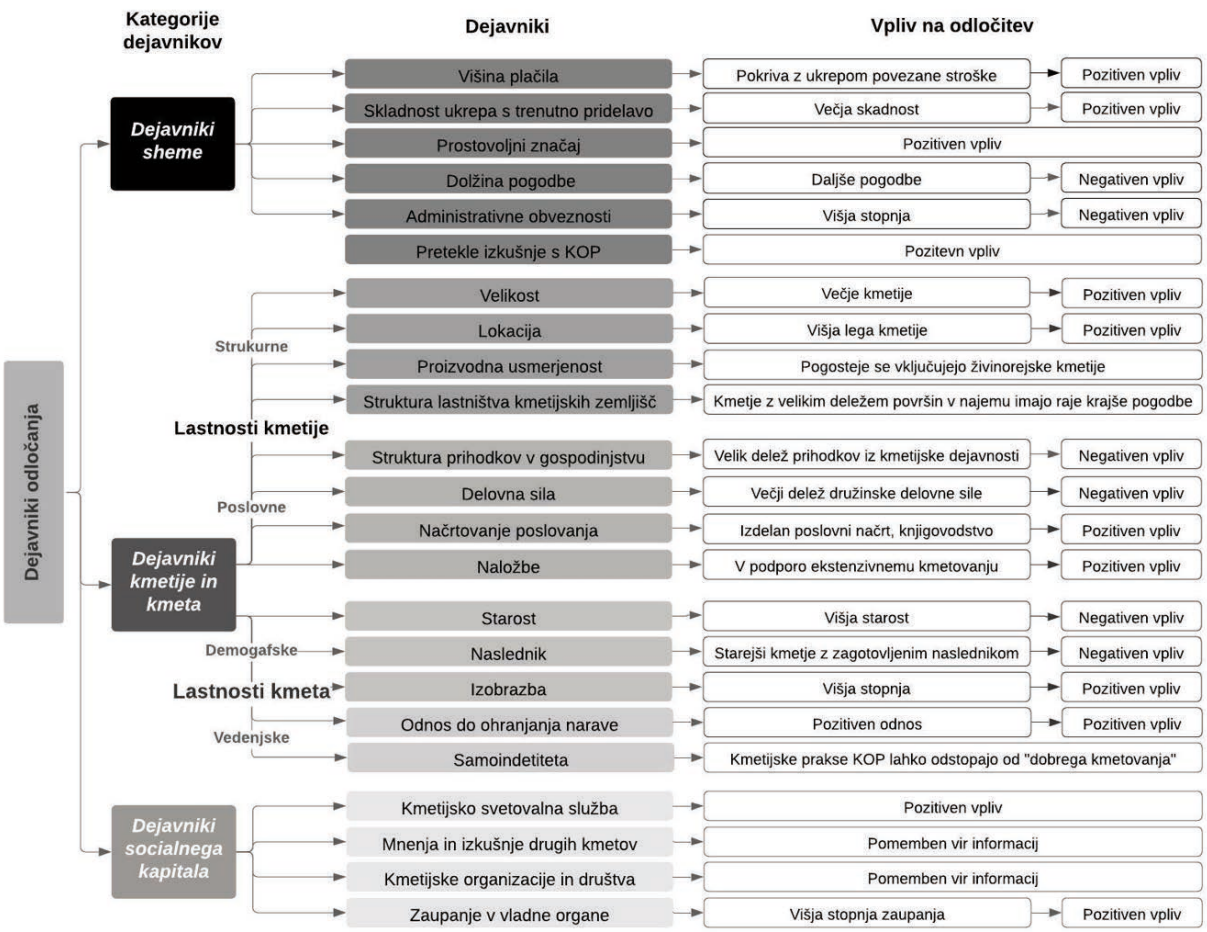
V literaturi avtorji razvijajo različne razvrstitve de-

javnikov odločanja za vključitev v kmetijsko-okoljske ukrepe (npr. Brotherton, 1989; Wilson in Hart, 2000; Ruto in Garrod, 2009; Lastra-Bravo in sod., 2015), ki jih lahko v grobem razdelimo v dejavnike, ki so povezani z zasnovo ukrepa, strukturnimi in poslovnimi značilnosti kmetije, demografskimi in vedenjskimi lastnosti kmeta ter socialnim kapitalom (Slika 1).

Najpomembnejša dejavnika, ki vplivata na preference kmetov do pogojev in zahtev ukrepov, sta višina plačila in skladnost zahtev ukrepa z obstoječim načinom kmetijske pridelave na kmetiji (Brown in sod., 2020). Kmetije, ki menijo, da ponujeno finančno nadomestilo v celoti pokriva z ukrepi povezane stroške, se lažje odločijo za sodelovanje, kar lahko do določene mere usmerja vedenje kmetov. Finančna nadomestila pa lahko tudi oslabijo notranje motive za izvajanje kmetijsko-okoljskih praks, s čimer prej samoumevna vedenja lahko postanejo zgolj finančno motivirana (Burton in Paragahawewa, 2011; de Snoo in sod., 2013). V splošnem velja, da manjši kot bo zahtevan obseg sprememb obstoječih kmetijskih praks, večja bo verjetnost, da se bodo kmetje odločili za vstop v kmetijsko-okoljske ukrepe (Defrancesco in sod., 2007).

Prostovoljni značaj ukrepov se je izkazal kot pozitiven dejavnik, ki praviloma spodbuja in pospešuje pripravljenost kmetov za vključitev (Wilson, 1997). Kmetje, predvsem starejši, v povprečju preferirajo čim krajšo dolžino pogodbe (v smislu trajanja obveznosti). Zaželena je tudi čim manjša obremenitev z vidika administracije in nadzora (Ruto in Garrod, 2009). Na vključitev praviloma spodbudno vplivajo tudi pretekle izkušnje kmetov s KOU in drugimi okolju prijaznimi kmetijskimi praksami (Wilson in Hart, 2000; Defrancesco in sod., 2007)

Izmed strukturnih značilnosti kmetije so pomembne predvsem velikost, lokacija in proizvodna usmerjenost kmetije ter struktura lastništva kmetijskih zemljišč. Raziskave kažejo, da se za vključitev raje odločajo večje (Wilson in Hart, 2000; Siebert in sod., 2006), višje ležeče (Defrancesco in sod., 2007; Capitanio in sod., 2011) in živinorejsko usmerjene kmetije (Peerlings in Polman,




**Slika 1**: Diagram dejavnikov odločanja kmetov za vključevanje v kmetijsko-okoljske ukrepe (povzeto po Wilson, 1997; Ruto in Garrod, 2009; Lastra-Bravo in sod., 2015)

2009; Capitanio in sod., 2011). Zaradi raznolikosti okoljskih in socio-ekonomskih značilnosti kmetij na preučevanih območjih ter tudi zaradi različnih opredelitev strukturnih značilnosti kmetij (npr. majhne in velike kmetije), ni povsem jasne in enoznačne ugotovitve, kako ti dejavniki vplivajo na vključitev v KOU (Lastra-Bravo in sod., 2015).

Med poslovnimi značilnostmi kmetij lahko na odločanje vpliva struktura prihodkov v gospodinjstvu, pri čemer velja, da se z večanjem deleža prihodkov iz kmetijske dejavnosti pripravljenost kmetov za sodelovanje v ukrepih zmanjšuje (Defrancesco in sod., 2007; Barreiro-Hurlé in sod., 2010). Druge poslovne značilnosti kmetije s pomembnim vplivom na odločanje kmetov so še delež družinske delovne sile, načrtovanje poslovanja (npr. izdelan poslovni načrt, knjigovodstvo in vrsta naložb na kmetiji) (Lastra-Bravo in sod., 2015).

Razumevanje demografskih in vedenjskih lastnosti kmeta na odločanje je precej kompleksnejše od prej navedenih (Wilson, 1997). Dosedanje raziskave kažejo, da so mlajši kmetje bolj pripravljeni izvajati programe KOU kot starejši kmetje (Lastra-Bravo in sod., 2015). Vključevanje slednjih je povezano tudi s tem, ali imajo zagotovljenega naslednika (Potter in Lobley, 1992), saj kmetje naslednikov pogosto ne želijo obremenjevati z že sklenjeno pogodbo in se zato ne odločijo za sodelovanje (Ruto in Garrod, 2009). Ugotovljeno je bilo tudi, da višja stopnja formalne izobrazbe poveča verjetnost, da se bo kmet odločil za vključitev v KOU (Lastra-Bravo in sod., 2015).

Vse več novejših raziskav (npr. Thomas in sod., 2019; Cullen in sod., 2020) prepoznava pomen vedenjskih in psiholoških dejavnikov pri odločanju kmetov, kot sta samoidentita in odnos do ohranjanja narave. Pri prepoznavanju teh dejavnikov je uporaben koncept »dobrega kmeta« (Burton in sod., 2008). Gre za kolektivno prepoznan in deljen zbir idej o pravilnem, pričakovanem in zaželenem vedenju kmeta, ki je rezultat vseživljenske socializacije v določenem družbenem prostoru. V lokalnem okolju »dobri kmetje« večinoma veljajo za tiste, ki dosegajo velike donose v kmetijski pridelavi in prireji, ohranjajo »urejene« kmetijske površine, so dobro umeščeni in prepoznavni na trgu, imajo vzorno urejeno rejo živine in podobno (Silvasti, 2003; Burton in Paragahawewa, 2011; Sutherland in Darnhofer, 2012). Kmetje zato kmetijske prakse, ki jih spodbujajo KOU (npr. ekstenzivna reja živine, puščanje strnjenih nepokošenih pasov), pogosto težje sprejmejo, saj v njihovi skupnosti niso prepoznane kot pokazatelji pričakovanega vedenja »dobrega kmeta«, to pa jih lahko odvrne od vključitve v tovrstne ukrepe (Burton in Paragahawewa, 2011; Slovenc, 2019).

Zadnja skupina dejavnikov se nanaša na socialni kapital. Ta združuje dejavnike, ki upoštevajo vključenost kmeta v družbene vezi in omrežja ter predstavljajo pomemben vir informacij o KOU (Mathijs, 2003). Kmetje, ki so dobro informirani o KOU, so običajno bolj zainteresirani za vključitev (Wilson in Hart, 2000). Informacije lahko pridobijo iz različnih virov, kot so svetovalna služba, sosednji kmetje, kmetijske organizacije in društva ter kmetijski mediji (Lastra-Bravo in sod., 2015). Med temi je zlasti pomembna svetovalna služba, ki praviloma spodbudno vpliva na odločitev kmetov za vstop v KOU (Lastra-Bravo idr., 2015). Pri tem imajo večje kmetije običajno več stikov s svetovalno službo, zato so lahko o KOU bolje informirane kot majhne kmetije (Wilson, 1997).

## 2 MATERIALI IN METODE

### 2.1 RAZISKOVALNO OBMOČJE

Raziskava je potekala na območjih Nature 2000 Haloze-vinorodne in Boč - Haloze - Donačka gora v skupni velikosti 171,8 km$^2$, kjer so se zaradi posebnih geoloških, podnebnih in drugih dejavnikov razvili nekateri naravovarstveno pomembni habitatni tipi. Mednje sodi habitatni tip polnaravna suha travišča in grmiščne faze na karbonatnih tleh (6210*), ki pokriva približno 30 % vseh negozdnih površin obravnavanega območja (Jakopič in Trčak, 2006). Za ohranjanje tovrstnih travišč je treba vzdrževati ekstenzivne načine kmetovanja, ki vključujejo pozno košnjo brez dosejevanja travnih mešanic in dodatnega gnojenja (Jakopič in Trčak, 2006). Naravovarstvene grožnje so na območju povezane predvsem z opuščanjem rabe in posledičnim zaraščanjem zemljišč (Žiberna, 2012; Ivanjšič in sod., 2020), v manjši meri pa je problem tudi preveč intenzivna raba travnikov in pašnikov, ki prav tako povzroča izginjanje varstveno pomembnega travinja.

Večino (56,0 %) raziskovalnega območja je v letu 2018 pokrival gozd, medtem ko je trajno travinje zavzemalo 24,3 % površine (4176 ha). Pomemben delež (8,5 %) površine pokrivajo tudi kmetijska zemljišča v različnih stopnjah zaraščanja (tipi rabe 1410–1600) in trajni nasadi (4,7 %), izmed katerih je bilo največ vinogradov (1,7 %). Njivskih površin je bilo v letu 2018 relativno malo (3,0 % oziroma 522 ha). V evidenci kmetijskih zemljišč (GERK) je bilo v letu 2018 zajetih okrog 47,2 % kmetijskih zemljišč, ki so lahko upravičena do prejemanja različnih podpor Skupne kmetijske politike in lahko sklepamo, da na njih poteka aktivna kmetijska raba. Nekoliko boljše je stanje na travinju, saj ga je bilo v sistem podpor kmetijske politike vključenih 60,7 %.

Z zemljišči je na obravnavanem območju leta 2018 upravljalo skupaj 986 kmetijskih gospodarstev, med katerimi prevladujejo majhne kmetije. Dobra polovica (58,8

%) kmetijskih gospodarstev upravlja z manj kot petimi hektarji kmetijskih zemljišč, okrog četrtina (26,3 %) pa s 5 do 10 hektarji. Velikih kmetij, ki upravljajo z več kot 50 hektarji kmetijskih zemljišč, je zgolj 1,0 %.

V kmetijsko-okoljske ukrepe, ki so se izvajali v okviru Programa razvoja podeželja 2014-2020 (v nadaljevanju KOPOP), je bilo v letu 2017 vključenih 10,2 % haloških kmetijskih gospodarstev. V ukrepu Posebni travišči habitati (HAB), ki se izvaja v okviru KOPOP, pa je sodelovalo zgolj 1,9 % (19) kmetij. Območje sodi med območja z omejenimi dejavniki za kmetijsko pridelavo, zato so kmetijska gospodarstva upravičena tudi do izravnalnih (dohodkovnih) plačil. Analizo stanja kmetijstva za potrebe opisa raziskovalnih območij smo izvedli s prostorsko analizo v programskem okolju ArcGIS (ESRI, verzija 10.5). Podatke smo pridobili iz evidence dejanske rabe kmetijskih in gozdnih zemljišč in atributnih podatkov iz zbirnih vlog za leti 2017 in 2018.

Glede na podatke iz zadnjega popisa kmetijskih gospodarstev iz leta 2010 na širšem območju Haloz prevladuje mešana pridelava (35,7 %) in specializirana reja pašne živine (34,7 %). Okrog petina kmetijskih gospodarstev je pridelovalcev poljščin (21,4 %), medtem ko je gojiteljev trajnih nasadov 7,6 %. Prevladujejo kmetije (73,8 %), ki pretežno pridelujejo za lastno porabo (SURS, 2020).

## 2.2 RAZISKOVALNI PRISTOP

Z namenom pridobitve natačnejšega vpogleda v proces vključevanja kmetov v kmetijsko-okoljske ukrepe na območju Haloz smo uporabili mešane metode raziskovanja (angl. mixed method research; Tashakkori in Teddlie, 1998), ki jih literatura navaja kot primeren pristop k raziskovanju družboslovnih vidikov ohranjanja narave (Torkar in sod., 2011; Lastra-Bravo in sod., 2015). Tako smo kvantitativni del raziskave, ki je zajemal statistično analizo podatkov, pridobljenih iz strukturiranega vprašalnika (258 anketirancev), podprli s kvalitativno analizo, ki je temeljila na analizi pogovorov s kmeti, posnetimi med anketiranjem (40 pogovorov).

### 2.2.1 Anketiranje in kvantitativna analiza

Anketiranje kmetov je potekalo v marcu in aprilu 2019 na Ptuju in v Slovenski Bistrici v času letne oddaje zbirnih vlog za kmetijske podpore. K anketiranju so bila povabljena vsa registrirana kmetijska gospodarstva, ki imajo na območju Natura 2000 Haloze v upravljanju vsaj 0,3 hektarjev trajnega travinja in zbirne vloge oddajajo na sedežu Javne službe kmetijskega svetovanja na Ptuju in v Slovenski Bistrici, ne glede na predhodno sodelovanje v KOU. Od 680 kmetov, ki so ustrezali opisanim pogojem, jih je v raziskavi sodelovalo 258 oziroma 37,9 %.

Anketiranje je izvajalo šest usposobljenih anketark, ki so z vsakim kmetom individualno izpolnile spletni vprašalnik. Vprašalnik je bil sestavljen iz štirih delov. Prvi del je vključeval vprašanja o poznavanju in sodelovanju v kmetijskih ukrepih ter površini in lastniški strukturi obdelovalnih površin. Drugi del je bil sestavljen iz vprašanj o indikatorskih vrstah suhih ekstenzivnih travišč ter o pomenu in koristih ohranjanja narave. Sledil je del, ki se je nanašal na ukrep Posebni travišči habitati (ukrep HAB), kjer so kmetje glede na razlago anketarja izbirali med različnimi alternativnimi zasnovami ukrepa HAB. Zadnji del vprašalnika je zajemal vprašanja o značilnostih kmetijskega gospodarstva in o demografskih značilnostih kmeta. Vprašalnik smo predhodno testirali na vzorcu 22 kmetov.

Opisna analiza pridobljenih podatkov in kvantitativna analiza sta potekali v programskem okolju STATA (StataCorp, verzija 16.1). Homogenost nominalnih spremenljivk smo preverili s testom hi-kvadrat. Za preverjanje povprečnih številskih spremenljivk smo uporabili enosmerni ali dvosmerni test ANOVA, kot neparametrično alternativo pa Mann-Whitneyev U-test. Slednji test smo uporabili tudi v primeru ordinalnih spremenljivk (Acock, 2014). Statistično analizo smo izvedli za pet skupin anketirancev, in sicer tiste, ki so kot pomemben dejavnik pri odločanju izpostavili višino plačila, vpliv ukrepa na pridelano krmo, administrativne obveznosti in nadzor, dolžino pogodbe in mnenje kmetijskega svetovalca (Preglednica 1).

### 2.2.2 Kvalitativna analiza

Če je anketiranec v to privolil, smo pogovor med izvajanjem ankete snemali, saj se je izkazalo, da so kmetje svoje odgovore pogosto dodatno utemeljili in podajali svoja mnenja, ki so pomembna za podrobnejšo analizo njihovih stališč. Pogovori so trajali od 30 do 100 minut.

Za potrebe kvalitativne analize smo izmed 160 posnetkov pogovorov izbrali 40 daljših posnetkov, ki so vključevali največ dodatnih pojasnil anketirancev. Po poslušanju smo za vsak posnetek najprej pripravili transkripcijo pogovorov, ki smo jih nato večkrat prebrali in analizirali s pomočjo kodiranja (Saldana, 2015). V procesu kodiranja smo posameznim relevantnim delom besedila o obravnavani temi pripisali pojme (kode). Besedila, ki smo jim pripisali isti pojem, smo zbrali in jih ločili od besedil, ki spadajo pod drug pojem. Sledila je organizacija besedila, kjer smo združili pomensko sorodne podatke oziroma pojme. Kodiranje nam je omogočilo zmanjšanje

**Preglednica 1**: Opisna statistika za anketirance zajete v kvantitativni del raziskave

| Spremenljivka | n | SD |
|---|---|---|
| Število | 258 | |
| Povprečna starost (leta) | 57 | 12,0 |
| Povprečna velikost kmetije (ha) | 6,1 | 4,1 |
| | % | |
| Reja živine | 81 | |
| Delež dohodkov iz kmetijstva v gospodinjstvu | | |
| < 25 % | 78 | |
| > 75 % | 3 | |
| Prihodnjost kmetije | | |
| - nadaljevanje kmetijske dejavnosti | 83 | |
| - opustitev živinoreje, vendar nadaljnja obdelava zemljišč | 13 | |
| - opustitev kmetijske dejavnosti | 4 | |
| Sodelovanje v KOPOP | | |
| - trenutno vpisani | 17 | |
| - vpisani pred 2015 | 26 | |
| - ukrep poznajo, vendar se niso vpisali | 44 | |
| - ukrepa ne poznajo | 14 | |

obsega podatkov in povezavo razdrobljenih pomenov raziskovalne tematike v vsebinsko in pomensko zaključene celote. Rekonstrukcija dobljenih podatkov v nove zaključene pomenske celote je omogočala novo poglobljeno razumevanje podatkov (Roblek, 2009). Pogovore s kmeti smo analizirali v programskem okolju ATLAS.ti (Cleverbridge, verzija 8).

### 2.3 OPISNA ANALIZA VZORCA

Od 258 anketirancev je bilo 60 % moških in 40 % žensk. Povprečna starost anketiranih je bila 57 let. Večina anketirancev (53 %) je imela zaključeno srednješolsko izobrazbo, formalno kmetijsko izobrazbo pa 8 % anketirancev.

V povprečju so anketiranci upravljali s 6,1 ha kmetijskih zemljišč oziroma 4,81 ha trajnega travinja. Prevladovale so kmetije, ki so imele upravljana zemljišča v celoti v svoji lasti (69,0 %). Na večini kmetij so redili živino (80,6 %) in najpogosteje so imeli na kmetijah mešano kmetijsko proizvodnjo (67,4 %). Prevladovale so izključno samooskrbne kmetije (37,6 %) in kmetije, ki pridelujejo pretežno za lastno porabo (34,5 %). Več kot tri četrtine anketiranih je odgovorilo, da dohodki iz kmetijske in gozdarske dejavnosti (vključno s kmetijskimi subvencijami) predstavljajo manj kot 25 % celotnih dohodkov gospodinjstva. Velika večina anketirancev je bila mnenja, da bodo v naslednjih desetih letih nadaljevali s kmetijsko dejavnostjo, vendar na večini kmetij naslednik trenutnega gospodarja kmetije (še) ni bil predviden (55 %). V prostovoljne KOU je bilo v času anketiranja ali že kdaj v preteklosti vključenih slaba polovica anketirancev. Večina anketirancev je ukrep HAB poznalo, vendar se niso odločili za vključitev (Preglednica 1).

Anketiranci, vključeni v kvalitativni del raziskave (v nadaljevanju sogovorniki), so bili po demografskih značilnostih, strukturi kmetijskih gospodarstev in po poznavanju oziroma vključenosti v kmetijsko-okoljske ukrepe primerljivi s celotnim vzorcem.

## 3 REZULTATI IN RAZPRAVA

Rezultati raziskave so predstavljeni v treh kategorijah dejavnikov, ki vplivajo na vključevanje kmetov v KOU: dejavniki ukrepa, kmetije in kmeta ter socialnega kapitala. Rezultati so dodatno podkrepljeni z izjavami kmetov, ki ilustrirajo njihovo razmišljanje o posameznih dejavnikih odločanja.

### 3.1 DEJAVNIKI UKREPA

#### 3.1.1 Višina plačila

Kmetje vpliv KOU na dohodek kmetije prepoznavajo preko višine plačila, zato je ta med ključnimi dejavniki, ki vplivajo na odločanje kmetov za vključitev v KOU (Brown in sod., 2020). To je razvidno tudi iz odgovorov anketiranih kmetov v raziskavi na Halozah, saj sta pomembnost plačila in dohodka pri odločanju poudarili skoraj dve tretjini anketirancev (65 %). Anketiranci, ki so kot pomemben dejavnik pri odločanju izpostavili višino plačila, so bili v povprečju nekoliko mlajši, njihovo gospodinjstvo pa je v povprečju štelo več članov, upokojencev in otrok, imeli pa so tudi nekoliko višje dohodke (preglednica 2), kar kaže na osebe v aktivni delovni dobi in z družinskim življenjem. Njihova kmetija je bila v zadnjih 10 letih tudi pogosteje investicijsko aktivna. Kar se tiče kmetijsko-okoljskih ukrepov so bila ta kmetijska gospodarstva pogosteje že kdaj vpisana v KOU in v ukrep HAB.

Sogovorniki, ki so upravljali predvsem z intenzivnejšimi kmetijami, so višino plačila običajno presojali z vidika pokritja stroškov dela in izgubljene krme zaradi pozne košnje, ki jo zahteva ukrep HAB. Izpostavili so, da je bilo plačilo trenutnega ukrepa HAB premajhno in zato ni odtehtalo, da bi se odločili za vstop. Večje plačilo so

**Preglednica 2**: Uporabljen statistični test in statistična značilnost primerjanih skupin anketirancev

| | | VPLAČ | | KRMA | | ADMIN | | DOLŽ | | SVET | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Izpostavljena lastnost | | Stat. | Znač. | Stat. | Znač. | Stat. | Znač. | Stat. | Znač. | Stat. | Znač. |
| Kmet. gospodar | Starost | F - | ** | F - | ** | F + | ** | | | | |
| | Spol - ženske | | | | | | | $\chi^2$ + | * | | |
| | Dosežena izobrazba | | | | | | | | | | |
| | Št. članov gospodinjstva | F + | * | F + | ** | | | | | | |
| | Št. upokojencev | F - | * | | | | | | | F - | * |
| | Št. otrok | F + | * | | | | | | | | |
| | Višina dohodkov gospodinjstva | M-W + | ' | M-W + | ** | | | M-W - | *** | | |
| | Delež dohodkov iz kmet. dejavnosti | | | $\chi^2$ + | *** | | | | | | |
| Kmetija | Velikost kmetije | | | F + | ** | | | | | F - | * |
| | Delež površin v najemu | | | M-W + | * | | | | | | |
| | Živinorejske kmetije | | | $\chi^2$ + | *** | | | | | | |
| | Tržna usmerjenost kmetije | | | $\chi^2$ + | * | | | | | | |
| | Investicijska aktivnost | $\chi^2$ + | ** | $\chi^2$ + | ** | | | | | | |
| Info | Poznavanje ukrepov SKP | | | M-W + | *** | | | M-W - | * | | |
| | Predhodne izkušnje s KOU | $\chi^2$ + | ** | $\chi^2$ + | * | | | | | | |
| | Predhodne izkušnje s HAB | $\chi^2$ + | ** | | | | | | | | |
| Dejavniki ukrepa | Vpliv višine plačila | | | | | $\chi^2$ - | *** | | | | |
| | Vpliv na krmo | | | | | $\chi^2$ - | * | | | | |
| | Administracija in nadzor | | | $\chi^2$ | * | | | | | | |
| | Vpliv ukrepa na okolje | | | | | $\chi^2$ - | ** | $\chi^2$ - | ** | | |
| | Dolžina pogodbe | $\chi^2$ - | * | | | | | | | $\chi^2$ - | ** |
| | Mnenje kmetijskega svetovalca | | | $\chi^2$ - | *** | $\chi^2$ - | *** | | | | |
| | Izkušnje drugih kmetov | | | $\chi^2$ | ** | | | | | | |

Legenda: VPLAČ – kmetje, ki so kot pomemben dejavnik pri odločanju izpostavili višino plačila, KRMA – vpliv ukrepa na pridelano krmo, ADMIN – administrativne obveznosti in nadzor, DOLŽ – dolžino pogodbe, SVET – mnenje kmetijskega svetovalca; F – ANOVA, M-W – Mann-Whitneyev U-test in $\chi^2$ – test hi-kvadrat; + pozitiven vpliv, - negativen vpliv
*** $p < 0,05$, ** $p < 0,01$, * $p < 0,001$, ' $p < 0,1$

pričakovali predvsem zato, ker je za ohranjanje površin na območju Haloz potrebnega veliko ročnega dela, saj uporaba kmetijske mehanizacije zaradi strmih naklonov pogosto ni mogoča.

> »Ni problem se vpisati v kakšen ukrep za kmetijsko-okoljske spremembe. To se mi bi. Mi smo naklonjeni zmanjšanju GVŽ in ohranjanju suhega travinja pa vstopanju v okoljske ukrepe, samo pod pogojem, da nam povišajo [plačila za] te ukrepe. Mi se ne strinjamo s temi slabimi subvencijami… s tem denarjem, ki nam ga oni ponujajo, naravovarstveniki.« (intervju št. 22, Haloze)

Primerna višina plačil zagotovo spodbuja kmete za vstop v KOU, so pa rezultati kvalitativne analize skladno z ugotovitvami preteklih raziskav (npr. Siebert in sod., 2006; Schenk in sod., 2007) pokazali, da kmetje pri odločanju upoštevajo tudi druge dejavnike. Nekateri kmetje niso bili pripravljeni vstopiti v kmetijsko-okoljske ukrepe

ne glede na višino plačila, saj jim je bila pomembnejša njihova neodvisnost. Mnogi sogovorniki so poudarili, da ne bodo spremenili načina kmetovanja in se prilagodili potrebam ukrepa v zameno za plačilo. Pri tem je potrebno vzeti v ozir, da so imeli kmetje pogosto občutek, da so bili v preteklosti samostojnejši in se jim ni bilo treba prilagajati veliko zahtevam, zato se kmetje, predvsem starejši, pogosto težje prilagodijo večjemu številu predpisov in zahtev (Schenk in sod., 2007).

### 3.1.2 Pričakovani učinki KOU na pridelano krmo za potrebe živinoreje

Kmetje običajno verjamejo, da je za zagotovitev zadostnega zaslužka treba dosegati velike pridelke z intenzivnejšim kmetovanjem, zato je zagotavljanje velikih pridelkov eden od osrednjih simbolov koncepta »dobrega kmeta« oziroma gospodarja (Sutherland in Darnhofer, 2012). Možnost zaslužka kmetje pogosto ne povežejo z ohranjanjem narave ali z izvajanjem kmetijsko-okoljskih ukrepov, zato so ekonomski motivi izrazitejši od motivov ohranjanja narave (Ahnström in sod., 2009). Podobno razmišljanje je prisotno tudi med anketiranci na raziskovalnem območju. Sogovorniki so pogosto poudarili, da je zanje »osnova krma« oziroma z drugimi besedami, da se niso pripravljeni vključiti v tovrstne ukrepe, ker bi zaradi zahtev ukrepa izgubili kvaliteto in količino krme ter s tem dohodek, ki ga pridobijo z rabo travinja preko živinoreje.

> »Dejansko nimaš nič od tiste trave, ki jo pokosiš. Mi rabimo travo za krmo živali. Zaenkrat je košnja že v začetku maja ali že prej in potem preveč izgubiš, da bi se vključil. Ni rentabilno.« (intervju št. 30, Haloze)

Dobra tretjina (38 %) anketirancev je vpliv ukrepa na količino in kakovost pridelane krme in s tem na dohodek kmetije označilo kot enega izmed najbolj pomembnih dejavnikov, ko razmišljajo o vstopu v kmetijsko-okoljske ukrepe. Ti anketiranci so bili statistično značilno mlajši in so prihajali iz gospodinjstev, ki imajo višje dohodke in večji delež dohodkov iz kmetijske in gozdarske dejavnosti. V primerjavi z drugimi kmetijami pa je bilo tudi statistično značilno večje število članov gospodinjstva, ki aktivno pomagajo pri delu na kmetiji.

Statistično značilne so tudi razlike v značilnostih kmetijskega gospodarstva, s katerim so upravljali, in sicer so v povprečju upravljali z večjim obsegom kmetijskih zemljišč in trajnega travinja, prav tako je bil večji delež zemljišč, ki so ga imeli v najemu. Pogosto je šlo za živinorejska kmetijska gospodarstva, ki so usmerjena v prodajo in so v zadnjih desetih letih tudi pogosteje izvedla nove investicije na kmetiji. Ti anketiranci so ukrepe kmetijske politike v splošnem poznali bolje kot drugi kmetje, prav tako so bili pogosteje že kdaj vpisani v KOU (Preglednica 2).

Zanimivo je, da je pri odločanju o vstopu v kmetijsko-okoljske ukrepe ta skupina kmetij redkeje izpostavljala administrativne obveznosti in kontrolo, dolžino pogodbe (5 let) ter mnenje kmetijskega svetovalca in izkušnje drugih kmetov (Preglednica 2). Predvidevamo torej lahko, da so ti kmetje pri odločanju o ukrepih dokaj samostojni, pogodbene obveznosti pa jim ne predstavljajo večje ovire, saj gre pogosto za (pol)profesionalna kmetijska gospodarstva.

### 3.1.3 Administrativne obveznosti in nadzor

Raziskave kažejo, da administrativne obveznosti, ki so povezane s sodelovanjem v ukrepu, kot so oddaja vloge, vodenje evidenc in nadzor nad izvajanjem ukrepa, praviloma negativno vplivajo na odločitev za sodelovanje v KOU (Ruto in Garrod, 2009; Lastra-Bravo in sod., 2015; Pavlis in sod., 2016). Podobno se kaže tudi iz odgovorov haloških kmetov, saj je administrativne obveznosti in izvajanje nadzora nad izvajanjem zahtev ukrepa 39 % anketiranih opredelilo kot pomemben dejavnik odločanja. Administrativne obveznosti predstavljajo oviro predvsem najstarejšim kmetom, saj ti, kot se je izrazil eden izmed sogovornikov, »s težavo dohajajo in vodijo evidence« (intervju št. 6, Haloze).

Večini sogovornikov se je vodenje zahtevanih evidenc zdelo dodatno nepotrebno in nekoristno delo. Dvomili so tudi v verodostojnost vodenih evidenc, saj sami vedo, da jih običajno ne pišejo redno oziroma jih napišejo pred napovedano kontrolo.

> »Pa saj ni težko zapisati, kdaj si kosil, kdaj pognojil… včasih pa že moraš tudi malo lagati. Včasih gre skozi, včasih pa ne.« (intervju št. 2, Haloze)

Ob prejemu plačila za izvajanje ukrepa KOU se kmetje večinoma počutijo odgovorne, da izpolnjujejo zahteve in dosegajo želene rezultate, zato so bili sogovorniki mnenja, da je nadzor nad izvajanjem zahtev ukrepa do določene mere dobrodošel in pozitiven del ukrepa. Nekateri sogovorniki pa so bili mnenja, da je zanje vključitev v ukrepe preveč omejujoča, saj izgubijo svojo neodvisnost, zato se pogosto niso bili pripravljeni vključiti v ukrep ne glede na višino plačila. To ugotovitev potrjuje tudi statistična analiza, saj so anketirani kmetje, ki so kot pomemben dejavnik pri odločanju izpostavili administrativne obveznosti in nadzor, hkrati redkeje izpostavili pomen višine plačila in dohodka kmetije, vpliva ukrepa na pridelano krmo in na okolje (preglednica 2).

> » […] ljudje smo tudi radi na udobno, da nimaš preveč nekih obremenitev. Ker že tako moraš delati,

potem pa je še administracija in vedno več vsega zahtevajo. In potem je včasih bolje nič.« (intervju št. 10, Haloze)

#### 3.1.4 Pretekle izkušnje s kmetijsko-okoljskimi ukrepi

Pretekle izkušnje s KOU naj bi pozitivno vplivale na odločitev za vključitev (Wilson in Hart, 2000; Defrancesco in sod., 2007), vendar odgovori anketiranih kmetov na Halozah razkrivajo kvečjemu obratno. Kar četrtina (26 %) anketiranih kmetov je bilo v KOU vključenih pred letom 2015, vendar se kasneje niso odločili za ponoven vpis, zgolj 10 % anketirancev pa se je odločilo, da nadaljujejo z izvajanjem ukrepov KOU tudi po letu 2015.

Sogovorniki so izpostavili kar nekaj negativnih izkušenj s preteklimi KOU. Nekateri so poudarili, da niso bili pravočasno obveščeni o terminih obveznih usposabljanj oziroma so ta potekala v terminu, ki se ga niso mogli udeležiti, in so posledično morali vračati prejeta sredstva. Odločitev anketirancev, da niso nadaljevali z izvajanjem KOU, je povezana tudi z drugimi razlogi, kot sta starost in prenizko plačilo. Nekaj sogovornikov pa je poudarilo, da so jim zahteve preteklih ukrepov KOU predstavljale preveliko obveznost in se zato niso odločili za ponoven vpis.

### 3.2 DEJAVNIKI KMETIJE IN KMETA

#### 3.2.1 Velikost kmetije

Ugotovitev tujih raziskav, da se večje kmetije praviloma pogosteje odločajo za sodelovanje v kmetijsko-okoljskih ukrepih kot majhne (Schramek in sod., 1999, cit. po Siebert in sod., 2006; Hynes in Garvey, 2009), se deloma kaže tudi med kmetijami anketirancev na Halozah. Med anketiranimi kmeti so tako obstajale razlike v velikosti kmetijskih gospodarstev glede na poznavanje in izkušnje tako s KOU (F: $p < 0{,}001$) kot tudi konkretno z ukrepom HAB (F: $p < 0{,}01$), in sicer so bile kmetije anketirancev, ki KOU in HAB niso poznali in z njimi tudi niso imeli izkušenj, statistično značilno manjše.

#### 3.2.2 Starost in nasledstvo

Starost kmetov igra pomembno vlogo pri odločitvi za sodelovanje v KOU. Večina raziskav kaže, da so mlajši kmetje bolj pripravljeni izvajati programe KOU kot starejši kmetje (Burton, 2014), kar se je izkazalo tudi na območju Haloz. Eden izmed razlogov, zakaj starejši anketiranci večinoma niso razmišljali o vstopu v KOU, so obveznosti ukrepa. Dodatna administracija, nadzor in druge zahteve so jim predstavljale veliko oviro, kar jih je pogosto odvrnilo od tega, da bi se vključili v KOU.

»Jaz bi delal enako, kakor sem delal do sedaj, drugo pa za mene ne pride v poštev. Ne, ne… za mene pri teh letih ni. Če bi pa to bilo pred 20 leti, pa bi seveda delali.« (intervju št. 26, Haloze)

Na odločitev kmetov vpliva tudi njihovo zdravje (Hounsome in sod., 2006), kar se je izkazalo za pomemben dejavnik predvsem pri starejših anketirancih. Ti se namreč v bojazni, da zaradi poslabšanja zdravstvenega stanja ali poškodbe morda ne bodo mogli izpolniti pogodbenih obveznosti, pogosto raje niso odločili za vključitev v ukrep. Poleg zdravstvenega stanja na vključevanje starejših kmetov vpliva tudi, ali imajo zagotovljenega naslednika (Ruto in Garrod, 2009). Mnogi starejši anketiranci namreč niso želeli obremenjevati svojih prevzemnikov kmetije z že sklenjeno večletno pogodbo, zato se niso odločili za sodelovanje v ukrepu.

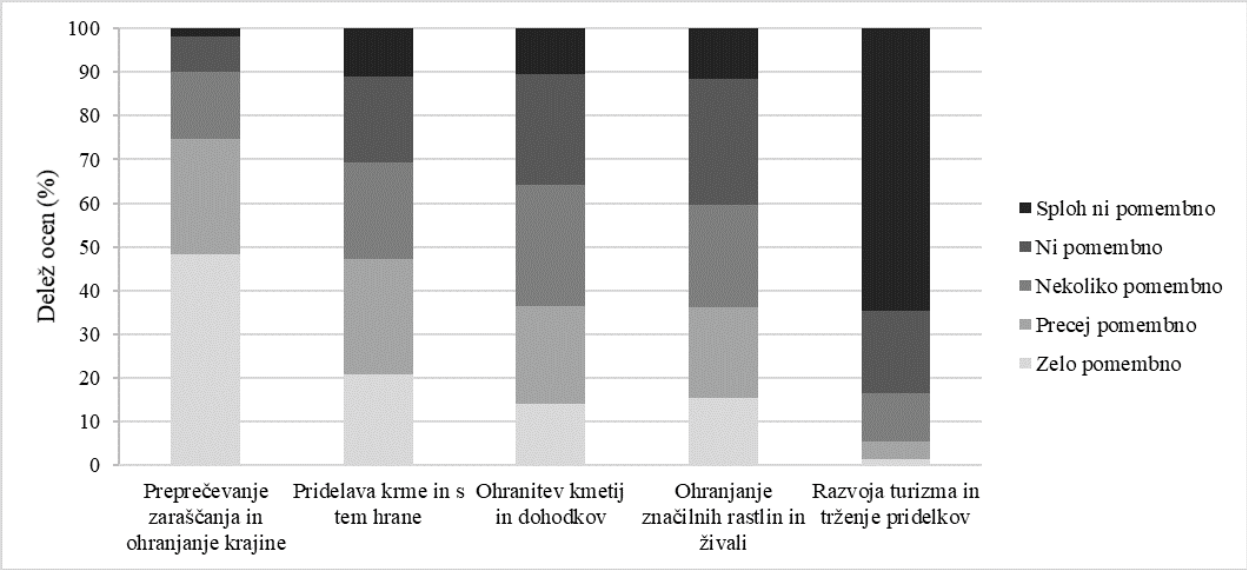
»Teh pet let [trajanja pogodbe]. Kaj hočem jaz razmišljati in neko pogodbo sklepati pri vas, če pa bo naslednjo leto [kmetijo prevzel] vnuk in bo potem vse prišlo nekam drugam. To je to. Saj sem rekel, da sem letos zadnje leto vložil, naslednje leto pa bo vnuk. Potem pa nima smisla, da bi jaz kaj delal, kar njemu ne bi odgovarjalo in bi ga obremenil.«(intervju št. 35, Haloze)

V raziskavi mlajši sogovorniki dolžine pogodbe praviloma niso izpostavljali kot dejavnik, ki bi jih odvrnil od vključitve v KOU. Petletna dolžina pogodbe se jim je zdela primerna in jim ni predstavljala ovire za vključitev.

#### 3.2.3 Odnos do ohranjanja narave in samoidentiteta

Kot pomemben dejavnik odločanja se je izkazal odnos kmeta do ohranjanja narave, ki ga kot ključen dejavnik prepoznavajo tudi drugi avtorji (npr. Morris & Potter, 1995; Brown idr., 2020) in je v nekaterih primerih lahko celo pomembnejši od finančnih spodbud (Battershill in Gilg, 1997, cit. po Schmitzberger in sod., 2005). Sogovorniki so imeli na splošno pozitiven odnos do narave, saj spoštujejo njene zakonitosti in cenijo dobrine, ki jim jih zagotavlja, zato so imeli večinoma tudi pozitiven odnos do sprejemanja okolju prijaznejših kmetijskih praks. Kar 76 % vprašanih je bila mnenja, da je ohranjanje ekstenzivnega travinja na območju Haloz precej ali zelo pomembno z vidika ohranjanja značilnih rastlinskih in živalskih vrst. Prav tako je po mnenju 94 % vprašanih smiselno, da država financira ukrepe, ki so namenjeni ohranjanju takšnih travnikov in pašnikov.

Pri tem je pomembno razumeti, da kmetje varstvo




**Slika 2**: Koristi ohranjanja ekstenzivnega travinja glede na njihovo pomembnost za kmete na Halozah leta 2019 (n = 258)

okolja in narave presojajo v skladu z lastnim vrednostnim sistemom. Ohranjanje narave je na primer kmetom pogosto pomembno predvsem zato, ker se s tem preprečuje zaraščanje (Ahnström in sod., 2009), kar so anketirani kmetje na Halozah v povprečju navedli kot najpomembnejši motiv za ohranjanje ekstenzivnega travinja (slika 2). To stališče verjetno temelji na prepričanju, da »dober kmet« ohranja svoje površine »čiste« in urejene, zato lahko kmetje travnike v zaraščanju povezujejo s slabim upravljanjem (Burton, 2004).

> »Glavno je, da je travnik pokošen pa čist. Da je zgled. Tako kot človek: če se lepo oblečeš, boš lep. Lahko si grd človek, pa če se lepo urediš, boš lep. Tako je tudi s travnikom, ko ga pokosiš in pospraviš na roke, pa ga potem pogledaš, kako je lep.« (intervju št. 20, Haloze)

Želja po ohranjanju »čistih« in »urejenih« travnikov pojasni mnenje nekaterih sogovornikov, da je trenutna zasnova ukrepa HAB nesmiselna, saj naj bi kmetje na travnikih pustili strnjen nepokošen pas, ki ga pokosijo šele v naslednjem letu. To se jim je zdelo še dodatno nerazumno, saj se v okolici zarašča veliko površin. Osnovnega namena puščanja nepokošenih pasov, ki je ohranjanje biotske prstrosti na travniku, večina ni poznala ali pa ta po njihovem mnenju ni bil dovolj utemeljen. Podobno so ugotovili na zahodu ZDA, kjer so se kmetje v večji meri odločali za vključitev v tiste naravovarstvene programe, ki so podpirali urejena kmetijska zemljišča (Ryan in sod., 2003). Kmetje razumejo in cenijo količino dela, ki je potrebna za ohranjaje »lepih in čisto obdelanih« površin, zato so ta v njihovih očeh vrednejša od površin, ki so vključena v naravovarstvene programe, saj te površine vrednotijo podobno kot nerodovitna zemljišča, ki jih ne morejo v polnosti uporabiti v proizvodne namene (Silvasti, 2003).

Proizvodno naravnano mišljenje anketiranih kmetov se zrcali v tem, da jim je bila zagotovitev zadostne pridelave krme za živino precej pomembnejša skrb kot ohranjanje narave. Motiv pridelave krme in s tem hrane so namreč postavili takoj za motivom zaraščanja (Slika 3).

> »Delno že mogoče [je smiselno financiranje ukrepov za ohranjanje narave], saj je lepo videti malo barvitih travnikov. Mi pa gledamo bolj za preživetje živine.« (intervju št. 11, Haloze)

Sklepamo torej lahko, da je kmetom ohranjanje narave pomembno predvsem v kontekstu urejene krajine in kmetijske pridelave. Dodatno to tezo podkrepi način, kako so sogovorniki opisovali indikatorske rastlinske vrste suhih ekstenzivnih travišč, ki so jih večinoma povezovali z načinom rabe in opisovali njihovo vrednost v smislu kmetijske pridelave, razmeroma redko pa iz ekoloških, kulturnih in estetskih vidikov.

> »Te sivke je malo manj, ker zdaj, ko se na eni parceli pasejo živali, jo iztrebijo. To je žal tako. Tam, kjer kosimo, pa se je zelo nazaj vrnila, samo tam je samo enkrat košnja, ker je v bregu in je strmina.« (intervju št. 6, Haloze)

Do podobnih ugotovitev so prišli na Poljskem, kjer so raziskovali, kako kmetje vrednotijo kmetijsko krajino

(Włodarczyk-Marciniak in sod., 2020). Ti so jo dojemali predvsem z vidika njene uporabnosti in produktivnosti, pa tudi specifične estetske vrednosti. Največjo vrednost so tako kmetje pripisali obdelanim poljem in travnikom, majhno vrednost pa mejicam, posamičnim drevesom, gozdnim zaplatam in vodnim telesom (Włodarczyk-Marciniak in sod., 2020).

Veliko sogovornikov, predvsem starejši kmetije, ki so prihajali iz manjših in ekstenzivno usmerjenih kmetij, svojega pristopa h kmetovanju niso dojemali kot razloga za izginjanje habitatov in spreminjanja narave. Takšno miselnost kmetov ponazarja tudi mnenje o ekološkem kmetovanju, saj je veliko sogovornikov odgovorilo, da v ta ukrep sicer niso vpisani, vendar kljub temu kmetujejo na ekološki način. Raziskave kažejo, da je med kmeti miselnost o usklajenosti njihovih kmetijskih praks z naravo precej običajna (Silvasti, 2003; Schenk in sod., 2007; Ahnström in sod., 2009). Kmetje posledično delujejo kontradiktorno, saj po eni strani svoje delo obravnavajo kot usklajeno in spoštljivo do narave, po drugi strani pa na njihov odnos do narave močno vpliva proizvodno naravnan vidik kmetovanja, kar lahko negativno vpliva na okolje in naravo ter jih ovira pri vstopu v naravovarstvene ukrepe, kot so KOU (Silvasti, 2003).

Velika večina anketiranih kmetov je tako imela pozitiven odnos do ohranjanja suhih travnikov, vendar je pri tem vidik ohranjanja biotske pestrosti v smislu ohranjanja rastlinskih in živalskih vrst manj pomemben. Podobno so ugotovili tudi v raziskavi na Goričkem, kjer pozitivni odnos do koristi od ohranjanja rastlin in živali na travnikih ni imel statistično značilnega vpliva na sodelovanje v KOU (Špur in sod., 2018).

## 3.3 DEJAVNIKI SOCIALNEGA KAPITALA

### 3.3.1 Informiranost kmetov o kmetijsko-okoljskih ukrepih

Kmetje, ki so dobro informirani o KOU, so običajno bolj zainteresirani za vključitev v ukrepe (Wilson in Hart, 2000). Kvantitativna analiza odgovorov kaže, da anketiranci precej dobro poznajo KOU, saj jih je poznalo 86 % anketirancev, ukrep HAB pa 73 %. Vendar se je to poznavanje glede na kvalitativno analizo izkazalo za vsebinsko precej skromno, še posebej v primeru ukrepa HAB. Večinoma so za ukrep samo slišali ali pa so samo približno poznali zahteve in pogoje za vpis, zgolj nekaj sogovornikov pa je poznalo naravovarstven pomen ukrepa in njegove cilje. Ob upoštevanju, da se ukrep HAB na območju Haloz izvaja že več kot desetletje (MKGP, 2015), je bilo še vedno razmeroma veliko kmetov (27 %), ki ukrepa sploh ni poznalo.

> »V: Ali ste že slišali za KOPOP – kmetijsko okoljska plačila? O: To je neki dodatek. […] Če sem odkrit, te kratice malo slabše poznam. Slišal sem zanjo, ampak si je ne znam razložiti.« (intervju št. 34, Haloze)

Informacije o kmetijsko-okoljskih ukrepih lahko kmetje pridobijo iz različnih virov, kot so svetovalna služba, drugi kmetje, kmetijske interesne in gospodarske organizacije in mediji (Lastra-Bravo in sod., 2015). Na mnenje kmetijskega svetovalca in drugih kmetov so se v veliki meri zanašali tudi anketirani kmetje. Anketiranci, ki so izpostavili, da jim je pri odločanju zelo pomembno mnenje svetovalca (35 %), so v povprečju upravljali z manjšimi kmetijskimi gospodarstvi in z manjšim obsegom primernega trajnega travinja (Preglednica 2).

Med anketiranimi kmeti jih je bilo 76 % vključenih v vsaj eno kmetijsko organizacijo ali podeželsko društvo, ki lahko glede na pretekle raziskave pomembno vplivajo na oblikovanje stališč kmetov o KOU (Peerlings in Polman, 2009; Capitanio in sod., 2011). Kmetje so bili najpogosteje vključeni v lokalni strojni krožek, kmetijsko zadrugo in razna podeželska društva. Med njimi je izstopalo lokalno društvo, v katerega je bilo včlanjenih veliko anektiranih kmetov, in se je aktivno zavzemalo za oblikovanje KOU, ki bi bili po njihovem mnenju bolj prilagojeni potrebam haloških kmetij. Kmetje, ki so sodelovali v omenjenem društvu, so bili precej dobro informirani o ukrepih kmetijske politike. Nekateri sogovorniki so omenjali stališča in pripomembe o ukrepu HAB, ki so jih oblikovali v okviru društva, pri čemer so izpostavili predvsem problem premajhne finančne spodbude za pokritje stroškov, povezanih z ukrepom. To stališče, ki so ga oblikovali znotraj društva, je bilo pogosto predvsem med kmeti, ki so prihajali iz nekoliko večjih in bolj proizvodno usmerjenih kmetij ter so bili najverjetneje tudi bolj aktivni v lokalnem okolju, zato vpliv takšnih organizacij na stališča in odločanje kmetov verjetno ni zanemarljiv.

### 3.3.2 Zaupanje v vladne organe

Sogovorniki so izražali precejšnje nezaupanje v vladne organe in pristojne institucije, kar lahko vpliva na to, da se kmetije v manjši meri odločajo za vključitev v kmetijsko-okoljske ukrepe (Peerlings in Polman, 2009). Predstavnike vladnih organov so nazivali kot »tiste iz Ljubljane« in »iz pisarne«, ki da ne poznajo razmer v Halozah. Posledično so bili kmetje mnenja, da ukrepi niso zasnovani tako, da bi bili dobro prilagojeni potrebam lokalnega okolja. Nekateri sogovorniki so poudarili, da bi si želeli, da bi pri oblikovanju ukrepov upoštevali tudi njihovo mnenje. Sodelovanje med različnimi deležniki, kot so kmetje, kmetijske organizacije in naravovarstvene institucije, se je namreč v preteklih raziskavah izkazalo

za pomemben dejavnik pri zasnovi uspešnih ukrepov (Niens in Marggraf, 2010; Whittingham, 2011).

## 4 SKLEPI IN PRIPOROČILA

### 4.1 DEJAVNIKI ODLOČANJA IN TRAJNOSTNI KMETIJSKO-OKOLJSKI UKREPI

V raziskavi smo s kombinacijo kvantitativnih in kvalitativnih metod preučili dejavnike, ki vplivajo na odločanje kmetov za vključitev v kmetijsko-okoljske ukrepe za ohranjanje ekstenzivne rabe travinja na območju Haloz. Izbrani pristop se je izkazal kot primeren način raziskovanja teh vprašanj, saj je kvalitativni del raziskave omogočil dodatno in celovitejše razumevanje določenih dejavnikov odločanja, ki jih je zgolj s kvantitativnimi metodami običajno težje raziskati (Schenk in sod., 2007).

Ekonomski razlogi, kot so višina plačila in vplivi ukrepov na obseg pridelane krme in dohodkovne potrebe kmetij, so se izkazali kot ključni dejavniki za (ne) vključitev v kmetijsko-okoljske ukrepe. Anketirani kmetje so bili prepričani, da s pozno košnjo, ki je potrebna za ohranjanje habitatov, izgubijo na kvaliteti krme in s tem tudi na obsegu prireje in dohodku iz živinoreje. Višino plačila so zato presojali predvsem v smislu izgube dohodkov zaradi izgubljene krme, vendar ponujeno plačilo te izgube ni ustrezno nadomestilo. Za kmete tako živinoreja, ki temelji na intenzivnejši pridelavi krme, predstavlja boljšo možnost za zagotovitev zaslužka kot vstop v KOU, kar je pomemben razlog, da se kmetje ne odločijo za vključitev. Ugotovitve so skladne z raziskavo iz Radenskega polja, kjer se kmetje niso bili pripravljeni vključiti v ukrepe, namenjene ekstenzivni reji goveda, predvsem zaradi njihove usmerjenosti v intenzivno živinorejo, ki jim prinaša večjo ekonomsko korist (Žgavec in sod., 2013).

Primerna višina plačil lahko motivira kmete, da se odločijo za vključitev v kmetijsko-okoljske ukrepe ter se s tem zavežejo k upoštevanju predpisov in kratkoročni spremembi kmetijskih praks, ki pa niso nujno skladne z njihovim osebnim prepričanjem (Schenk in sod., 2007; Ahnström in sod., 2009). Vedenjski dejavniki, kot so odnos kmetov do ohranjanja narave in njihova samoidentiteta, so tako pri odločanju izredno pomembni (Morris in Potter, 1995; Schmitzberger in sod., 2005), kar je razkrila tudi ta raziskava. Anketirani kmetje so imeli v splošnem do ohranjanja narave in okolja pozitiven odnos, vendar ta ni bil nujno povezan z ohranitveno etiko in večinoma ni bil zadosten razlog, da bi se odločili za vstop v kmetijsko-okoljske ukrepe (KOU).

Med haloškimi kmeti je bil splošno uveljavljen proizvodno naravnan pogled na kmetijstvo, ki zagovarja, da je kmetovanje primarno namenjeno pridelavi hrane. Ta pogled je bil pomemben del njihovih temeljnih vrednot in identitete, kar ima lahko vpliv na izbiranje KOU. Smiselnost vsebine ukrepov namreč kmetje praviloma niso presojali z vidika učinkov na biotsko prestrost, kar lahko vodi v nerazumevanje namena in zavračanje samih ukrepov. Kmetijsko-okoljske prakse, kot sta puščanje nepokošenih pasov na travnikih in pozna košnja, ki vodijo v manjšo prirejo živine na račun izgubljene krme, lahko zato kmetje pogosto ocenjujejo kot pokazatelj slabega upravljanja, ki odstopa od njihovega pojmovanja »dobrega kmeta« (Burton in Paragahawewa, 2011). Tovrstne prakse so zato vrednostno nezaželene.

Med dejavniki, ki so povezani z lastnostmi kmetije in kmeta, sta se poleg vedenjskih kot pomembna dejavnika izkazala tudi velikost kmetije in starost kmeta. Kmetje, ki so prihajali iz večjih kmetij, so bili v povprečju bolje informirani o ukrepih KOU in so se pogosteje vključevali v le-te, kar je skladno z nekaterimi preteklimi raziskavami (npr. Wilson in Hart, 2000; Hynes in Garvey, 2009). Vpliv starosti na pripravljenost kmetov za sodelovanje v KOU se je izkazal kot kompleksen in je običajno pogojen še z drugimi dejavniki odločanja. Predvsem starejšim anketirancem so obveznosti izvajanja ukrepa, kot so sprememba kmetijskih praks, vodenje evidenc in dodaten nadzor, pogosto predstavljale veliko oviro, zaradi katere se niso vključili v KOU. Starejši anketiranci so bili v primerjavi z mlajšimi tudi slabše informirani o ukrepih. Dodatno se je v povezavi s starostjo kmetov kot negativen dejavnik izkazala prisotnost prevzemnika, saj starejši kmetje pogosto niso želeli obremeniti svojega naslednika z že podpisano pogodbo.

Na pripravljenost kmetov za vključitev pomembno vpliva tudi informiranost kmetov o KOU (Wilson in Hart, 2000), ki je bila med anketiranimi kmeti na splošno dobra, vendar je podrobnosti o ciljnih ukrepih (HAB), kot je poznavanje okoljskega namena in ciljev ukrepov, poznalo razmeroma malo kmetov, kar je lahko eden izmed pomembnih razlogov, da se kmetje za vključitev v KOU niso odločali v večji meri.

### 4.2 PRIPOROČILA ODLOČEVALCEM

Ključen izziv, ki ga je razkrila ta raziskava in bi ga bilo treba naslavljati pri oblikovanju prihodnjih ukrepov, je postopno spreminjanje vrednostnega sistema kmetov in njihovih socialnih norm in prioritet do ohranjanja narave in okolju prijaznih kmetijskih praks. V raziskavi smo namreč zaznali neskladja med cilji kmetijsko-okoljskih ukrepov za ohranjanje ekstenzivne rabe travinja in prepričanji kmetov, ki jim je bilo ohranjanje travinja pomembno predvsem z vidika preprečevanja zaraščanja in za dosego proizvodnih in ekonomskih ciljev, medtem ko

je bilo njihovo ohranjanje z vidika biotske pestrosti za veliko večino kmetov manj pomembno.

Spreminjanje vrednot in prepričanj kmetov je dolgotrajen proces, ki se mu je treba posvečati na vseh ravneh, od kmetov do vladnih institucij. Spodbuditi ga je verjetno mogoče predvsem z uvedbo novih zasnov kmetijsko-okoljskih ukrepov ter krepitvijo prenosa znanja in dialoga med deležniki. Na območju Haloz bi bilo zato za namene ohranjanja ekstenzivnega travinja smiselno razmisliti o uvedbi rezultatskih shem, kjer kmetje namesto za izvajanje predpisanih praks prejmejo plačilo za dosego okoljskih in naravovarstvenih rezultatov (Herzon in sod., 2018; Šumrada in Erjavec, 2020). Iz kulturnega vidika imajo rezultatske sheme prednost, da so kmetje primorani razviti nove in edinstvene rešitve za dosego okoljskih ciljev in se hkrati naučiti prepoznati povezave med svojimi praksami in vplivi teh na biotsko pestrost (Birge & Herzon, 2019). Ta znanja lahko delijo z drugimi kmeti, kar lahko prispeva k ustvarjanju družbenega statusa in ugleda znotraj kmečke skupnosti ter s tem okrepi njihov socialni kapital (Burton in Paragahawewa, 2011). Tovrstni ukrepi imajo zato potencial, da kmetje začnejo okoljske rezultate obravnavati kot proizvode, primerljive z drugimi proizvodi na kmetiji (Matzdorf in Lorenz, 2010; Burton in Schwarz, 2013), in da hkrati sonaravne prakse začnejo vrednotiti kot prakse »dobrega kmetovanja« (Burton in sod., 2008; Burton in Paragahawewa, 2011).

Za naslavljanje naštetih izzivov in oblikovanje uspešnih rezultatskih in drugih naravovarstvenih ukrepov je zato potreben bolj lokalen in individualen pristop (Herzon in sod., 2018). Ta lahko na eni strani spodbudi boljše razumevanje pomena in ciljev KOU, po drugi strani pa bodo kmetje lahko tudi izboljšali izvajanje ukrepov, saj dobro poznajo lokalne razmere in družbeno okolje. V ta proces se lahko vključijo tudi lokalne in naravovarstvene organizacije, ki lahko kasneje sodelujejo pri upravljanju ukrepa (Šumrada in Erjavec, 2020).

Poleg primernih finančnih spodbud je ključno, da se krepi izobraževalna in svetovalna podpora, ki je usmerjena predvsem v izboljšanje kmetovega razumevanja pomena in ciljev varovanja okolja in ohranjanja narave (Kleijn in Sutherland, 2003; Ahnström in sod., 2009; Mack idr., 2020). V prvi vrsti je pomembno, kako so ukrepi kmetom predstavljeni (Riley, 2011), nato pa je pomemben tudi način nadaljnje komunikacije na izobraževanjih o ukrepih. Varovanje narave in okolja tako od kmetov kot tudi kmetijskih svetovalcev zahteva nova, celovitejša in multi-disciplinarna znanja in spretnosti, ki presegajo znanja, ki so potrebna zgolj za pridelavo hrane (Bergeå in sod., 2008), zato se kaže večja potreba po širitvi kompetenc svetovalcev in uporabi novih pristopov prenosa znanja, ki bodo praktično in lokalno naravnani (Ingram, 2008; Faure in sod., 2012).

## 5 ZAHVALE

Prispevek je nastal v okviru Ciljnega raziskovalnega projekta (CRP V4-1814) Analitične podpore za večjo učinkovitost in ciljnost kmetijske politike do okolja in narave v Sloveniji in programa Ekonomika agroživilstva in naravnih virov (P4-0022) s finančno podporo Javne agencije za raziskovalno dejavnost in Ministrstva za kmetijstvo, gozdarstvo in prehrano.

Vsem sodelujočim kmetom se zahvaljujemo za čas, ki so ga posvetili naši raziskavi, in vpoglede v njihovo delo in razmišljanje. Za pomoč pri vzpostavitvi kontakta s kmetijami se zahvaljujemo kmetijskim svetovalcem na Kmetijsko-gozdarskem zavodu Ptuj.

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# Investigation in physicochemical characteristics of jujube (*Ziziphus jujuba* Mill.) extract cake

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**Investigation in physicochemical characteristics of jujube (*Ziziphus jujuba* Mill.) extract cake**

**Abstract:** In spite of freshly eaten, Jujube (*Ziziphus jujuba* Mill.) might be dried or processed into confectionary recipes in cakes, as one of the important products in food industries. The experiment was conducted based on complete randomized design with three replications. Treatments were control and three levels of jujube extraction (2, 4, and 6 percent) which added to cake's formulation and sampling in different time periods. In the present study, the process of baking was in four steps. First of all, dried jujube fruit was mixed with water and its brix was reached 15 using direct heat. In the second step, a final brix of 60 was obtained by rotary evaporator. Then, appropriate amounts of egg, sugar, yogurt, oil, 2, 4, or 6 % jujube extract was mixed using a blender. Finally, baking powder, flour, and vanilla were mixed and the mixture was placed in the oven set at 160 °C for 20 minutes. Physicochemical analysis showed that the cakes containing 4 and 6 % of jujube extract were the best treatments at all time periods. However, analyze of sensational test results revealed that especially panelists evaluations that 6 percentage extract sample was significantly different from the others.

**Key words:** fruity jujube cake; jujube extract; confectionary

**Preučevanje fizikalno kemijskih lastnosti dodatkov izvlečkov žižole (Ziziphus jujuba Mill.) pri izdelavi peciva**

**Izvleček:** Čeprav se plodovi žižole (*Ziziphus jujuba* Mill.) uživajo sveži, se lahko posušijo in predelajo v različnih slaščičarskih receptih v pecivo, ki je pomemben izdelek v živilski industriji. Poskus je bil izveden kot popolni naključni poskus s tremi ponovitvami. Obravnavanja so obsegala kontrolo in tri različne odmerke izvlečka žižol (2, 4, in 6 %), ki so bili dodani pri pripravi peciva in vzorčeni v različnih časovnih obdobjih. V raziskavi je priprava peciva potekala v štirih korakih. Najprej so pusušene plodove žižole zmešali z vodo in jih segrevali dokler njihova sladkost ni dosegla 15 briksov.V drugem koraku je bila končna sladkost 60 briksov dosežena s krožnim evaporatorjem. Nato so 2, 4, ali 6 % izvlečkom žižole dodali primerno količino jajc, sladkorja in jogurta ter zmes zmešali z mešalnikom. Nazadnje so dodali pecilni prašek, moko in vanilijo, vse dobro premešali in dali v pečico za 20 minut pri 160 C. Fizikalno kemijske analize so pokazale, da je bilo pecivo, ki je vsebovalo 4 in 6 % izvlečka žižole najboljše pri vseh časih obravnavanja. Kljub temu je analiza senzoričnega preiskusa odkrila, da so preiskuševalci ugotovili značilno različnost peciva s šestimi odstotki izvlečka žižol.

**Ključne besede:** sadno pecivo iz žižol; izvlečki žižole; izdelovanje slaščic

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## 1 INTRODUCTION

Interests have been raised about the potential effects of diet hobbies on controlling or preventing various diseases, during the last few decades. Therefore, practical foods play an undeniable role in decreasing health problems and improving health quality. The impact of some vegetable-based foods in decreasing chronic illnesses have been documented, at least in secondary metabolites derived from biologic activities (Crisosto et al., 2003).

As one of the productions in food industries, cake, has been considered by people principally children and teenagers. This product is a form of sweet dessert that is typically baked and consists of flour, oil (except sponge cake), sugar, and eggs (National standard of Islamic Republic of Iran, 2006). (*Ziziphus jujuba* Mill), commonly called jujube is a member of the buckthorn family (Rhamnaceae) originated from China. Jujube is known as tasty fruit and an effective herbal remedy (both fresh and dried). Jujube is one of the native plants of Iran, which is used for curing various diseases, digestive problem, feeling weak, fatness, urination issues, and diarrhea in traditional medical education in Iran and some other middle east countries. Also, in Iran, this plant is well-known as anti-diabetes medicine (Delfan et al., 2014). This fruit is high in vitamin C and on the other hand, its glucose quantity is 36.74 and between 65 and 71.77 percent in fresh and dried mode, respectively. Hence, it has been suggested as a compote and sweet production (Pareek, 2017). Nutritional comparison of jujube and apple showed that jujube consisted of higher phosphor (fivefold), potassium (twofold) and ascorbic acid (tenfold) than to apple (Qinqin et al., 2015). FAO suggested daily use of jujube, even one per day, for obtaining necessary vitamins such as C and B complex. Results of a study showed that dried form of jujube possessed 100 to 600 nanomole per gram and on the other hand, fresh form showed 100 to 150 nanomole per gram for AMP (adenosine monophosphate), GMP (guanosine 3′:5′-monophosphate) (Qing-Han et al., 2012). Dried jujube is a rich snack and can be regarded as an impressive substitution for raisin and date in the cooking industry. Productions such as jujube's cake, jujube's butter, jujube's candy, and jujube's beverage have been commercialized. Moreover, jujube is also processed as sweetened fruit, smoked fruit, juice, jam, wine, mixed drinks, powder, and tea in China and South East Asia, (Esteki and Urooj, 2012).

Researchers have found various compounds such as cycloplex, alkaloid (Suksamrarn et al., 2005; Han et al., 2011), languid, and tryptoid in jujube fruit (Choi et al., 2011). Another study (Wang et al., 2010) discovered this fruit contains fatty acids, beta-carotene, alpha-tocopherol, seven phenolic compositions such as catein, caffeic acid, AP katchin, folic acid, routine, P-hydroxy benzoic, and chlorogenic acid (Wang et al., 2011). Knowledge and technology on functional foods were first identified in the 1860s in Japan. Accordingly, governments decided to control different factors that lead to some illnesses among the population through increasing production and use of certain foods. Consequently, they effectively managed expenses related to health care and cure section. Functional foods industry was found through adding or thickening useful and omitting ineffective or harmful compounds, and their production and consuming market have become greater immediately (Bigliardi and Galati, 2013).

Functional foods are known as foods, which have beneficial effects on health. They play a vital role in decreasing health risks and improving health quality. Barley is one of the well-known cases of functional foods since it naturally has soluble fiber and is effective in decreasing cholesterol level (Zhen-Yu et al., 2011). For reaching to such situation, as mentioned earlier, a couple of food materials have been modified; for instance, orange juice fortified with calcium is considered a remarkable substance for bones. These metabolites overall, as active biological compounds, are less effective than medicines. However, if they are used regularly in considerable amounts as a part of daily diet, should have noticeable physiological effects for a long time (Hasler, 2009). Methanol and water have been used with flavonoids, saponite containers, antioxidant activity, and also two experiments were used with 2.2 diphenylene and 1.1 picryl hydrazil (DPPH), in order to control free radicals activities, and reducing strength methods for measuring antioxidant activity of juice (Xiaohong et al., 2014).

Anti-cancer and immunologic effect of polysaccharides in jujube has been documented (Xie et al., 2016). Ethanol extract, which is existed in jujube, shows protection effect against liver damages caused by CCL4 and also antioxidant mechanism in rats. Moreover, the significant impact of jujube juice was reported on improving liver damages in the result of ischemia/perfusion (Dongying et al., 2012). Chen et al., (2010) investigated the effects of aqueous extract of jujube fruit on liver damage in rats and concluded that high antioxidant effect of its extract (Chen et al., 2010). Since jujube grows as a native plant in Iran and there is no information about jujube-based products, therefore, the present study was aimed to produce a new fruity jujube cake. To this end, an experiment was outlined to test the effect of various factors on some physicochemical properties of fruity jujube cake.

## 2 MATERIALS AND METHODS

### 2.1 MATERIALS

Dried jujube was purchased from local market of Mashhad (Khorasan-e-Razavi province in Iran) and was kept in outdoor condition. White flour, with extraction degree of 81 percent, was bought from Golmakan Flour Factory (Mashhad, Iran). For this purpose, flour needed for all examinations were provided at one time and was kept under cold storage. Other ingredients for experiences such as sugar, liquid oil, and vanilla were provided from a confectionary, and also, fresh eggs and yogurt were purchased one day before daily baking of cakes and kept in the refrigerator.

### 2.2 METHODS

#### 2.2.1 Providing jujube's extract

At first, jujubes were washed carefully. The water as a solvent was added in scales of 1:4. The temperature for extraction and final brix was 80 ± 5 °C and 60 °C, respectively. After washing, some scratches were made on fruit and then the fruit was extracted for 3 hours within water in 1:4 scales in above temperature. After reaching to brix 15, extraction was filtered with its pulps using quilted fabric. In order to improve the efficiency, remained pulps were washed and heated again and achieved extract was filtered by Buchner hopper and filter paper under vacuum condition. Eventually, for increasing density under vacuum, rotary evaporator was used with 45 °C and under 72 mbar vacuum.

#### 2.2.2 Improving density by rotary evaporator

For reaching appropriate density and brix 60 in dried jujube, after extraction and achieving brix 15, rotary evaporator machine (model 4003 made in Heidolph Company in Germany) was used for 2 hours in 45 °C and under 72 mbar vacuum.

#### 2.2.3 Providing and producing cake dough

Cake dough consisted of 225 g wheat flour, 175 g sugar, 150 g oil, 4 eggs, 150 g yogurt or milk, 2 g vanilla (Dimitra et al., 2011) and 2, 4, and 6 % jujube's extract for different samples. Sugar, eggs, oil, and jujube extract were mixed by an electric mixer for making cake dough (Electra EK-230M, made in Japan) with speed of 128 RPM for 4 minutes, and cream with bubbles was achieved. Then, vanilla was added to wheat flour and product added slowly to the cream. In this study, the treatments were the concentration of jujube's extract (in 3 levels of 2, 4, and 6 %) which was added as mentioned earlier. After that, 40 grams of provided dough was placed into special papers that were placed in casts with the aid of fabric cloth. Finally, baking took place in a laboratory oven with hot air (Zucchelli Forni, made in Italy) at a temperature of 170 °C for 20 minutes. After cooling, each of samples was put in PE bags and kept in room temperature for measuring different specifications.

#### 2.2.4 Cake's physicochemical tests

##### 2.2.4.1 Measuring pH

The pH of cake was measured by the method of (Arunepanlop et al., 1996) using a pH meter (Metrohm 691, made in Switzerland) (Arunepanlop et al., 1996).

##### 2.2.4.2 Measuring cake moist percentage

To perform this experiment, standard AACC, 2000 number 44-16 was used (AACC, 2000). For this purpose, samples were put in the oven (Jet Tech OF-O2G, made in South Korea) with 100-105 °C in 2 hours, 3 days, and 6 days periods.

##### 2.2.4.3 Measuring cake $a_w$

For determining $a_w$, an equal mass of each sample was completely smashed in certain periods (2 hours, 3 days, and 6 days after baking) and their water resistance was measured by $a_w$ meter device (Novasina, MS1 model, made in England).

##### 2.2.4.4 Measuring cake special mass

For measuring cake special mass, mass substitution method with rapeseed was used according to AACC standard, 2000, number 72-10 (AACC, 2000). For doing so, a slice of cake in 2 x 2 centimeter from geometric center of cake was cut in specific periods (2 hours, 3 days, and 6 days after baking) and its special mass was measured.

#### 2.2.4.5 Hardiness of cake

Hardiness of cake at the time intervals (2 hours, 3, and 6 days after baking) was measured using a tissue texture instrument (QTS model 25 made in the UK) based on (Zhang et al., 2016). The maximum force required to penetrate a cylindrical tip (2 cm in diameter, 2.3 cm in height) at a speed of 60 mm $min^{-1}$ from the cake center was calculated as a hardiness index. The starting point and target point were 0.05 N and 25 mm, respectively.

### 2.3 DATA ANALYSIS AND EXPERIMENTAL DESIGN

The experiment was conducted based on complete randomized design with three replications. Treatments were control and three levels of jujube extraction (2, 4, and 6 percent) which added to cake's formulation and sampling was performed in different time periods (2 hours, 3 days, and 6 days after baking). One-way analysis of variance (ANOVA) was used to test the difference between the means of treatments and the mean data were compared according to Duncan's Multiple Range Test (DMRT) at 5 % level using SPSS ver. 23. Graphs were drawn by Microsoft Excel software ver. 2013.

## 3 RESULTS AND DISCUSSION

### 3.1 WATER ACTIVITY ($A_W$)

Effect of sampling method on $a_w$ was significant ($p \leq 0.01$) (Table 1). The average of 12 different treatments for $a_w$ has been categorized using Duncan method (Table 2). The maximum $a_w$ was observed from control sampling in 2 hours after baking and the sample of 6 % extraction in 6 days after baking had the minimum $a_w$ (Table 2).

The (Figure 1) shows that as time passes, increasing in extraction content increased and $a_w$ decreased. The evaluation of jujube extraction on physicochemical properties of buns, achieved the same results about cake. However, the slope was less in bun than cake (Qing-Han et al., 2013).

### 3.2 SPECIAL VOLUME

The results illustrated that the effect of sampling method was significant on special volume ($p \leq 0.01$) (Table 1). Comparison of means showed that the maximum special volume was observed from sample of 4 % extraction in 2 hours after baking while the minimum special volume was related to control sample in 6 days after baking (Table 2). The average of 12 different treatments

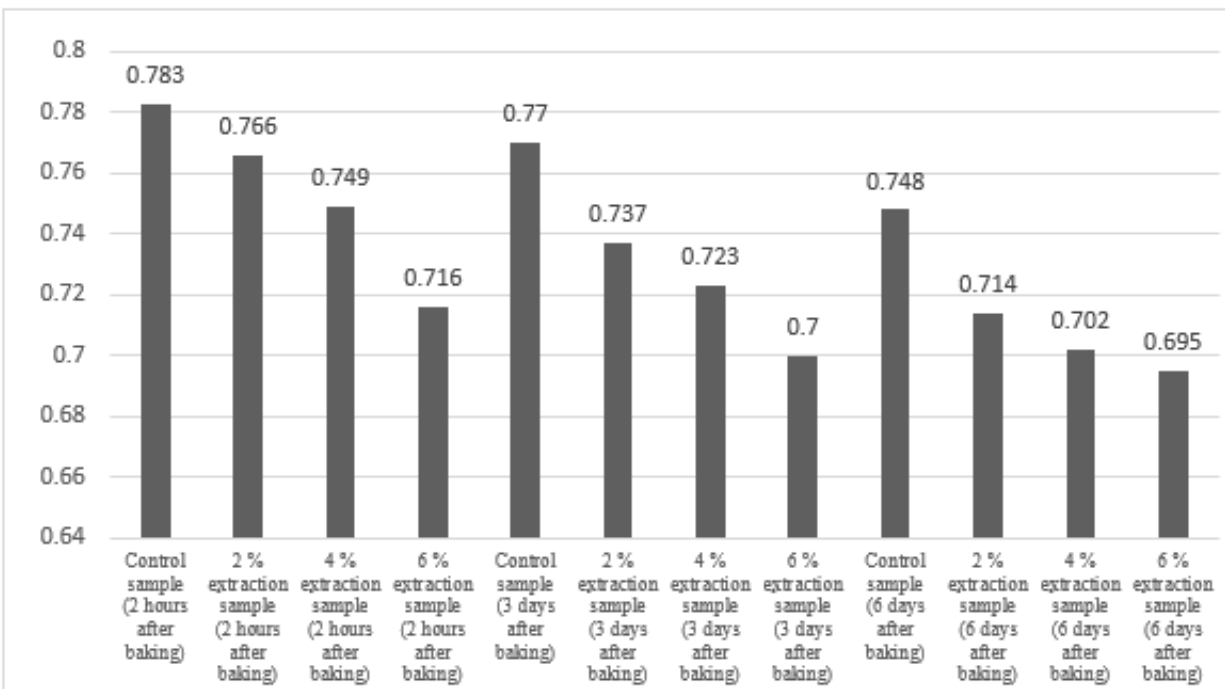


**Figure 1:** The comparison of $a_w$ on different levels of sampling using Duncan method

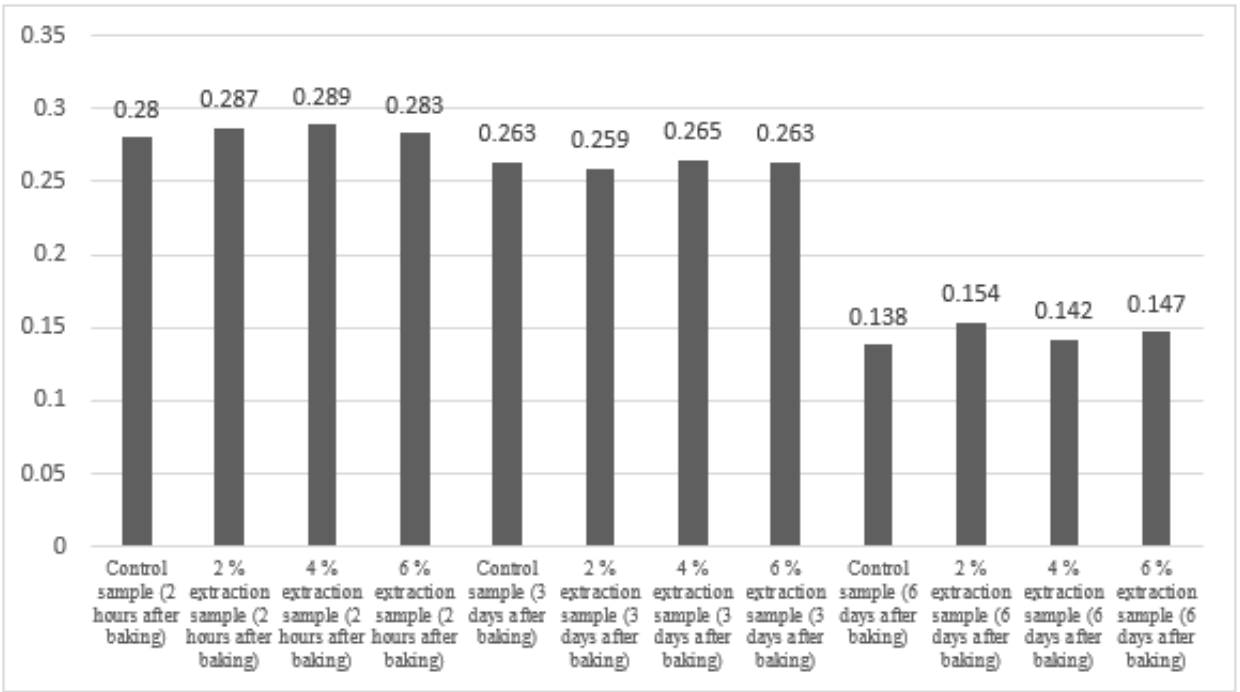


**Figure 2:** Comparison of special volume in different levels of sampling using Duncan method

for special volume has been categorized using Duncan method. Means with similar letters are not significantly different at $p \leq 0.05$ (Figure 2). As results indicated, as time passes, increasing in extraction content led to a decrease in special volume. According to another study that was conducted on the evaluation of the effect of ju-

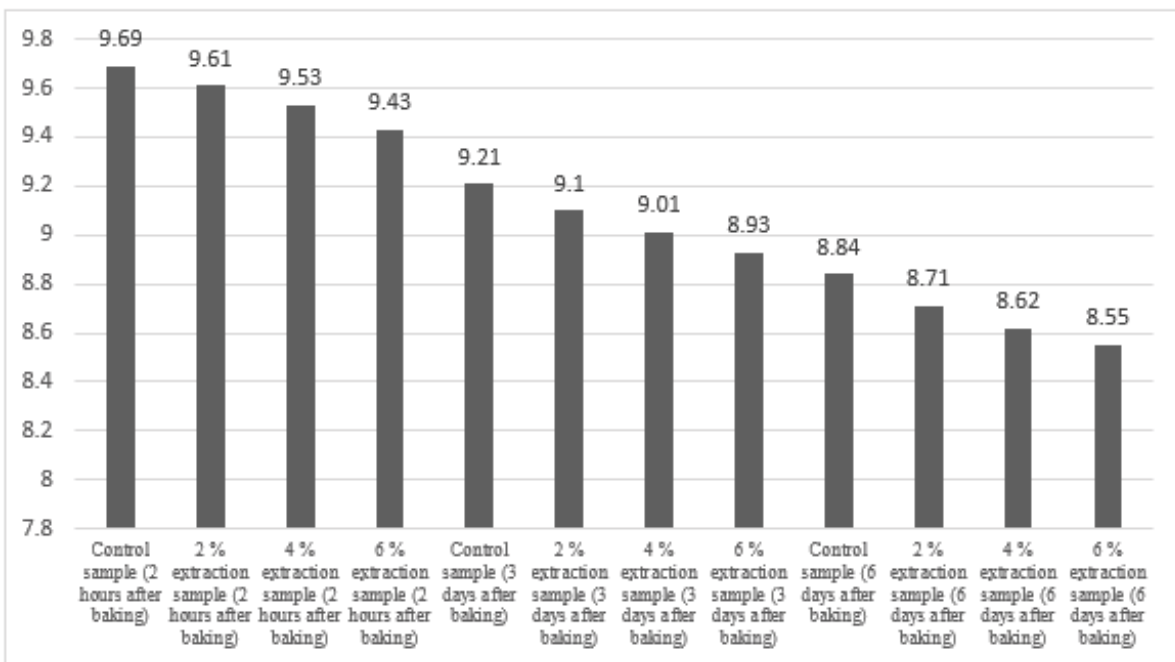


**Figure 3:** The comparison of pH on different levels of sampling using Duncan method

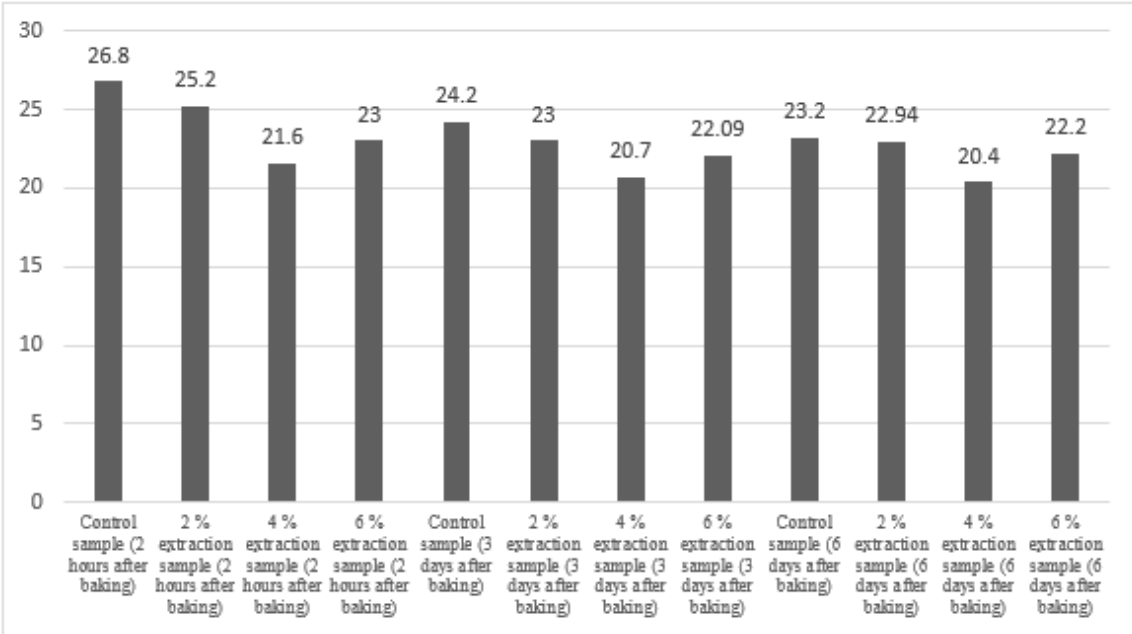


**Figure 4:** The mean moisture content of samples compared by Duncan method

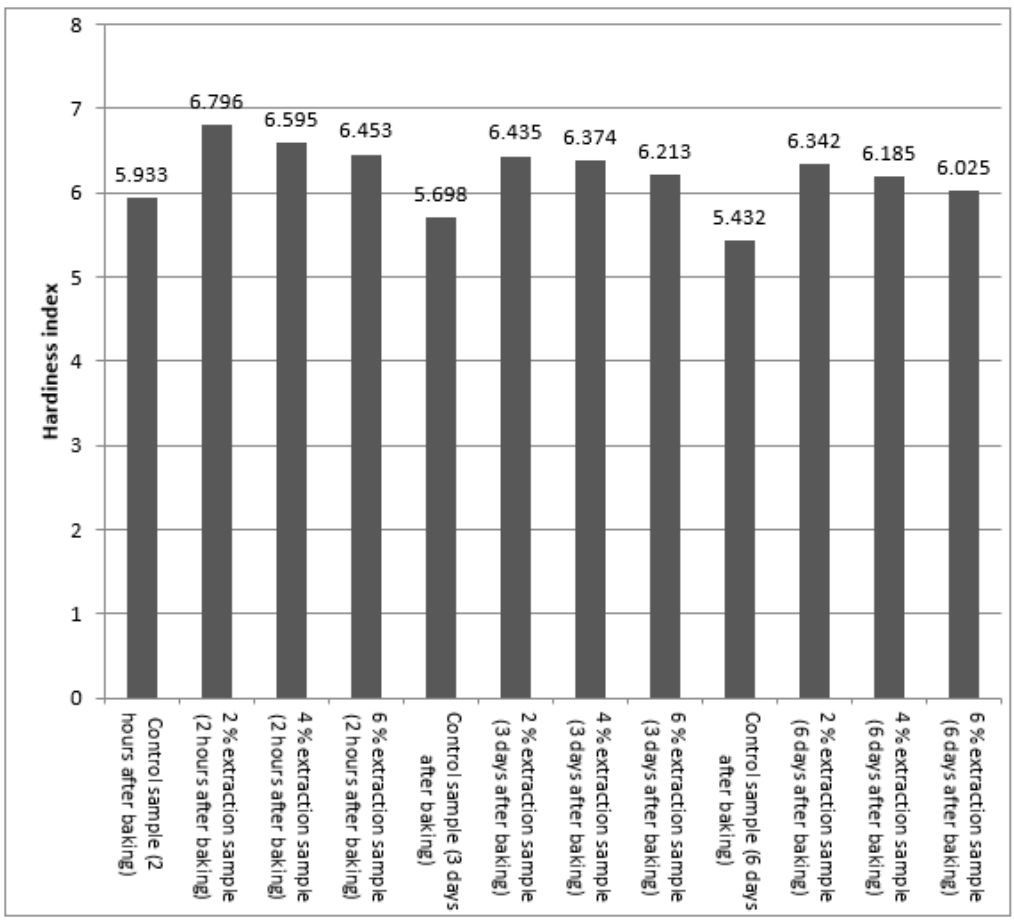


**Figure 5:** Comparison of hardiness index in different levels of sampling using Duncan method

**Table 1:** Analysis of variance (mean square) for the effects of sampling treatments on physicochemical characteristics of jujube extracted cake

| Source of variation | df | $a_w$ | Special Volume | pH | Moisture content | Hardiness |
|---|---|---|---|---|---|---|
| Treatments | 11 | 0.003** | 0.012** | 0.465** | 9.874** | 0.443** |
| Error | 24 | 0.00015 | 0.000007 | 0.00042 | 0.0503 | 0.000007 |
| C.V. (%) | | 1.72 | 1.17 | 0.23 | 0.98 | 0.04 |

** Significant at $p \leq 0.01$

**Table 2:** Mean comparison for the effects of sampling treatments on physicochemical characteristics of jujube extracted cake

| Treatments | $a_w$ | SpecialVolume | pH | Moisture content | Hardiness |
|---|---|---|---|---|---|
| Control sample (2 hours after baking) | 0.7825 f | 0.280 f | 9.69 l | 26.80 g | 5.933 c |
| 2 % extraction sample (2 hours after baking) | 0.7655 ef | 0.287 gh | 9.61 k | 25.20 f | 6.796 l |
| 4 % extraction sample (2 hours after baking) | 0.7485 de | 0.289 h | 9.53 j | 21.60 b | 6.595 k |
| 6 % extraction sample (2 hours after baking) | 0.7155 ab | 0.283 fg | 9.43 i | 23.00 d | 6.453 j |
| Control sample (3 days after baking) | 0.7695 f | 0.263 de | 9.21 h | 24.20 e | 5.698 b |
| 2 % extraction sample (3 days after baking) | 0.7370 cd | 0.259 d | 9.10 g | 23.00 d | 6.435 i |
| 4 % extraction sample (3 days after baking) | 0.7230 bc | 0.265 e | 9.01 f | 20.70 a | 6.374 h |
| 6 % extraction sample (3 days after baking) | 0.7000 a | 0.263 de | 8.93 e | 22.09 c | 6.213 f |
| Control sample (6 days after baking) | 0.7477 de | 0.138 a | 8.84 d | 23.20 d | 5.432 a |
| 2 % extraction sample (6 days after baking) | 0.7140 ab | 0.154 c | 8.71 c | 22.94 d | 6.342 g |
| 4 % extraction sample (6 days after baking) | 0.7020 ab | 0.142 a | 8.62 b | 20.40 a | 6.185 e |
| 6 % extraction samples (6 days after baking) | 0.6950 a | 0.147 b | 8.55 a | 22.20 c | 6.025 d |

Means with the similar letters are not significantly different at $p \leq 0.05$

jube extraction on physicochemical properties of buns, same results about cake bun were achieved (Dairou et al., 2014). However, special volume after six days from baking was considerably lower than other cake samples.

### 3.3 PH

The results indicated that the sampling method was significant on pH ($p \leq 0.01$) (Table 1). The average of 12 different treatments for pH has been categorized using Duncan method (Table 2). Comparison of means indicated that the maximum pH was observed from control sampling in 2 hours after baking while the minimum pH was related to the sample of 6 % extraction in 6 days after baking (Table 2). This study results indicated that as time passes and increasing in extraction content pH decreased (Figure 3). According to another study that was conducted on the evaluation of the effect of jujube's extraction on physicochemical properties of buns, same results about cake bun were achieved (Huan-xia et al., 2015).

### 3.4 MOISTURE CONTENT

Analysis of variance showed that the different methods of sampling had significant effects on moisture content ($p \leq 0.01$) (Table 1). Duncan multiple range test was used to compare moisture contents of 12 different baking method and indicated means categories (Table 2). Results illustrated that as time passed and extraction increased, the moisture content decreased (Figure 4). Another study was conducted on the evaluation of the effect of jujube extraction on physicochemical properties of buns showed the same results about cake bun (McFarlane, 2005).

### 3.5 HARDINESS INDEX

Analysis of variance showed that the different methods of sampling had significant effects on hardiness index ($p \leq 0.01$) (Table 1). Duncan multiple range test was used to compare hardiness of cake for 12 different baking method and indicated means categories (Table 2). Results

showed that as time passed and extraction increased, the hardiness index decreased (Figure 5). Another study was conducted on the evaluation of the effect of jujube extraction on physicochemical properties of buns showed the same results about cake bun (Guynot et al., 2003).

## 4 CONCLUSIONS

In this study, physicochemical characteristics and sensory properties of samples were analyzed. Cakes with 4 % and 6 % jujube extraction were chosen as best samples in every period (2 hours, 3 days, and 6 days after baking) for physicochemical tests. For sensory properties, specially panelists, 6 % jujube extraction sample in all time periods (2 hours, 3 days, and 6 days after baking) was chosen as the best sample compared with other samples. Furthermore, for keeping produced cakes for more than six days, adding preservatives seems to be essential to prevent corruption.

## 5 CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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# Morphological and molecular characterization and new distributional record of *Tetrastichus miser* (Nees, 1834) (Hymenoptera: Chalcidoidea: Eulophidae) from Kashmir

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**Morphological and molecular characterization and new distributional record of *Tetrastichus miser* (Nees, 1834) (Hymenoptera: Chalcidoidea: Eulophidae) from Kashmir**

**Abstract:** *Tetrastichus miser* (Nees, 1834) (Hymenoptera: Eulophidae: Tetrastichinae) is a parasitoid of Curculioninae and Scolytinae infesting various trees of economic importance. In the present study, it was collected from dried *Cedrus deodara* (Roxb.) G. Don (Pinaceae) infested with *Scolytus* beetles using sweep net and aspirator. The species is reported first time from Kashmir valley. Identification of a parasitoid is of paramount significance for studying its behavior, ecology, life cycle and usage in various biological control programmes. In addition to morphological description, molecular analysis using Cytochrome C Oxidase Subunit I was carried out to complement morphotaxonomy and to facilitate its easier identification for future studies. Phylogenetic analysis by Bayesian inference (BI) and Maximum Likelihood (ML) method showed Isolates of *Tetrastichusmiser* species clustering in same clade and separated from its closest match Tetrastichinae sp.Inter-specific divergence between *Tetrastichusmiser* and Tetrastichinae sp. was evident and ranged from 0.09 to 0.10 % (0.05 % mean). No overlap was observed between maximum distance within species and minimum distance between species.

**Key words:** *Tetrastichus miser*; *Cedrus deodara*; beetle; Cytochrome C Oxidase Subunit I; Clade; morphotaxonomy; molecular analysis

**Morfološka in molekularna določitev ter novi podatki o razširjenosti vrste *Tetrastichus miser* (Nees, 1834) (Hymenoptera: Chalcidoidea: Eulophidae) v Kašmirju**

**Izvleček:** Vrsta *Tetrastichus miser* (Nees) (Hymenoptera: Eulophidae: Tetrastichinae) je parazitoid hroščev iz podružin Curculioninae in Scolytinae, ki napadajo različne gospodarsko pomembne drevesne vrste. V raziskavi je bil parazitoid nabran s stresalnimi mrežami in aspiratorjem na posušenih himalajskih cipresah, *Cedrus deodara* (Roxb.) G.Don (Pinaceae), ki so bile napadene s hrošči iz rodu *Scolytus*. O vrsti poročajo prvič iz doline Kashmir. Določitev parazitoida je zelo pomembna za preučevanje njegovega obnašanja, ekologije, življenskega kroga in pri njegovi uporabi v različnih programih biotičnega zatiranja škodljivcev. Poleg morfološkega opisa je bila za lažjo določitev v bodočih raziskavah uporabljena molekularna analiza na osnovi podenote I citohrom C oksidaze. Filogenetska analiza z metodama Bayezinove inference (Bayesian inference, BI) in največje verjetnostni (Maximum Likelihood, ML) je pokazala, da so se izolati vrste *Tetrastichus miser* združevali v istem kladu, ločeno od najbližjih, ki se ujemajo s predstavniki poddružine *Tetrastichinae*. Ločitev vrste *Tetrastichus miser* in predstavnikov poddružine *Tetrastichinae* je bila očitna in je znašala od 0,09 do 0,10 % (v povprečju 0,05 %). Opaženega ni bilo nobenega prekrivanja med maksimalno razdaljo znotraj vrste in minimalno razdaljo med vrstami.

**Ključne besede:** *Tetrastichus miser*; *Cedrus deodara*; hrošč; podenota I citohrom C oksidaze; klad; morfotaksonomija; molekularna analiza

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## 1 INTRODUCTION

Eulophidae (Hymenoptera: Chalcidoidea) is one of the largest chalcid families consisting of about 300 genera and 5000 speciesworldwide (Shree &Singh, 2015; Noyes, 2014). Eulophids have cosmopolitan distribution (Noyes, 2002) and are known to be entomophagous attacking insects and other arthropods like spiders and mites. Most eulophids are small in size (3 mm, average 1.5 mm). Family Eulophidae comprises of four subfamilies i.e. Eulophinae, Entedoninae, Euderinae and Tetrastichinae (Graham, 1975, Bouček & Graham, 1978). Eulophids are distinguished from other chalcidoid families by the presence of four segmented tarsi in both sexes and a short, straight protibial spur (as opposed to a generously proportioned, curved spur in most other chalcidoids) (Schauff, 1997). Species of eulophidae are mostly primary parasitoids or hyperparasitoids, parasitizing a wide range of hosts like cotton boll weevil, beetles, caterpillars of borers, midges, leaf miners, scale insects which are notorious pests of various horticultural, agricultural crops and forest plantations. Some eulophids are known to attack gall forming insects, mites (Bouček& Askew, 1968), eggs of spiders in silken egg sacs (LaSalle, 1990, 1994) and nematodes (van den Berg et al., 1990). Insect species belonging to more than 100 families and 10 orders are recorded as hosts for various eulophid chalcids (Talebi et al., 2010, 2011). Besides, most of the eulophids are parasitoids of insects hidden in plant tissue, such as wood borers, leaf miners, leaf rollers and gall makers.

The genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae: Tetrastichinae) is far and wide distributed worldwide containing 518 species worldwide (Noyes, 2014). *Tetrastichus* species virtually occur in all terrestrial habitats in all geographic realms, and constitute a vital component of terrestrial ecosystems. Taxonomic work on Tetratsichinae was started by Burks (1943), who provided key to North American species of *Tetrastichus*. Tetrastichinae fauna of India includes 34 genera and 272 species (Hayat & Shah, 2004; Narendran, 2007). Still many species are yet to be explored and employed for various pest management programmes. The main diagnostic character include a submarginal vein with one seta (rarely 2-4), propodeum with inverted Y shaped paraspiracular carina and hind coxa with strong reticulations. *Tetrastichus miser* (Nees, 1834) was first reported from India by Narasimham (1984). The specimens of the present study were collected during surveys in Botanical garden of University of Kashmir from dried *Cedrus deodara* (Roxb.) G.Don tree infested with *Scolytus* beetles. Asthe species is new faunal record from Kashmir valley, the present study provides a brief diagnosis and photographic illustration to authenticate the new record. In addition, molecular identification via DNA barcoding of cytochrome C oxidase subunit I (COI) was also carried to complement morphotaxonomy and to facilitate its easier identification.

## 2 MATERIAL AND METHODS

Sampling on the prevalence of pest infestation was conducted in Botanical garden, University of Kashmir (34°08'09" N 74°49'14" E; 1590 m). Dead and dried *Cedrus deodara* was found infested with Scolytid beetles. 11 parasitoids were collected by hand picking, aspirator and using sweep net with ethyl acetate used as killing agent. Collection after proper isolation and separation was preserved in vials in 70 % alcohol for further taxonomic studies. After morphological identification, specimens which were needed for molecular analysis were preserved in 90 % alcohol and then frigid at -20 °C. For morphological studies, Card mounted specimens were examined under Leica M205A stereozoom microscope (Leica Microsystems, Germany).

### 2.1 DNA EXTRACTION AND SEQUENCING

#### 2.1.1 DNA extraction

Before isolation, frigid samples were thoroughly washed with alcohol and formaldehyde to avoid contamination. The Genomic DNA was extracted from legs using DNA extraction kit (Nucleospin Insect DNA kit from Macheray Nagel, Germany) following manufacturer's protocol. PCR reaction mixture of 25 µl was prepared with following composition: 2.50 µl (10 x) of Taq assay buffer, 2.5 µl of dNTPs (each in 10 mM concentration), Forward primers and reverse primers each 0.2 µl (10 picomoles $\mu l^{-1}$), $MgCl_2$ buffer (1.5mM) 1.5 µl, Taq Polymerase 0.2 µl (1 U), DNA template 3 µl and Mili Q or sterilised water 14.9 µl. Universal primers (LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' (forward) and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (reverse)) were used to amplify the COI region (Folmer et al., 1994). Amplification was done by using a Thermal Cycler (Biorad Laboratories, California) programmed to 98 °C for 5 minutes, followed by 30 cycles of 95 °C for 30 seconds, 45 °C for 45 seconds and 72 °C for 45 seconds and a final extension at 72 °C for 10 minutes. Amplified products were gel eluted in 0.8 % agarose stained with ethidium bromide and visualized using a UV trans-illuminator. Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator

v 3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol at Rajiv Gandhi Centre for Biotechnology, Kerala, India.

#### 2.1.2 DNA sequence analysis

Trace files of sequences were subjected to quality check by recording Phred score with a minimum of 20Q assigned to qualify as actual and further edited and trimmed at ends using Chromas 2.2.2 and Sequence scanner V2.The Phred score logarithmically represents error probabilities in base calling, hence this algorithm is used by majority of sequence analysis softwares (Ewing et al., 1998). Sequences were also checked for indels and numts using BioEdit 7.2 (Hall et al., 2011). The homology search was performed for the resulting consensus sequences using Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) and identification option of BOLD systems (Ratnasingham and Hebert 2013) against sequences in GenBank to confirm the corresponding sequence taxonomy. The generated COI consensus sequences were deposited in NCBI GenBank database and the corresponding accession numbers generated are MK419323, MT012501, MT017888, MT012523 and MT012515.

#### 2.1.3 Phylogenetic analysis

Phylogenetic analysis was done for the homologous COI sequences obtained from Genbank database by performing similarity searches using BLASTn search algorithm (Altschulet al. 1990).A threshold of 3 % variation between individuals of Chalcid wasps for COI gene was used for differentiating putative species (Hebert et al., 2004; Santos et al., 2011; Smith et al., 2018).Only top hits (sequences) with high similarity score and E-values in BLASTn were considered and non-redundant species sequences were retained for further analysis. Our sequences did not reveal perfect matches, so a set of top 20 sequences were chosen for phylogenetic analysis and were aligned using Clustal W(Thompson et al., 1994) multiple alignment program inbuilt in MEGA X with the default alignment parameters (Kumar et al., 2018). Pair wise distance between each sequence was calculated using distance option of MEGA software. In addition, variable sites analyses from the alignment of the dataset were performed in MEGA X (Kumar et al., 2018).

#### 2.1.4 Abbreviations and Acronyms

AIC: Akaike Information Criterion
BI: Bayesian Inference
BLAST: Basic Local Alignment Search Tool
BOLD: Barcode of Life Data System
CC: costal cell
COI: Cytochrome c Oxidase Subunit 1
DNA: Deoxyribonucleic acid
MCMC: Markov Chain Monte Carlo
MEGA: Molecular Evolutionary Genetic Analysis
ML: Maximum Likelihood
MV: marginal vein
NCBI: National Centre for Biotechnology Information
NJ: Neighbor Joining
SLG: sublateral groove
SMG: submarginal groove
SMV: submarginal vein
♀: Female

## 3 RESULTS

### 3.1 MORPHOLOGICAL IDENTIFICATION

#### 3.1.1 Diagnosis

Body black with copper bronze metallic reflections; mesocutum with median line weaker towards pronotum, 5 long adnotaular setae on either side, in addition two rows of three setaeon either side of median line of mesonotum; eye with long pubescence; clava (0.72 x) distinctly shorter than scape; antennal formula 1 : 1 : 1 : 3 : 3; ratio of length of antennal segments 0.79: 0.32: 0.35 : 0.32 : 0.25 : 0.57. CC subequal to MV; SMV with one dorsal seta; malar space O.62 x length of eye; SMG of scutellum 1.7x from each other than from SLG, enclosed space 1.9 x as long as broad; callus with 4-7 setae arranged in two groups, one near the spiracle and the other near hind corner of propodeum; metasoma excluding ovipositor sheath a little shorter than mesosoma (2.5 : 2.6); hypopygium reaching 0.6 x length of gaster. The specimens slightly varies in the number and pattern of setae on mesonotum, the other characters are matching the redescription by Graham, 1991 (Fig. 1).

### 3.2 MATERIAL EXAMINED AND HOST ASSOCIATION

Card mounted ♀, INDIA: Jammu & Kashmir, Botanical garden, University of Kashmir (34°08'50.5"N, 74°52'00.9"E), elevation 1600 m), collected by Ajaz Rasool, May 2018, Graham (1991) reported it from *Rhynchaenus alni* (Linnaeus, 1758), *Rhynchaenus fagi*

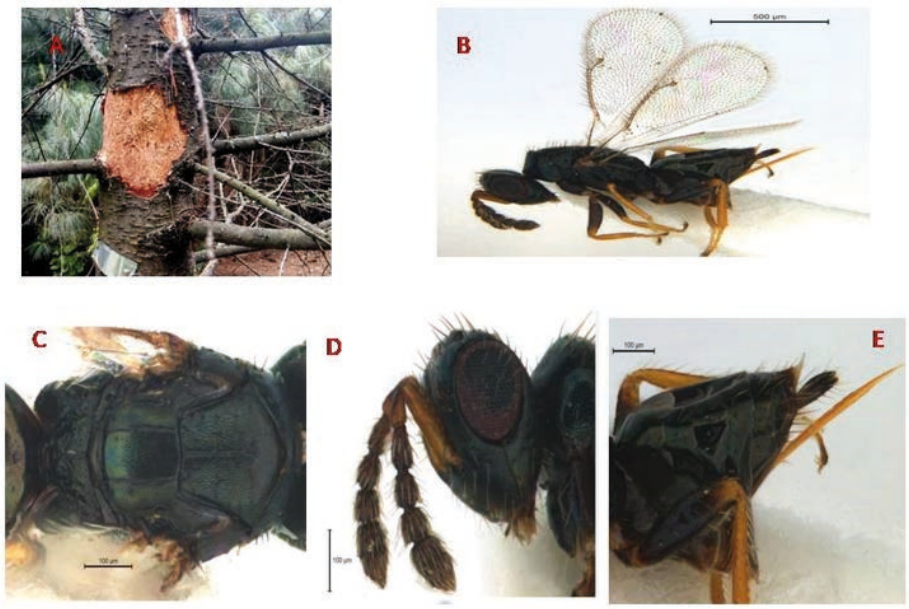


**Figure 1:** *Tetrastichus miser*, female: A infested *Cedrus deodara* tree, B Habitus, lateral view; C Mesosoma; D Head, Antennae; E Abdomen

(L., 1758), *Rhynchaenus pilosus* (J.C. Fabricius, 1781), *Rhynchaenus quercus* (L., 1758), *Rhynchaenus salicis* (L., 1758), and *Rhynchaenus oxyacantha* (Curculonidae). In the present study, it was reported from *Cedrus deodara* (Roxb.) G.Don trunk infested by Scolytidae (Coleoptera: Scolytidae).

### 3.3 DISTRIBUTION

Austria, Czech republic, Slovakia, Denmark, Finland, France, Germany, Hungary, India (Bangalore and J&K), Italy, Netherlands, Spain, Sweden, Ukraine, UK. Host records are in Universal Chalcidoidea Database (www.universalchalcidoideadatabase) (Noyes, 2014).

### 3.4 GENETIC DATA AND PHYLOGENETIC ANALYSIS

The accession numbers of sequences so generated areMK419323, MT012501, MT017888, MT012523 and MT012515. Barcoding of *Tetrastichus miser* from Kashmir has been carried for the very first time. Our sequences did not reveal perfect hits and hence were submitted to GenBank only after morphological identification. Nuclear copies of mitochondrial DNA (NUMT) contamination were fortified by performing amino acid translation by checking for the stop codons in the sequences. Translation of frame 3 of amino acid sequence containing 180 amino acids was performed using ExPASy bioinformatics resource portal. No haplotype diversity was seen in the *Tetrastichusmiser* isolates. All 5 isolates were collected from same host tree (*Cedrus deodara*) at same location. The final alignment of the data set resulted in 567 nucleotide sites having 488 conserved sites, 121 variable sites, 97 parsim-info sites and 24 singleton sites. The mean A+T content was revealed as 74.69 %.

After receiving accession numbers, final alignment of top 20 hits was done each roughly 567bp long of which 5 sequences represent the current study, 6 represent *Baryscapus* sp., 2 represent *Aprostocetus* sp., 4 *Tetrastichinae* sp. and 3 eulophid species. The nucleotide composition revealed high A-T content (74.69 %) which is common for arthropods. The nucleotide frequencies include 34.25 % (A), 40.44 % (T/U), 12.61 % (C), and 12.70 % (G). Phylogenetic trees for *Tetrastichus miser* species were constructed by Bayesian inference (BI) and Maximum Likelihood (ML) methods. Both methods were used to confirm the evolutionary history of *Tetrastichus* species. For BI method, model selection was based on the Akaike information criterion (AIC) computed by Partitionfinder version 2.1.1 software (Lanfear, 2012). The subset partitions with positions 1, 2 and 3 were done and the best fit substitution models were predicted. The BI analyses was performed using MrBayes version 3.2.2 (Ronquist et al., 2012), a stop rule convergence value of 0.01 was set, which occurred on the 1140000 Markov chain Monte Carlo (MCMC) generation and two incrementally heated chains. MCMC started from a random tree, sampling

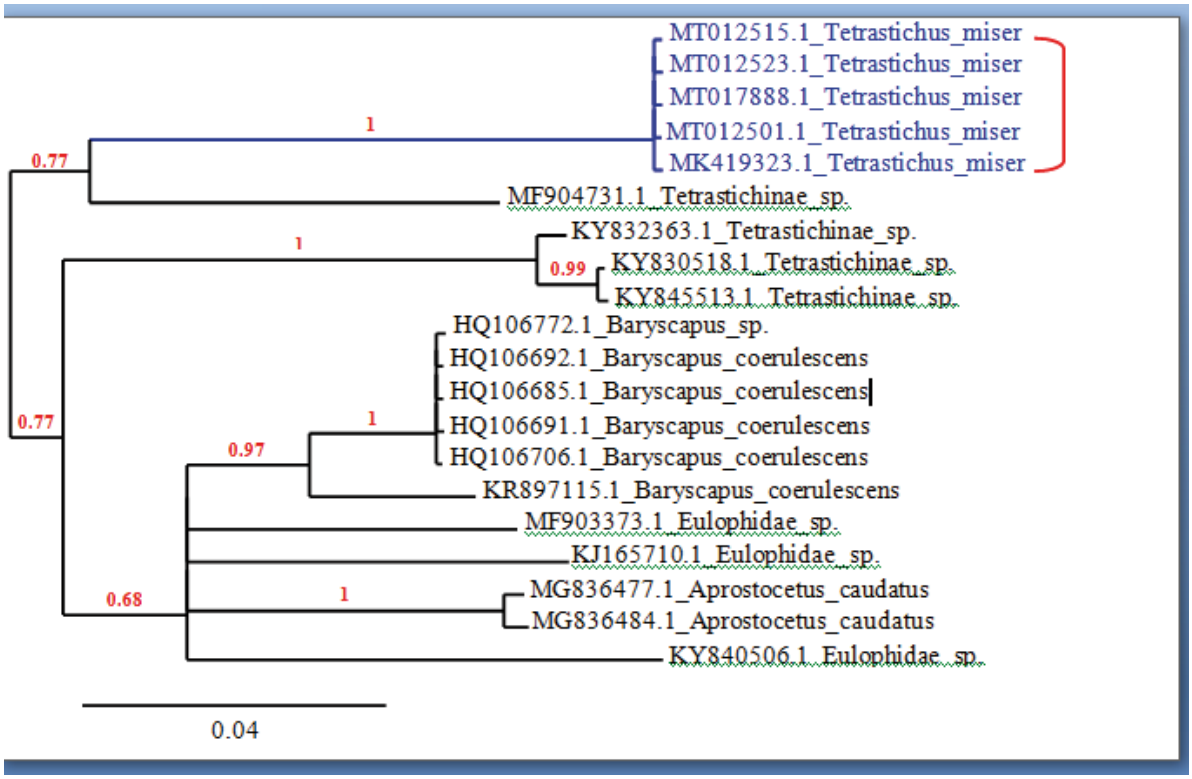


**Figure 2:** Bayesian inference phylogenetic tree of COI gene sequences of *Tetrastichus* spp. The scale bar indicates the number of substitutions per nucleotide position

one of every 500 generations, with the first 550 (25 %) of the trees discarded as burn-in out of 2200. The resulting tree was imported, edited and visualized using TreeDyne (Chevenet et al., 2006) inbuilt in Phylogeny.fr (Dereeper et al., 2008) (Fig. 2).For ML method, evolutionary history was inferred based on the Kimura 2-parameter model (Kimura 1980) in MEGA X (Kumar et al., 2018). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985) (Supplementary file). In both trees, similar typology was found and *Tetrastichus miser* isolates clustered in a single clade with strong bootstrap value (100 %) and separated from other species.

### 3.5 INTRA- AND INTER- SPECIFIC EVOLUTIONARY DIVERGENCE

Pairwise Kimura-2-parameter (K2P) distance was calculated to comprehend the evolutionary divergence rate among the species based on COI gene (Kimura 1980). Datasets which were used for phylogenetic analysis were used for estimation of Pairwise intra and inter species K2P distances among the species using option implemented in MEGA X (Kumar et al., 2018). Among *Tetrastichus miser* isolates, no divergence was reported as such with intraspecific nucleotide difference of 0.00 %. Tetrastichinae sp. showed intraspecific divergence of 0.01 to 0.09 % whereas among *Baryscapus coerulescens* (Ashmead, 1898) species it ranged between 0.00 to 0.03 %. Interspecific divergence between *Tetrastichus miser* and Tetrastichinae sp. ranged from 0.09 to 0.10 % (0.05 % mean). Between *Baryscapus coerulescens* and *Tetrastichus miser* species interspecfic divergence of 0.08 to 0.09 % (0.05 % mean) was reported.

## 4 DISCUSSION

Identification of a parasitoid is of paramount significance for studying its behavior, ecology, life cycle and usage in various biological programmes. *Tetrastichus miser* parasitizes a wide range of Curculionid beetles infesting various deciduous trees (Graham, 1991). Management of various bark beetles therefore requires information of natural enemies associated with them for future biological control programs. Morphologically, it has been described in India but owing to new record from Kashmir, current study was complemented by molecular analysis

for future taxonomic purposes. There have been only few molecular studies on chalcid wasps from India (Rasool et al., 2018). Employing both morphological and molecular analysis for identification and characterization of *T.miser* species has been carried out for the first time. COI gene was used as marker gene for barcoding purposes for this species. Card pointed specimens were observed under LeicaM205A stereozoom microscopefor morphological studies. Key morphological characters like single SMV dorsal setae, black body with metallic reflections, were consonance with studies of Narasimham (1984).The specimens slightly varies in the number and pattern of setae on mesonotum, the other characters are matching the re-description by Graham,(1991).

The final alignment of the data set resulted in 567 nucleotide sites having 488 conserved sites, 121 variable sites, 97 parsim-info sites and 24 singleton sites. The mean A+T content was revealed as 74.69 %. The data was analyzed for sequence divergence at different taxonomic levels in MEGA X software. Pairwise distance using K2P parameter in MEGA X was used to calculate the distance matrix (Kimura, 1980). Interspecific divergence between *Tetrastichus miser* and Tetrastichinae sp. was noticeable and ranged from 0.09 to 0.10 % (0.05 % mean). No overlap was observed between maximum K2P distance within species and minimum distance between species. High nucleotide variations indicate geographical isolation and hence limited gene flow between species (Santos et al., 2011; Powell et al., 2019).

For phylogenetic analysis, Maximum likelihood method and Bayesian Inference methods were used to infer evolutionary history so as to look for clustering of clades in different trees. Both methods resulted in somewhat similar typology. In both the methods congeneric species cohesively clustered together with closely related genera. Besides, taxa belonging to a particular species more often than not formed a coherent cluster indicating that CO1 gene sequences are useful in identification of species. The bootstrap consensus tree inferred from 1000 replicates is taken to correspond to the evolutionary history of the taxa analyzed (Felsenstein, 1985). The taxa belonging to the same species, genus or family clustered together with healthy bootstrap support. It was also found that sequences from same country and genus or species clubbing in the same clade in both trees. Along with low support values at nodes, some high values were also reported when nodes include sister or conspecific sequences. The reason for this is that the COI gene fragment has been reported to best resolve shallow species-level relationships in arthropod fauna but showed poor results when family level and beyond relationships were taken into consideration (Waugh, 2007) and same was the case with our sequence data. Nevertheless, molecular analysis supported morphological results, besides adding its barcode to the databases for further exploration. Considering the role of *T. miser* in biological control programmes, molecular data of this study will serve as an elite DNA barcode for species identification, future molecular studies, better understanding of bio-control services and other related taxonomic studies in future.

## 5 CONCLUSION

*Tetrastichus miser* is well known parasitoid of scolytid beetles infesting various tree species of economic importance. Morphologically, species has been defined and described in India, but molecular taxonomy was missing. There was rarely an entry in the GenBank database for this species. This study was carried to add to knowledge of chalcid wasps from Kashmir valley and also complement its morphologically defined taxonomy. COI gene of mitochondrial genome was used as DNA barcode for the molecular analysis. The sequenced segment was found to be 567 bp long and was found to be AT rich in content. The sequence showed low percentage of match in NCBI and BOLD database systems with the closest match being Tetrastichinae sp. (91 %).Phylogenetic analysis inferred close match between *Tetrastichus* isolates, clustering into same clade with good bootstrap support. No overlap was observed between maximum K2P distance within species and minimum distance between species. Considering the obscurity in identification of diverse insect fauna, this exercise will complement taxonomic analysis and sequence data will serve as an elite DNA barcode for species identification and other related taxonomic studies in future.

## 6 CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

## 7 ACKNOWLEDGEMENT

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# The effects of temporary occupation of agricultural land by gravel deposits and construction on selected soil properties

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**The effects of temporary occupation of agricultural land by gravel deposits and construction on selected soil properties**

**Abstract:** We addressed the condition of restored soil on alluvial plain in the south-eastern Slovenia after they have been given for the gravel deposit easement during construction. According to pre-investigation using soil probes, two soil profile pits were dug: Profile 1 on the area where excavated soils were deposited over original soils; and Profile 2 on the area where topsoil had been removed before gravel deposition and reapplied after the easement. Undisturbed and disturbed soil samples were collected and analyzed for physical and chemical properties. The results show that chemical properties were generally not the limiting factor for soil fertility. Compaction of the soil reduced hydraulic conductivity and resulted in water stagnation. The bulk density on the area where the material was deposited directly on the soil surface ranged from 1.41 to 1.77 g $cm^{-3}$. The hydraulic conductivity of the saturated soil was practically impermeable at depths of 10, 20, and 30 cm, indicating compaction due to high mechanical load. At the area where topsoil was removed before deposition and restored after easement the hydraulic conductivity of the saturated soil was low to moderate. Removal of the topsoil before construction began was an appropriate action, but reclamation measures are also required.

**Key words:** fluvisols; soil degradation; soil restoration; soil physical properties; soil chemical properties

**Vpliv začasne zasedbe kmetijskih zemljišč z deponijo gramoza ob gradbenih posegih na lastnosti tal**

**Izvleček:** Namen raziskave je bil preveriti lastnosti tal na območju spodnje Save po rekultivaciji zaradi začasne zasedenosti zemljišč za deponijo gramoza. Po pregledu območja (sondiranje) smo na dveh mestih izkopali talna profila; profil 1 na delu, kjer je bila na obstoječa tla odložena odstranjena rodovitna zemljina; profil 2 na delu, kjer je bil vrhnji sloj tal pred deponiranjem gramoza odstranjen in nato ponovno vzpostavljen. Odvzeli smo neporušene in porušene talne vzorce za merjenje fizikalnih in kemijskih lastnosti tal. Izmerili smo teksturo, volumsko gostoto tal, nasičeno hidravlično prevodnost, pH, vsebnost organske snovi, parametre kationske izmenjalne kapacitete in rastlinam dostopna hranila. Ugotovili smo, da kemijske lastnosti v splošnem niso ovirale rodovitnosti tal. Zbitost tal je omejevala hidravlično prevodnost in povzročila zastajanja vode. Na območju, kjer je bil deponiran material neposredno na površino tal, je bila gostota tal od 1,41 do 1,77 g $cm^{-3}$. Tla so bila na tem delu praktično neprepustna na globinah 10, 20 in 30 cm, kar kaže na veliko zbitost zaradi mehanskih obremenitev. Tla, na območju, kjer je bila vrhnja plast tal odstranjena in po odstraniti začasne deponije ponovno nanesena, so bila manj zbita. Odstranitev zgornje plasti tal pred deponiranjem gramoza je bil ustrezen ukrep, vendar so potrebni tudi melioracijski ukrepi po zaključku gradbenih del.

**Ključne besede:** obrečna tla; degradacija tal; rekultivacija tal; fizikalne lastnosti tal; kemijske lastnosti tal

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## 1 INTRODUCTION

Alluvial plains are important agricultural areas due to favourable soil properties, topography and the vicinity of water resources. Even though several soil types occur, Fluvisols and Cambisols are the most important. Fluvisols are young soils formed by frequent deposition of sediments along river courses and streams. In the upper reaches of the channel, the sediments are usually dominated by large boulders and angular stones, but downstream the particles increase in roundness, become smaller and represent a good basis for soil development. Soils of alluvial plains (Fluvisols, Cambisols) of the lower reaches of rivers or along streams are mostly under agricultural land use, less commonly under forest. The sediments are fine-grained (silty or clayey), and the epipedon may be thick and rich in humus (Vidic et al., 2015, Vrščaj et al., 2017). The lower part of a soil profile may contain gravel and sand; if finer, we usually find reductimorphic features as evidence of gleying, such as grey-brown mottling, which is a consequence of a changing levels of groundwater table and alternating reduction and oxidation processes. Alternation of these processes results from alternating wet and dry phases in the soils, which are associated with a seasonal distribution of precipitation. In Slovenia, wet autumn and spring periods lead to stagnant water in the soil profile alternating with dry winter and summer periods when soil pores fill with air and cause oxidation of Fe substances on the walls of pores and surface of soil aggregates. However, permanent water stagnation in Fluvisols and Cambisol is rare and occurs only in lower soil horizons, which can express predominantly grey color. The alluvial plains are typical for their distinct hydraulic properties (e.g. by higher hydraulic conductivities ranging from 2 to 180 m $day^{-1}$ in the subsoil) compared to upper laying parts of the watershed (Miller et al., 2016; Šípek et al., 2019). Soils are usually enriched with nutrients and characterized by high vertical and horizontal heterogeneity, which is explained with the varying characteristics of alluvial sediments, regime of deposition, age of formation (distance to the river), and land use (Kercheva et al., 2017). In some cases, Fluvisols may be subjected to contamination of deposits (Antić et al., 2006; Schwartz et al., 2006; Mabit et al., 2012).

The predominant land use of alluvial plains is agricultural, where high quality arable land for intensive crop and vegetable production (Maršić et al., 2012; Vrščaj et al., 2017) alternates with grassland (for livestock). The latter is more often found in areas with clayey soils and stagnating water. Fluvisols are of high importance because of their broad ecosystem functions, not only for agricultural production but also for their role in soil water (Zupanc et al., 2011; Zupanc et al., 2012, Zupanc et al., 2020) and flood water retention (Glavan et al., 2020; Bezak et al., 2021). The agronomic significance provokes long-standing interest in determining and mapping of soil physical and chemical properties for designing either drainage or irrigation system (Kercheva et al., 2017, Matičič and Steinman, 2007).

Alluvial plains are very often the subject of different interests of land use planners (Zupanc et al., 2011). Beside agricultural land use, the construction of urban and industrial infrastructure pose negative effects on soil resources (Grčman and Zupanc, 2018), not only directly with soil sealing but also due to the indirect influence of construction work on nearby land and siting of meliorative measures necessary for compensating natural habitats (e.g. flood protection measures, Bezak et al., 2021). As Fluvisols are young soils, soil morphological properties, namely soil structure aggregates are unstable and weakly expressed. Such soils are susceptible to compaction and their structure is not easily re-established after the disturbance (Zupanc et al., 2016, Schomburg et al., 2019), which leads to water logging and hampers soil tillage (Grčman and Zupanc, 2018).

As the areas of Fluvisols are very limited in Slovenia (5 % of Slovenian territory; Vrščaj et al, 2017), we have to pay attention to soil sealing and other degradation processes caused by construction works, which often require easement of the surrounding area. The aim of this study was to evaluate the soil properties on the alluvial plain of the lower Sava River, to assess its possible degradation after the construction of a hydropower plant, for which an easement for gravel deposits was required. We evaluated chemical and physical parameters crucial for soil fertility to establish possible degradation and causes of water stagnation.

## 2 MATERIALS AND METHODS

The study area is located in the alluvial plains of the lower Sava and Krka rivers (Figure 1). The area was affected by the construction of the Brežice hydropower plant, as part of the agricultural land was used for gravel deposition during the construction works. After the construction works were completed, the gravel deposits were removed and the land was returned to agricultural use (Figure 1). However, stagnant water was seen in some parts of the area, raising questions about the quality of the earthworks used to restore the land.

After detailed surface inspection and soil probing, two sites were selected for excavation of the soil profile pits (Fig. 1). One on the area where the excavated fertile topsoil was deposited directly on the agricultural land (Profile 1), and the other on an area where the fertile top-

soil was removed before the gravel was deposited up to the height of 2 - 6 meters and later restored (Profile 2).

The description of morphological properties was done according to the Guidelines for soil description (FAO, 2006) and disturbed soil samples were taken from each recognized horizon. Undisturbed soil samples (V = 100 $cm^3$) were taken in 10 cm increments. The soil samples were analysed for soil physical properties, i.e., texture, soil bulk density, and saturated hydraulic conductivity, as well as chemical properties, i.e. pH, plant available nutrients organic matter content and parameters of cation exchange capacity. Texture was measured by sedimentation pipette method (SIST ISO 11277), bulk density of soil was determined gravimetrically (ISO 11272, 1993). Saturated hydraulic conductivity was measured using a Darcy apparatus. Five measurements of water flow under saturated conditions were made for each sample and the average was calculated. Results for saturated hydraulic conductivity were interpreted using Bear's (1972) permeability classes (< 0.001 m $day^{-1}$ practically impermeable, 0.001–0.01 very low permeability, 0.01–1m $day^{-1}$ low permeability and from 1m $day^{-1}$ permeable soils). Organic matter content was measured by SIST ISO 14235 – modified method after Walkely-Black, total nitrogen after dry combustion (ISO 13878), cation exchange capacity according to Soil survey laboratory methods manual (1992), pH in extraction with $CaCl_2$ after SIST ISO 10390, and plant available phosphorous and potassium after ÖNORM L 1087 – modification – amonlactate extraction.

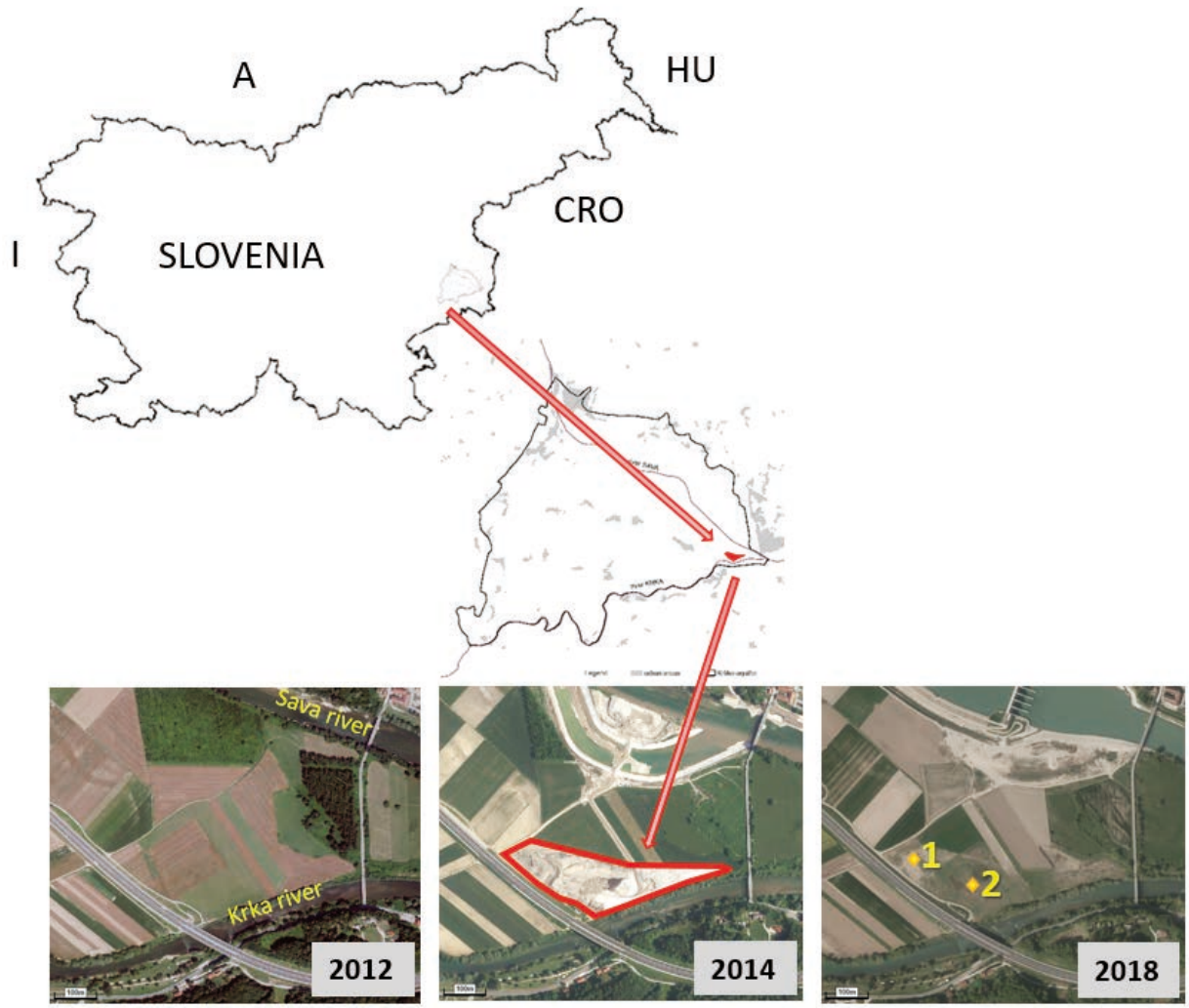


**Figure 1**: The land use on alluvial plain between the Sava River and the Krka River before, during and after hydropower plant construction; the location of two soil profile pits are marked on the right picture
**Slika 1**: Raba tal na aluvijalni ravnini med Savo in Krko pred, med in po izgradnji hidroelektrarne; na desni sliki sta označeni lokaciji profilov 1 in 2

## 3 RESULTS AND DISCUSSION

Both soil profiles were deep and had an anthropogenic influence. The soils on the western part of the formerly occupied land (Profile 1) have a sequence of horizons typical of the Fluvisols of the lower Sava River (Prus, 2000; Prus et al. 2015; Vidic et al., 2015; Vrščaj et al., 2017). Textural differences between the soil horizons were typical of sedimentation processes, but the structural aggregates which were angular-blocky in shape and weak in grade indicating that pedogenetic processes had already started, leading to the development of eutric brown soils (Eutric Cambisols). Morphological evidence of stagnant water, i.e. grey-brown mottling, was found throughout soil profile 1 and in two layers of profile 2, although to a small extent. No water occurred at the bottom of the profiles, although sampling was conducted several days after heavy rain, suggesting that textural discontinuities and soil compaction may be affecting water movement through the soil profile (Figure 2, Table 1).

Soil properties vary horizontally and vertically. These differences occur mainly in texture, which is a result of the different alluvial sediments. Variation in chemical properties was less pronounced (Tables 2 and 3). In =Profile 1, the soil texture varies, silt particles are the predominant soil texture fraction, after which the sand fraction increases to over 80 %. Cation exchange capacity reflects clay content and decreases with depth. Base saturation is high, greater than 90 %, with calcium being the predominant cation. The pH is high, ranging from 7.6 to 7.9. Organic matter decreases with depth, which is typical of undisturbed automorphic soil profiles. The soils are poor in plant-available phosphorus and potassium, indicating that the soils were extensively farmed in the past without the use of fertilizers.

In Profile 2 greater textural variability through the depth was measured. Organic matter content is much higher compared to Profile 1, with concentrations greater than 4 % to a depth of 89 cm. The texture, organic matter content, and soil color indicate that approximately 90 cm layer was removed and later reapplied as topsoil. The cation exchange capacity reflects the clay and organic

**Figure 2**: Two soil profile pits were dug;Pprofile 1 on the area where the excavated fertile topsoil was deposited directly on the agricultural land (left); Profile 2 on the area where the fertile topsoil was removed before the gravel was deposited and later restored (right)

**Slika 2**: Izkopana sta bila dva pedološka profila; profil 1 na območju kjer je bila začasno deponirana izkopana zemljina neposredno na površino kmetijskih tal (levo); profil 2, na kasneje rekultiviranem območju, kjer so pred začasno deponijo gramoza odstranili zgornji sloj tal (desno)

**Table 1**: Morphological characteristics of soil
**Preglednica 1:** Morfološke lastnosti tal

| Area/Profile | Hori-zon* | Soil depth (cm) | Colour*** | Structure | Consistency when moist | Roots | Pedogenetic forms |
|---|---|---|---|---|---|---|---|
| Topsoil was not removed | Ap | 0 - 22 | 2.5Y 4/2 | Angular blocky | Very firm | very few | few mottles |
| Profile 1 | II | 22 - 46 | 10YR 4/4 | Angular blocky | Firm | very few | few mottles |
| | III | 46 - 82 | 10YR 4/3 | Angular blocky | Firm/Friable | very few | few mottles |
| | IV | 82-102 | 10YR 4/4 | Angular blocky | Friable | very few | few mottles |
| | V | 102-138 | 10YR 5/3 | Angular blocky | Friable/Loose | very few | few mottles |
| | VI | 138-179 | 10YR 6/4 | Single grain | Loose | no | - |
| Topsoil was removed and later restored | I** | 0-45 | 2.5Y 3/3 | Angular blocky | Friable | few | no |
| | II** | 45-89 | 2.5Y 3/2 | Angular blocky | Firm/Friable | few | few mottles |
| Profile 2 | III | 89-119 | 10YR 5/3 | Angular blocky | Firm/Friable | very few | few mottles |
| | IV | 119-160 | 10YR 5/4 | Angular blocky | Friable | no | no |
| | V | 160-175 | 10YR 6/6 | Single grain | Loose | no | no |

*according to Slovenian national classification, horizons of Fluvisols and Technosols are marked with roman number (Prus et al., 2015)
**replaced layers
***soil colour was identified using Munsell soil colour chart

**Table 2**: Soil texture and chemical soil characteristics
**Preglednica 2**: Tekstura in kemijske lastnosti tal

| Area/ Profile | Hori-zon* | Soil depth | Sand | Silt | Clay | Texture | pH | Org. matter | C | N | C/N | $P_2O_5$ | $K_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cm | | % | | | | % | | | | mg/100 g | |
| Topsoil was not removed | A | 0-22 | 16.0 | 62.6 | 21.4 | SL | 7.6 | 2.6 | 1.5 | 0.16 | 9.4 | 0.7 | 7.9 |
| | II | 22-46 | 5.5 | 71.5 | 23.0 | SL | 7.7 | 1.8 | 1.0 | 0.13 | 7.7 | < 0.5 | 5.5 |
| Profile 1 | III | 46-82 | 6.4 | 70.9 | 22.7 | SL | 7.8 | 1.3 | 0.8 | 0.09 | 8.9 | < 0.5 | 4.7 |
| | IV | 82-102 | 20.6 | 64.5 | 14.9 | SL | 7.8 | 0.9 | 0.5 | 0.06 | 8.3 | < 0.5 | 3.5 |
| | V | 102-138 | 62.1 | 30.2 | 7.7 | Sl | 7.8 | 0.5 | 0.3 | 0.02 | 15.0 | < 0.5 | 2.1 |
| | VI | 138-179 | 87.4 | 8.0 | 4.6 | S | 7.9 | 0.2 | 0.1 | 0.01 | 10.0 | 0.6 | 1.6 |
| Topsoil was removed and later restored | I** | 0-45 | 50.8 | 34.5 | 14.7 | L | 7.5 | 4.5 | 2.6 | 0.14 | 18.6 | 1.4 | 6.1 |
| | II** | 45-89 | 43.0 | 41.4 | 15.6 | L | 7.6 | 4.2 | 2.4 | 0.14 | 17.1 | 1.4 | 6.4 |
| Profile 2 | III | 89-119 | 17.4 | 67.1 | 15.5 | SL | 7.7 | 1.2 | 0.7 | 0.07 | 10 | < 0.5 | 4.1 |
| | IV | 119-160 | 20.9 | 64.6 | 14.5 | SL | 7.8 | 0.9 | 0.5 | 0.06 | 8.3 | < 0.5 | 3.4 |
| | V | 160-175 | 62.1 | 30.7 | 7.2 | PL | 7.8 | 0.3 | 0.2 | 0.02 | 10 | < 0.5 | 2.7 |

*According to Slovenian national classification, horizons of Fluvisols and Technosols are marked with roman number (Prus et al., 2015)
** replaced layers

matter content and decreases with depth. Base saturation is high, greater than 90 %, with calcium being the predominant cation. The pH is high, ranging from 7.5 to 7.8. Similar to profile 1, the soils are poor in plant-available phosphorus and potassium.

The results show that chemical properties are generally not the limiting factor for soil fertility, especially high base saturation, high pH and high organic matter content were favorable characteristics. However, nutrient content could be increased by intensive fertilization.

The bulk density of the soil in Profile 1 (area, where topsoil has not been removed) ranged from 1.41 to 1.77 g $cm^{-3}$. Notable is a large difference between the soil density of the uppermost 30 cm and the depth from 40 cm, where soil bulk density was from 1.41 to 1.54 g $cm^{-3}$) (Figure 3). Soil bulk density of the upper 30 cm exceeds

**Table 3**: Parameters of cation exchange capacity
**Preglednica 3**: Izmenljivi bazični kationi in kationska izmenjalna kapaciteta

| Area/Profile | Hori-zon* | Soil depth | Ca | Mg | K | Na | H | CEC | Base saturat. |
|---|---|---|---|---|---|---|---|---|---|
| | | cm | | | $mmol_C\ 100\ g^{-1}$ | | | | % |
| Topsoil was not removed | Ap | 0-22 | 25.07 | 2.01 | 0.19 | 0.07 | 1.40 | 28.7 | 95.1 |
| Profile 1 | II | 22-46 | 27.14 | 2.17 | 0.12 | 0.12 | 1.05 | 30.6 | 96.6 |
| | III | 46-82 | 27.00 | 1.99 | 0.11 | 0.10 | 0.85 | 30.1 | 97.2 |
| | IV | 82-102 | 23.22 | 1.33 | 0.07 | 0.06 | 0.10 | 24.8 | 99.6 |
| | V | 102-138 | 18.89 | 0.70 | 0.04 | 0.04 | 0.10 | 19.8 | 99.5 |
| | VI | 138-179 | 17.60 | 1.41 | 0.03 | 0.03 | 0.10 | 19.2 | 99.5 |
| Topsoil was removed and later restored | I** | 0-45 | 27.25 | 1.44 | 0.15 | 0.06 | 1.85 | 30.8 | 94.0 |
| | II** | 45-89 | 25.58 | 1.41 | 0.15 | 0.05 | 1.65 | 28.8 | 94.3 |
| Profile 2 | III | 89-119 | 23.29 | 1.06 | 0.09 | 0.07 | 0.10 | 24.6 | 99.6 |
| | IV | 119-160 | 23.92 | 1.15 | 0.07 | 0.08 | 0.10 | 25.3 | 99.6 |
| | V | 160-175 | 19.71 | 0.68 | 0.05 | 0.05 | 0.10 | 20.6 | 99.5 |

*According to Slovenian national classification, horizons of Fluvisols and Technosols are marked with roman number (Prus et al., 2015)
** replaced layers

values, commonly found in the soils of alluvial plains (Kercheva et al., 2017). These results confirm the findings from the field, namely that the uppermost soil layer is highly compacted, hindering the flow of water to depth. The bulk density in profile 2 (area with removed and restored soil) ranged from 1.47 to 1.37 g $cm^{-3}$ (Table 3). In addition to the removal of topsoil prior to the placement of gravel, soil texture could also influence the bulk density. Soils with higher sand content are less susceptible to compaction.

Since hydraulic conductivity below 0.001 m $day^{-1}$ indicates practically impermeable soils (Bear, 1972), the top 30 cm layer was practically impermeable (Figure 4). This could also imply that no water would be infiltrating and percolating vertically and replenishing water storage below the root zone without meliorative measures (deep plowing, plant cover) potentially indefinitely.

In the area where topsoil was removed before deposition and later soils were restored (Profile 2), there were differences in the hydraulic conductivity of the saturated soil within individual soil layer and between soil layers (e.g. 10, 30 and 40 cm depth, Fig. 4). When the hydraulic conductivity of the upper layer is much larger compared to the hydraulic conductivity of the lower layer (factor 10 or larger), the effect of impervious layer occurs. This may cause water stagnation even between more permeable layers. However, large differences were observed between soil profiles at different depths, most likely due to the different soil texture typical for Fluvisols and heterogeneous consolidation of soil mass after soil restoration.

After construction and restoration works are completed, the soil must be rehabilitated to improve the physical properties of the soil (Krümmelbein et al., 2010; Krümmelbein et al., 2012). Generally, restoration cannot be done with construction measures alone (Krümmelbein et al., 2010; Zupanc et al., 2016), necessary time for soil rehabilitation depending on the extent of disturbance to the soil profile (Grčman and Zupanc, 2018). The reasonable approach is to leave the last phase to land users (farmers), who are better able to adapt to weather conditions and optimal soil moisture and consistency than construction companies (Zupanc et al., 2016). A high value of bulk density and poor hydraulic conditions expressed as stagnant water at the soil surface indicate that meliorative measures need to be taken to accelerate soil aggregation and thus improve soil structure. This can best be achieved with a suitable plant cover (e.g. *Medicago sativa* L.), where the roots of the plants can help to structure and loosen the compacted layers (Schomburg et al., 2019). It is important to establish plant cover as soon as possible, and we recommend that protective plants for reclamation remain for at least three years.

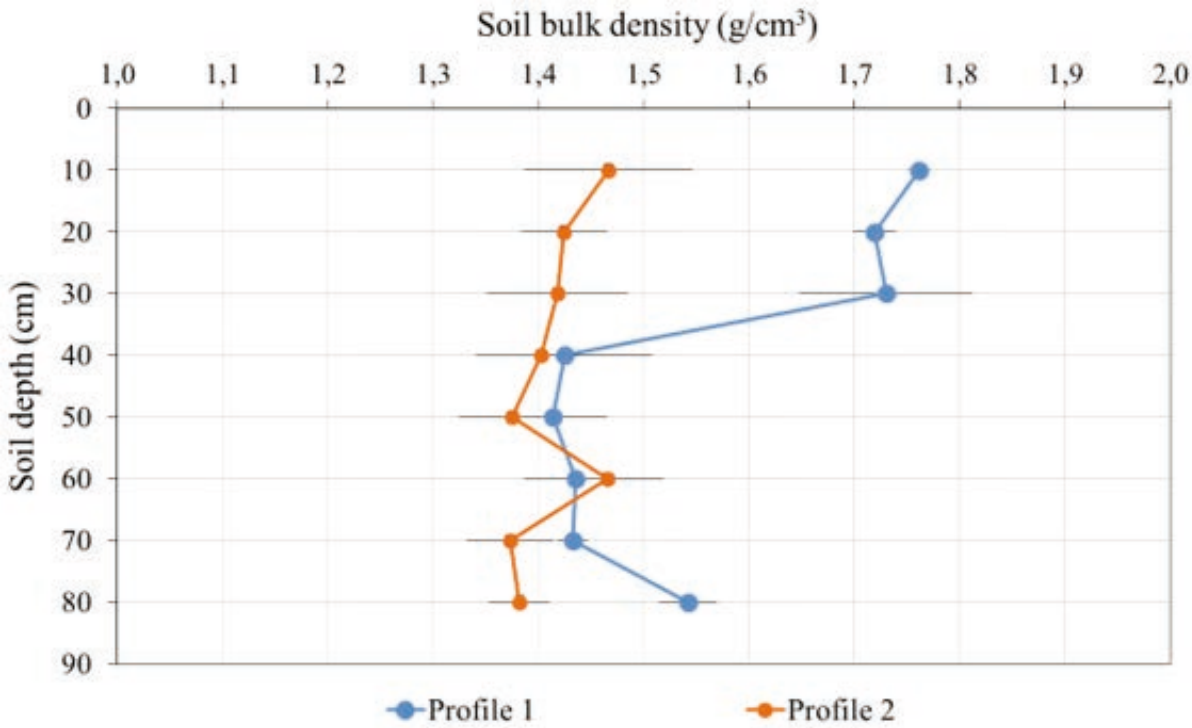


**Figure 3**: Average soil bulk density (g cm$^{-3}$) for soil Profile 1 and soil Profile 2
**Slika 3**: Povprečna volumska gostota tal (g cm$^{-3}$) v talnem profilu 1 in v talnem profilu 2

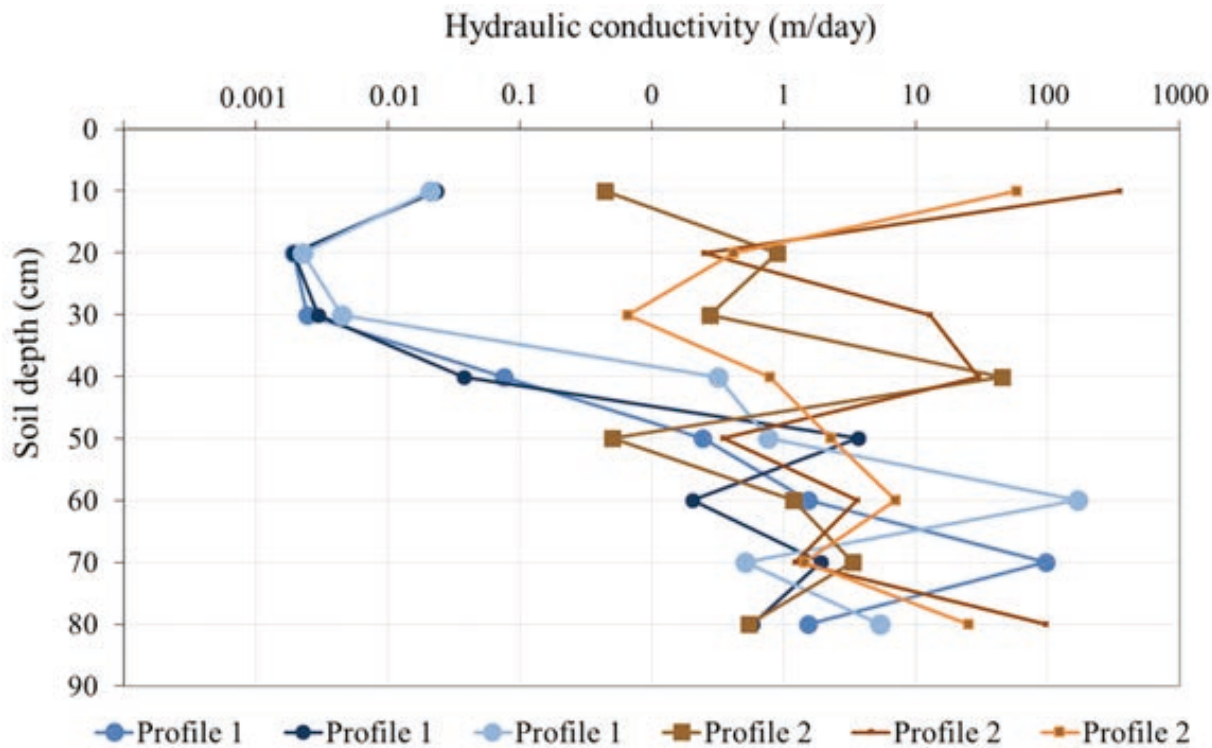


**Figure 4:** Saturated hydraulic conductivity (m day$^{-1}$) for soil Profile 1 and soil Profile 2 (three replicates)
**Slika 4:** Hidravlična prevodnost nasičenih tal (m dan$^{-1}$) v talnem profilih 1 in 2 (tri ponovitve)

## 4 CONCLUSIONS

The results of our study show that the temporary occupation of agricultural land by gravel deposits can have negative effects on soil functions. While chemical properties were not affected and were generally not the limiting factor for agricultural use, the bulk density and hydraulic conductivity of the soil showed serious consequences of mechanical stress. Hydraulic conductivity was reduced in the area where the topsoil was not removed prior to deposition (Profile 1) due to compaction in the upper 30 cm. The restored soils (Profile 2) and the lower soil layers of the both permanently occupied sites (profiles 1 and 2) had hydraulic conductivity typical of soils in alluvial plains. Removal of the topsoil prior to the start of construction was an appropriate measure.

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# Evaluation of biocidal activity of four Lamiaceae leaves on the black bean aphid *Aphis fabae* Scopoli, 1763 (Homoptera: Aphididae)

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**Evaluation of biocidal activity of four Lamiaceae leaves on the black bean aphid *Aphis fabae* Scopoli, 1763 (Homoptera: Aphididae)**

**Abstract:** The objective of our work was the valorisation of four aromatic plants growing spontaneously in Kabylia (Algeria) by the evaluation of their insecticidal activity against the black bean aphid *Aphis fabae* Scopoli, 1763. These are oregano, thyme, rosemary and lavender. The plants were subjected to a phytochemical analysis to determine their secondary metabolites composition. The results obtained showed that the four extracts are toxic to *A. fabae* and can significantly reduce its populations. LD50s of 7.76 %; 8.91 %; 9.72 % and 12.88 % were recorded for extracts of oregano, rosemary, thyme and lavender respectively. In addition, the phytochemical screening shows the presence of flavonoids, tannins, saponins and polyphenols. The polyphenols extraction indicates that the oregano extract is the richest with a polyphenol content of 218.73 (± 0.22) µg GAE $ml^{-1}$. This substance has significant biocidal power.

**Key words:** *Aphis fabae*; mortality rate; Lamiaceae; polyphenols; phytochemical screening

**Ovrednotenje biocidne aktivnosti izvlečkov listov štirih vrst ustnatic (Lamiaceae) na črno fižolovo uš, *Aphis fabae* Scopoli, 1763 (Homoptera: Aphididae)**

**Izvleček:** Predmet razikave je bilo ovrednotenje insekticidne aktivnosti štirih aromatičnih rastlin, ki rastejo samoniklo na območju Kabylije (Alžirija) na črno fižolovo uš *Aphis fabae* Scopoli, 1763. Izbrane rastline so bile dobra misel, timijan, rožmarin in sivka. V rastlinah so s fitokemičnimi analizami določili sestavo sekundarnih metabolitov. Rezultati so pokazali, da so bili vsi štirje izvlečki strupeni za črno fižolovo uš in so znatno zmanjšali njeno populacijo. Vrednosti LD50 so za izvlečke dobre misli, rožmarina, timijana in sivke znašale 7,76 %; 8,91 %; 9,72 % in 12,88 %. Fitokemične analize rastlin so pokazale prisotnost flavonoidov, taninov, saponinov in drugih polifenolov. Izvleček polifenolov je pokazal, da je bila na njih najbogatejša dobra misel z vsebnostjo 218,73 ( ± 0,22) µg GAE $ml^{-1}$, kar kaže na njeno znatno biocidno moč.

**Ključne besede:** *Aphis fabae*; smrtnost; Lamiaceae; polifenoli; fitokemijska analiza

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## 1 INTRODUCTION

Herbivorous insects are responsible for significant losses to agriculture due to food damage, but also by carrying pathogens such as viruses (Kortbeek et al., 2019). They have implemented a range of strategies allowing them to discover their host plants and then reproduce and develop at their expense (Huignard, 2013). Aphids are considered among the most important pests of crops. They cause significant financial losses, and are responsible for yield losses in many cultivated plants (Tagu et al., 2004).

These pests cause direct damage by sucking sap, and indirectly by transmitting phytopatogenic viruses and by secreting honeydew on which sooty mold is deposited, which decreases photosynthesis (Blackman and Eastop, 2000; 2006). The use of insecticides is one of the first methods used against aphids (Esmaieli-Vardanjani et al., 2013). However, as a result of excessive use of these products, some aphids have developed resistance to most of them. In addition, insecticides destroy beneficial organisms and disrupt ecosystems (Harmel et al., 2010; Sabahi et al., 2011).

It is therefore necessary to have an alternative control program and to search for new products, on the one hand, to ensure effective protection of agricultural production, and on the other hand, to contribute to sustainable management of the environment (Bouchelta et al., 2005). For this purpose, several control methods are recommended to limit the level of aphid outbreaks in crops (Harbaoui et al., 2008). Among these, the use of plant extracts endowed with insecticidal activities which offer a certain potential (Isman, 2000). In fact, plants have developed different mechanisms to defend themselves against insects, including the production of specialized metabolites that act as natural insecticides (Kortbeek et al., 2019).

According to Huignard (2013), plants are rich in phenolic compounds. The latter are of significant physiological and morphological importance in the plant kingdom; they are involved in growth, reproduction and give the plant some resistance against pathogens and pests (Maroun et al., 2013). The main objective behind this work is to study the insecticidal activity of four aqueous extracts of Lamiaceae: oregano (*Origanum floribundum* Munby), thyme (*Thymus numidicus* Poir.), rosemary (*Rosmarinus officinalis* L.) and lavender (*Lavandula stoechas* L.) on the black bean aphid *Aphis fabae* Scopoli, 1793 (Hemiptera: Aphididae) which can transmit 30 viral diseases to plants (Jahan et al., 2019), as well as the research of the main active elements and the content of polyphenols that exist in each plant.

## 2 MATERIALS AND METHODS

### 2.1 HARVESTING PLANT MATERIAL

During this experiment, four species of Lamiaceae were tested on the black bean aphid, these are rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus numidicus* Poir.), oregano (*Origanum floribundum* Munby) and lavender (*Lavandula stoechas* L.). Rosemary and lavender were harvested in a private garden located in the region of Draâ El Mizan at an altitude of 432 m (36° 32'8"N. 3° 50'3"E.). Whereas the thyme came from the mountains of Ait Bouadou (Ouadia) at an altitude of 828 m (36° 30'0"N. 4° 1'0"E.), and oregano was harvested in a private plot located in the Makouda region at an altitude of 458 m (36° 47'27"N. 4° 4'1"E.).

### 2.2 PREPARATIONS OF AQUEOUS EXTRACTS

For each tested plant, leaves are dried and then crushed. 50 g of powder for each plant were macerated in 1 l of distilled water for 24 hours. Resulting solutions were filtered then stored in vials and kept in the dark. 11 doses (1 %, 2 %, 3 %, 4 %, 5 %, 10 %, 20 %, 30 %, 40 %, 50 % and 100 %) whose concentrations vary according to the dilution of the stock solution in the distilled water were prepared.

### 2.3 EXPERIMENTAL DESIGN

An experimental design formed from 144 jars of infested beans are treated with the four plant extracts by means of contact. 12 batches including a control batch have been used for each extract. Aphids were given one dose per batch, with three repetitions for each dose. The three control jars were treated with distilled water.

### 2.4 APPLICATION OF TREATMENTS

After sowing the bean seeds ('Histal') in plastic pots, small seedlings obtained were infested with 40 aphids; larvae of different stages and adults. After three days of infestation, the treatment was applied by spraying different doses of the four aqueous solutions on the aphid colonies. In order to follow the chronological evolution of aphids mortality subjected to different extracts at different concentrations, observations were made daily after spraying. A magnifying glass has been used to count the dead and alive aphids.

### 2.5 CHARACTERIZATION OF MAIN CHEMICAL COMPONENTS

Phytochemical study enables the detection of bioactive secondary metabolites existing in the leaves of the plants tested. It is based on coloring and precipitation reactions by specific chemical reagents. The method used is the approach adopted by Tona et al. (1998) and Longaga et al. (2000).

Besides, total polyphenol content is obtained by spectrophotometry according to Folin-Ciocalteu (FCR) method (Singleton et al., 1999).

### 2.6 DATA ANALYSIS

Mortalities in the treated batches (Mo) were expressed according to Abbott's formula (1925) in corrected mortality (Mc), taking into account natural mortalities observed in the control batches (Mt). Mortality is expressed as a percentage calculated using the following formula:

$$Mc = (Mo\text{-}Mt / 100\text{-}Mt) \times 100$$

*Mc*: Corrected mortality rate
*Mo*: Mortality rate in the treated population
*Mt*: Mortality rate in the control population

### 2.7 KRUSKAL-WALLIS TEST

The Kruskal-Wallis non-parametric test has been used to compare the average mortalities obtained with different treatments. It is based on classification in ascending order of all observations (Legras and Kohler, 2007).

### 2.8 CALCULATION OF LETHAL DOSE (LD50) AND LETHAL TIME (TL50)

The method of Finney (1971) based on the regression of the probits of mortalities depending on the logarithms of the doses of solutions tested made it possible to determine the LD50. The 50 % lethal dose of each extract was estimated, after exposure of aphids to different concentrations tested. These values were determined from an experimental curve giving the variations in mortality according to increasing concentrations of the extracts. The 50 % lethal time (TL50) was also determined by the same method. The time is transformed into a logarithm and the percentage of mortality corrected into probit.

## 3 RESULTS

After spraying the leaf solutions of the four Lamiaceae on *A. fabae* populations, an increase in the percentage of mortality of the latter appeared as a function of time and dose (Fig. 1). Total mortality was observed after 12 days of treatment at the highest doses (50 % and 100 %) for oregano, thyme and rosemary extracts, and at the 100 % dose for lavender extract (Table 1).

### 3.1 KRUSKAL-WALLIS TEST

The mortality-dose boxplot shows that the mortality rate is dose dependent; as the dose increases, the mortality rate increases (Fig. 2). This is confirmed by the Kruskal Wallis test, with a p-value of 5.834e-14 for thyme, 3.891e-16 for oregano, 2.695e-12 for rosemary and 9.585e-14 for lavender. These values are less than 0.05. This means that there is a highly significant effect of dose on aphid mortality.

The Mortality-time boxplot shows that the mortality rate is higher at time T6 = 12 days after treatment (Fig. 3). This is confirmed by the Kruskal Wallis test with a p-value of 1.811e-14 for thyme, 9.912e-14 for oregano, 5.711e-16 for rosemary and 5.006e-16 for lavender. These values confirm that there is a very significant effect of time on aphid mortality.

### 3.2 DETERMINATION OF THE LD50 OF THE VARIOUS TREATMENTS TESTED ON *A. FABAE*

After spraying the aqueous extracts of the four plants tested on different batches of aphids, at doses ranging from 1 % to 100 %, the percentages of mortality transformed into probits were recorded and plotted in figure 4. The LD50 of each product confirms the results obtained in the tests. Indeed, the LD50 obtained showed that the solutions tested present a high degree of toxicity towards these insects. Oregano extract gave the lowest lethal dose (7.76 %). It is therefore the most toxic extract compared to the other biopesticides with LD50s of 8.91 %, 9.72 % and 12.88 % for rosemary, thyme and lavender extracts respectively.

### 3.3 DETERMINATION OF THE TL50 OF THE VARIOUS TREATMENTS TESTED ON *A. FABAE*

Calculation of the lethal time showed that the extracts tested on the black bean aphid had a fairly high

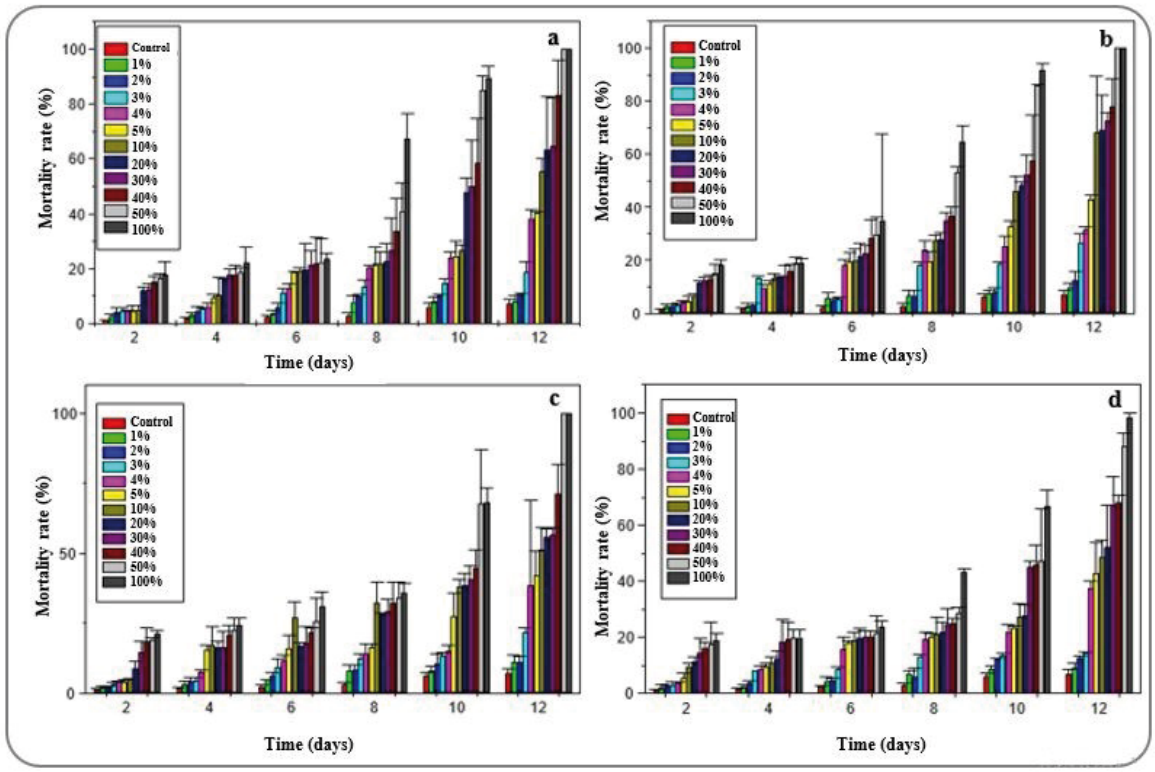


**Figure 1:** Aphid mortality rate (a: Thyme extract, b: Oregano extract, c: Rosemary extract, d: Lavender extract)

**Table 1**: Corrected mean mortality of populations of *Aphis fabae* after 12 days of treatment with the 4 aqueous extracts

| | Corrected mean Mortality (%) | | | |
|---|---|---|---|---|
| Dose (%) | Oregano | Thyme | Rosemary | Lavender |
| 1 | 3.03 | 0.88 | 2.58 | 1.76 |
| 2 | 5.61 | 2.95 | 4.13 | 5.46 |
| 3 | 20.7 | 12.61 | 15.51 | 7.81 |
| 4 | 26.1 | 33.63 | 33.57 | 32.86 |
| 5 | 38.42 | 35.66 | 37.34 | 38.57 |
| 10 | 65.79 | 51.83 | 47.68 | 44.93 |
| 20 | 66.53 | 60.64 | 52.25 | 48.09 |
| 30 | 70.29 | 61.81 | 53.44 | 64.81 |
| 40 | 76.06 | 82.25 | 69.18 | 65.94 |
| 50 | 100 | 100 | 100 | 87.29 |
| 100 | 100 | 100 | 100 | 100 |

biocidal activity that could reduce 50 % of the pest population on the 5th day after spraying the solutions based of oregano leaves (123.03 hours) and of thyme (128.82 hours) and after 6 days for those based on rosemary leaves (134.89 hours) and lavender (141.25 hours) leaf solutions (Fig. 5).

**Table 2:** Results of the chemical analysis of the four plants tested

| | Thyme | Oregano | Rosemary | Lavender |
|---|---|---|---|---|
| Total tannins | + | + | + | + |
| Catechetical tannins | + | + | + | + |
| Gallic tannins | + | + | + | + |
| Anthocyanins | - | - | + | - |
| Saponoside | + | + | + | + |
| Mucilages | + | + | + | + |
| Reducing sugars | + | + | + | + |
| Flavonoids | + | + | + | + |
| Total Polyphenols | +++ | +++ | +++ | +++ |
| Glucosides | + | + | + | + |
| Starch | + | + | + | + |
| Protein | + | + | + | + |

(-): absence of substance; (+): presence of substance; (+++): very high substance content

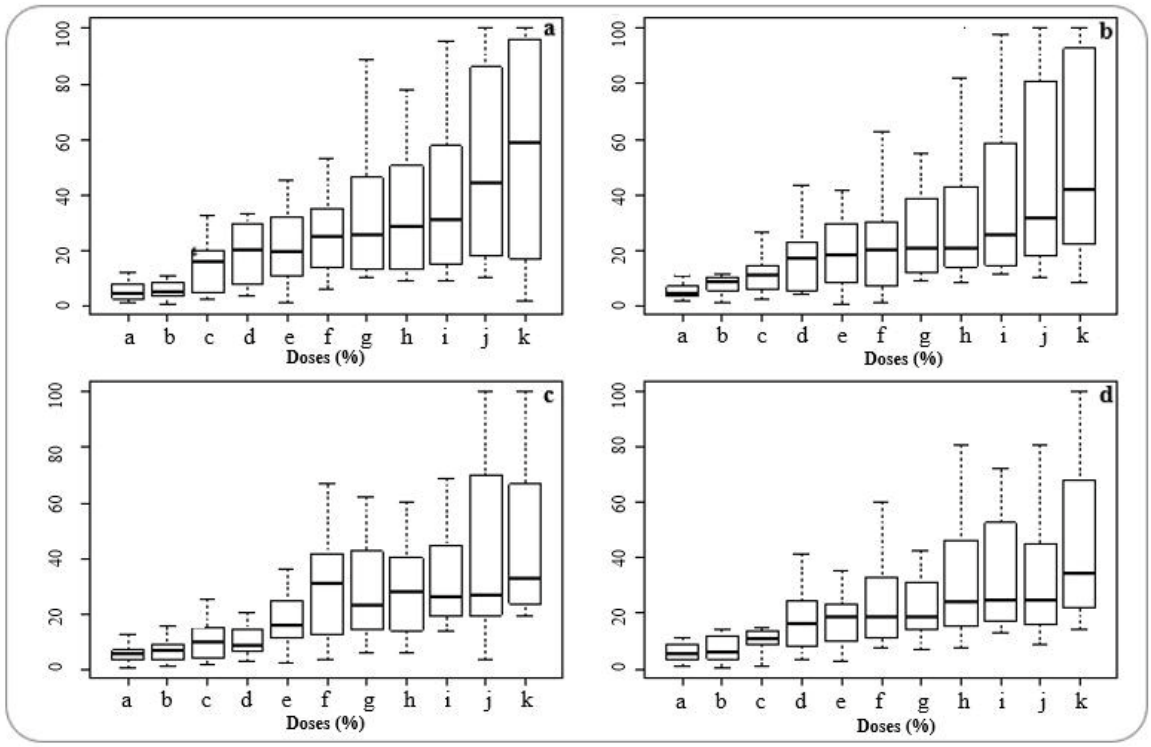


**Figure 2:** Mortality-dose boxplot for the four aqueous extracts (a: Oregano extract, b**:** Thyme extract, c**:** Rosemary extract, d: Lavender extract)

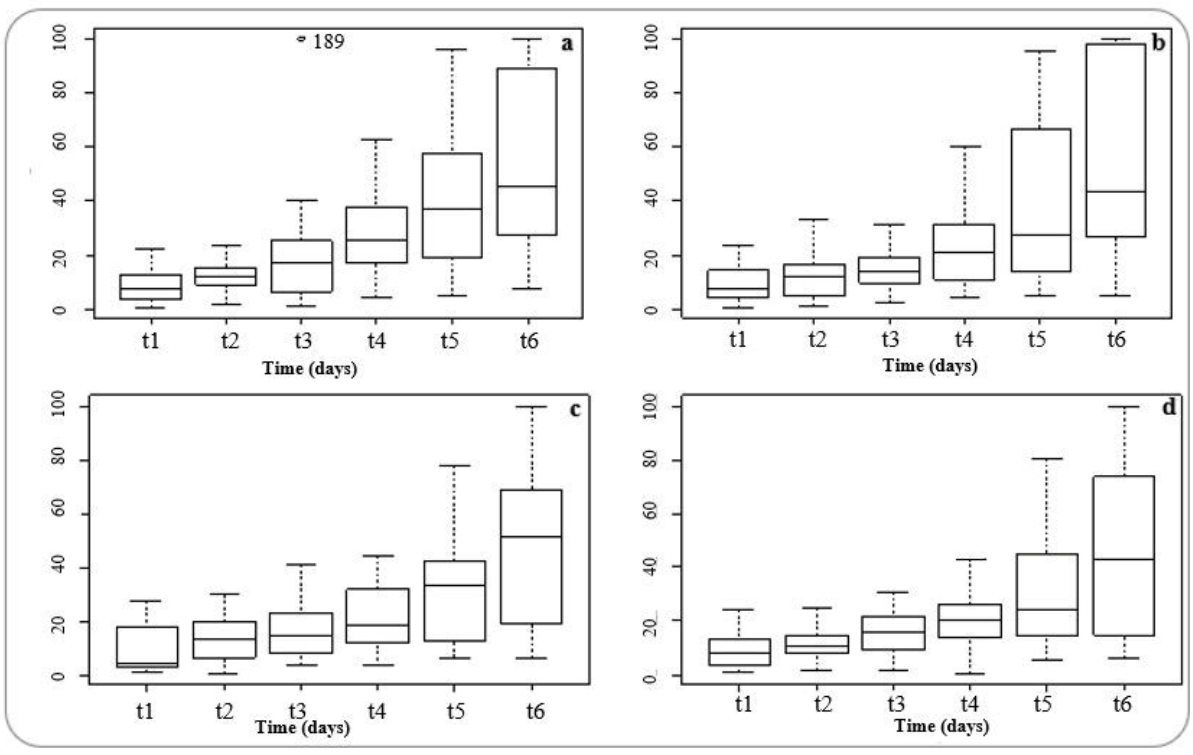


**Figure 3**: Mortality-Time boxplot for the four aqueous extracts (a: Oregano extract, b: Thyme extract, c: Rosemary extract, d: Lavender extract)

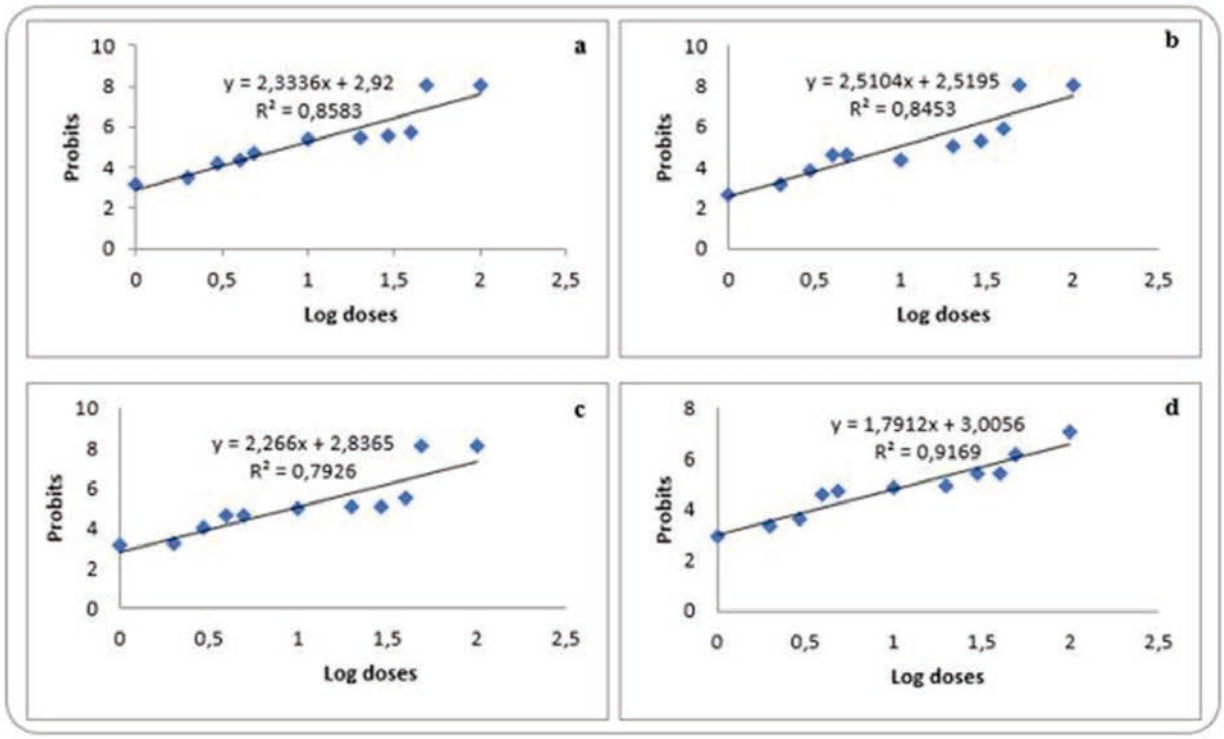


**Figure 4**: Aphid mortality rate as a function of the logarithm of the dose (a: Oregano extract, b: Thyme extract, c: Rosemary extract, d: Lavender extract)

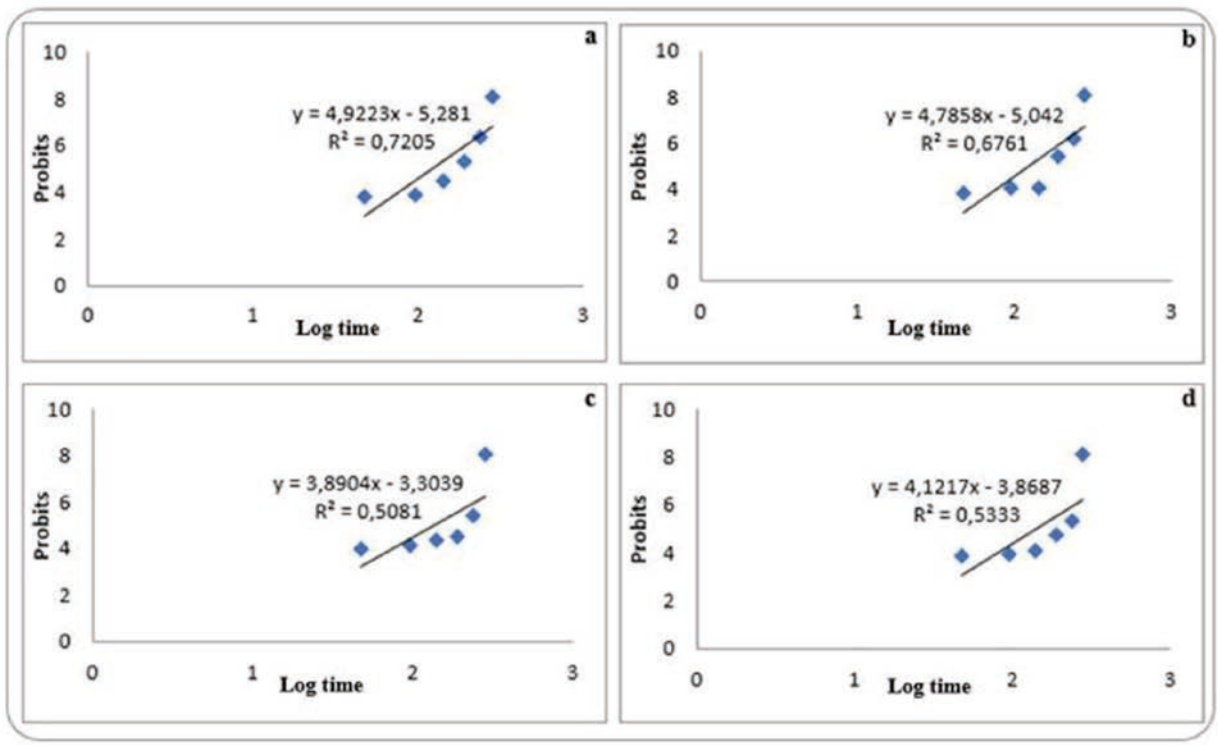


**Figure 5**: Aphid mortality rate as a function of the logarithm of time (a: Oregano extract, b: Thyme extract, c: Rosemary extract, d: Lavender extract)

### 3.4 CHEMICAL COMPOSITION OF THE EXTRACTS TESTED

The results of the phytochemical characterization tests for the four species made it possible to demonstrate the presence of several common compounds between them. These are polyphenols, flavonoids, tannins, saponosides, mucilges, reducing sugars, glucosides, starch and proteins (Table 2). The presence of anthocyanins was only observed in *Rosmarinus officinalis*. Our results also showed that all four plants have very high polyphenol content.

### 3.5 TOTAL PHENOL CONTENT

UV/Visible spectrophotometry made it possible to quantify the level of polyphenols present in the extracts prepared from the four plants. The results obtained are expressed in microgram equivalents of gallic acid standard used per ml of extract (µg GAE $ml^{-1}$ extract) and determined by the equation: $y = ax + b$. The results are reported in Figure 6.

The results obtained revealed that the four plants are rich in polyphenols with a total phenol content that varies between 64.2 µg GAE $ml^{-1}$ and 218.73 µg GAE $ml^{-1}$. From these results, it appears that oregano contains the highest concentration of polyphenols compared to the other Lamiaceae species.

## 4 DISCUSSION

Under the conditions of this study, aqueous extracts obtained from Lamiaceae leaves had an effect on the mortality of *A. fabae* populations. The results obtained after the bioassays with the 4 biopesticides showed a direct relationship between aphid mortality rates on the one hand and product concentration and exposure time on the other hand.

Aphids were very sensitive to the bioassays as extract concentrations increased and time passed. In the range of eleven concentrations tested, the 50 % and 100 % doses induced high toxicity on aphids after 12 days of contact. The reference control caused low mortality rates compared to the biopesticides tested.

Several studies have shown that the toxic effect of plant extracts is related to the concentration of the extract and the period of exposure.

The work of Habou et al. (2011) showed that the biocidal effect of the essential oil of Jatropha *Jatropha curcas* L. on the black bean aphid increases with the increase of the dose. They obtained a mortality rate of 100 % for the 15 % dose after 96 h. Authors indicate that the number of dead aphids increases with the duration of treat-

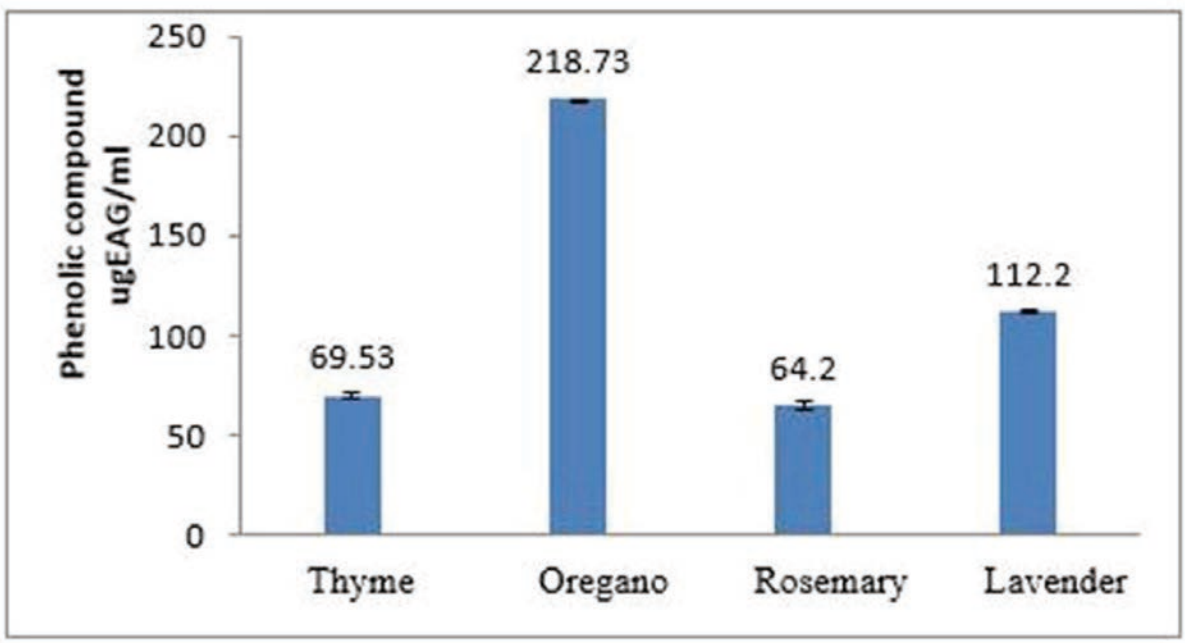


**Figure 6:** Polyphenol content of the four Lamiaceae expressed in micrograms of gallic acid equivalents per milliliter of extract (µg GAE $ml^{-1}$)

ment, it is after 72 hours that they observe a high mortality rate and which becomes stable between 96 and 120 hours. Besides, Akantetou et al. (2011) mentioned that the mortality rate of aphids subjected to different concentrations of oil of the whitish basil (*Ocimum canum* Sims) increased numerically in a linear manner according to the observation periods (1, 3, 5 and 24 hours). Similarly, Laznik et al. (2011) stated that the highest mortality rate of *Aphis pomi* populations occurred after the third day after treatment with *Symphytum officinale* and *Calendula officinalis* extract compared to the first and second day post-treatment.

The insecticide effect-dose relationship was also confirmed by the study conducted by Benoufella-Kitous et al. (2014) on *A. fabae*. According to these authors, the two aqueous extracts of nettle and fern showed a considerable insecticidal effect at the highest dose, i.e. 73.8 % and 75.1 % mortality respectively. The biocidal effect of nettle and fern on *A. fabae* increased during the days following the application of the aqueous extracts, reaching a maximum after 6 days. Our results are confirmed by those found by Baba-Aissa et al. (2017) who showed that the toxicity of the essential oil of sour orange is dependent on the dose, indicating that more the dose increases, more the formulation presents a greater biocidal effect which results in a reduction in the density of populations of *A. fabae*. In addition, Kulimushi's study (2014) showed that the degree of toxicity of the extracts is related to the dose used. After treatment of populations of *A. fabae* with aqueous extracts of garlic and papaya leaves and their combination, the average number of aphids decreased from 1 to 36 aphids and on average 86.92 % of aphids were controlled.

These results are in agreement with those Saïfi and Belhamra (2018) noted that the toxicity of the essential oil of *Thymus pallescens* de Noë depends on the concentration. These authors recorded on populations of *A. fabae*, after 24 h, a mortality rate of 34.75 % at the dose of 12 µl $ml^{-1}$. In a study on the insecticidal activity of sage against the black bean aphid, Benoufella-kitous et al. (2020) demonstrated that the effect of this plant with regard to this pest is the highest 9 days after treatment. Furthermore, Oulebsir-Mohand Kaci et al. (2015) reported that the mortality rate of *Myzus persicae* (Sulzer, 1767) treated with two plant extracts *Eucalyptus globulus* Labill. and *Thymus vulgaris* L. increases proportionally with increasing dose. The latter state that the D4 dose (8 µl $ml^{-1}$) shows a mortality rate of 65.4 % and 71.7 % respectively for the two extracts and that the aphid mortality rate is increasing over time. Likewise, in a study on the biological activity of santolina (*Santolina africana* Jord. & Fourr) against the aphid *Aphis craccivora* C.L.Koch, 1854[, Lebbal et al. (2017) demonstrated that the toxic effect of this plant with regard to this pest was proportional to the dose, with a mortality rate of 80 % at the dose of 15 % after 24 hours. According to Kumar and Patel (2017), the extract of *Cassia angustifolia* M.Vahl. showed a toxic effect on *Brevicoryne brassicae* (L., 1758) with a mortality rate of 100 % at doses of 7 % and 10 % after 72 hours of exposure.

On the other hand, Acheuk et al. (2017) mentioned that the crude ethanolic extract of *Artemisia judaica* L. revealed potent insecticidal effects against the black aphids, *A. fabae*. Total mortality (100 %) was reached 2 hours after treatment with the highest concentration. Besides, Lebbal et al. (2018) found that the extract obtained by maceration of *Thymus algeriensis* Bioss. & Reut. at a concentration of 25 % was the most effective, with a larval mortality rate of 70 % of *A. fabae* individuals after 24 h.

The toxic effects of the solutions could depend on their chemical composition and the sensitivity level of the insects. According to Saidj (2007), among the plants whose efficacy has been evaluated, aromatic plants of the Labiatae family were the most active as direct insecticides but also as inhibitors of oviposition and larval development of insects. Chiasson and Beloin (2007) suggested that biopesticides act directly on the cuticle of insects and mites, especially soft-bodied ones such as aphids.

The phytochemical study of the four aqueous extracts showed that these plants contain mainly flavonoids, tannins, saponosides and other polyphenols. These results confirm that plants of the Lamiaceae family are medicinal plants rich in secondary compounds that have a toxic effect against insect pests. Similarly, Asghari et al. (2017), in a phytochemical analysis study carried out on several plants of Lamiaceae used in medicine in Aligudarz region, in Iran, show that the three species of thyme: *Thymus daenesis* Celak, *Thymus eriocalyx* (Ronniger) Jalas, and *Thymus lancifolius* Celak are rich in flavonoids and tannins.

According to Huignard (2013), secondary compounds in plants can cause the death of insects that try to consume them by disrupting the functioning of the nervous system, the digestive system or by preventing larval growth. For instance, phenolic compounds are toxic when ingested by phytophagous insects (Kortbeek et al., 2019). They are present in all parts of higher plants: roots, stems, leaves, flowers, fruits and seeds (Medic-Saric et al., 2003; Boizot and Charpentier, 2006). According to Galeotti et al. (2008), secondary metabolites such as alkaloids, saponosides, polyphenols (flavonoids and tannins) have pharmacological and toxicological activity. Some alkaloids, anthocyanins, flavonoids, quinines, lignans, steroids, and terprnoids have commercial application in the pharmaceutical and biomedical fields and are included in

drugs, dyes, flavours, fragrances and insecticides (Teixeira Da Silva, 2004).

The results obtained revealed that the four plants are rich in polyphenols with a total phenol content varied between 64.2 µg GAE $ml^{-1}$ and 218.73 µg GAE $ml^{-1}$. These results of the quantitative analysis of polyphenols are close to those of several authors. Celiktas et al. (2007) noted polyphenol concentrations for the crude extract of *Rosmarinus officinalis* ranging from 34.1 to 119 mg GAE g-1. Cocan et al. (2018), in a study on the biological activity of rosemary officinale extracts, state that this plant has a polyphenol content that is 86.05 ± 0.40 mg GAE $g^{-1}$. Fadili et al. (2015) mentioned that *Rosmarinus officinalis* and *Thymus satureioides* Coss. are rich in polyphenols for all the fractions studied and the concentration of polyphenols varies between 21.66 ± 2mg GAE $g^{-1}$ to 185.71 ± 4 mg GAE $g^{-1}$. Kholkhal et al. (2013) reported that the polyphenol content of *Thymus ciliatus* Desf. is 64.23 mg GAE $g^{-1}$. In Morocco, Bachiri et al. (2016) noted that *Lavandula stoechas* and *L. dentata* L. are composed of polyphenol contents of 150.34 mg $g^{-1}$ and 184.02 mg $g^{-1}$ respectively.

In general, oregano extract was the most effective (LD50 = 7.76 %, TL50 = 123.03 hours) on aphid populations. Low LD50 values indicate strong insecticidal activity and low TL50 values indicate a rapid biocidal effect. The obtained results seem to show that the polyphenols with the highest concentration in this plant would be the active ingredient that plays a determining role in the biocidal activity of this plant. The richness of the aqueous extract in chemically active compounds could explain the traditional use of this plant in various fields. However, the synergistic additive effect of the different compounds may also be a factor explaining the remarkable activity revealed by the rosemary extract. The latter was found to be highly toxic to aphids (LD 50 = 8.91 %).

Lavender extract ranked last (LD50 = 12.88 %, TL50 = 141.25 hours) compared to the other solutions tested despite its high polyphenol content. This would suggest that the insecticidal activity of plants is not limited to some of their major constituents; it could also be due to some minority constituents. According to Akantetou et al. (2011), the difference in toxicity between the different extracts could be explained by the growing conditions, the harvesting period and the climatic and edaphic conditions. The distribution of secondary metabolites may change during plant growth. This may be related to conditions of high temperature, sun exposure, drought and salinity, which stimulate the biosynthesis of secondary metabolites such as polyphenols (Falleh et al., 2008; Zaouali et al., 2010).

Some works has shown that the toxic effect of extracts depends on the nature of the plant. According to Khalfi-Habes and Sellami (2010), oregano shows a stronger insecticidal action than rosemary and thyme. These results confirm those obtained in the present study. Similarly, Kumar and Patel (2017) state that *Curcuma angustifolia* Roxb. causes a higher toxic effect compared to the other plants tested (*Cercis gigantean* L., *Cannabis sativa* L., *Parthenuim hysterophorus* L., *Lobelia chinensis* Lour., *Solanum nigrum* L. and *Ageratum conyzoides* L.). In a study on the biological activity of nettle and fern on the black bean aphid, Benoufella-Kitous et al. (2014) showed that the most significant toxic effect was recorded for the second species. Benoufella-Kitous (2015) notes that among 8 plant species tested against *A. fabae*, the most toxic extracts were those from the leaves of toothed lavender with an efficacy of 99.4 % at the 10 % dose, sage with an efficacy of 98.5 % at the 40 % dose and garlic with an efficacy of 97.9 % at the 10 % dose.

## 5 CONCLUSION

The present study showed the relative importance of the use of botanical pesticides, namely aqueous extracts of oregano, rosemary, thyme and lavender leaves, against the black bean aphid.

The phytochemical study revealed the presence of the main groups of active chemical compounds in these plants (polyphenols, gall and tannins, saponosides, flavonoids). Fractionation of these extracts will probably allow the isolation of the active principles responsible for their biological activities.

These aromatic plants being very commonly found in Algeria could open up interesting prospects for their use in the production of biopesticides. They therefore appear as potentially usable for an integrated management of aphids after field tests to confirm their aphicidal activity.

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# Primer uporabe Griffiths-Taylorjevih diagramov za prikaz podnebnih sprememb, pomembnih za kmetijstvo

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**An example of the analysis of climate change in agriculture using Griffiths-Taylor diagrams**

**Abstract:** The climate clearly determines the characteristics of agriculture in terms of favourable conditions for the development of plants and animals. Climate change has a major impact on agriculture, and we need at least its analysis for past decades for effective adaptation. The annual scale of meteorological variables is quite rough, much more information is obtained from the monthly scale, for example when using bioclimatic indices and diagrams. Using Griffiths-Taylor diagrams, where each point shows the average monthly value of temperature and relative humidity or precipitation, we showed the change of 30-year averages (1961 to 2020) for six climate stations. Climate change can be seen from the shape of the diagram, an increase in average monthly air temperatures, a decrease in average relative humidity and changed precipitation patterns are visible. With the plot of favourable conditions for a certain plant / animal species, the diagram acquires ecological value. We have shown changes in the conditions for the development of the fruit fly (*Ceratitis capitata* (Wiedemann, 1824)) and the growth of sweet potatoes (*Ipomoea batata* L.). For the fruit fly, conditions improved due to higher temperatures, especially in colder locations. In Murska Sobota, which has the only suitable conditions for the growth of sweet potatoes, the period with favourable conditions is extended, in the last two periods in July and August, ideal conditions also appear. In further research, we recommend an analysis of weather-extreme years, which provides additional information on the variability of the conditions.

**Key words:** climate change; Griffiths-Taylor diagram; air temperature; precipitation; relative humidity; fruit fly; sweet potato

**Primer uporabe Griffiths-Taylorjevih diagramov za prikaz podnebnih sprememb, pomembnih za kmetijstvo**

**Izvleček:** Podnebje izrazito določa značilnosti kmetijstva glede na ugodne razmere za razvoj rastlin in živali. Podnebne spremembe imajo na kmetijstvo velik vpliv, za učinkovito prilagajanje pa potrebujemo vsaj analizo dosedanjih podnebnih sprememb. Pri tem je letna skala meteoroloških spremenljivk precej groba, veliko več informacij dobimo iz mesečne skale, na primer pri uporabi bioklimatskih indeksov in diagramov. Z Griffiths-Taylorjevimi diagrami, kjer posamezna točka prikazuje povprečno mesečno vrednost temperature in relativne vlage ali padavin, smo prikazali časovne spremembe 30-letnih povprečij (od 1961 do 2020) za šest podnebnih postaj. Podnebne spremembe vidimo iz oblike diagrama, vidno je povišanje povprečnih mesečnih temperatur zraka, zmanjšanje povprečne relativne vlage in spremenjeni vzorci padavin. Z vrisanimi ugodnimi razmerami za določeno rastlinsko/živalsko vrsto pridobi diagram ekološko vrednost. Prikazali smo spremembe razmer za razvoj breskove muhe (*Ceratitis capitata* (Wiedemann, 1824)) in rast sladkega krompirja (*Ipomoea batata* L.). Za breskovo muho so se razmere zaradi višjih temperatur izboljšale predvsem na hladnejših lokacijah. V Murski Soboti, ki ima edina primerne razmere za rast sladkega krompirja, se obdobje z ugodnimi razmerami podaljšuje, v zadnjih dveh obdobjih se julija in avgusta pojavijo tudi idealne razmere. V nadaljnjih raziskavah priporočamo analizo vremensko ekstremnih let, ki podajo dodatne informacije o variabilnosti razmer.

**Ključne besede:** podnebne spremembe; Griffiths-Taylorjev diagram; temperatura zraka; padavine; zračna vlaga; breskova muha; sladki krompir

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## 1 UVOD

Kmetijstvo je v veliki meri prilagojeno podnebju na določenem območju, saj so zaradi velike izpostavljenosti vremenskim razmeram infrastruktura in izbor poljščin pa tudi pojavnost škodljivcev in invazivnih rastlin odvisni od povprečnih značilnosti lokalnega podnebja (Gornall in sod., 2010). Projekcije kažejo, da bodo v zmernih geografskih širinah višje temperature tekom vegetacijske dobe pomenile povečanje produktivnosti in ustreznosti poljščin, še posebej žit in semenskih posevkov v jesensko-zimskem času (ARSO, 2017; Olesen in sod., 2007). Pri tem pa se spreminja tudi razširjenost in številčnost plevelov, škodljivcev in opraševalcev (Bocci in Smanis, 2019). Vplivi podnebnih sprememb bodo pozitivni in negativni, v vsakem primeru pa se kaže potreba po vlaganju v prilagoditvene strategije in tehnologije (Knox in sod., 2010).

Tako kmetijske kot tudi ostale rastline in živali se na podnebne spremembe različno odzivajo (Gornall in sod., 2010), predvsem v odvisnosti od za njih specifičnih idealnih in ugodnih razmer za razvoj. S podnebnimi spremembami povprečna letna temperatura zraka v Sloveniji narašča hitreje od evropskega in globalnega povprečja (ARSO, 2017). Od leta 1961 se je temperatura zraka v letnem povprečju za celo Slovenijo dvignila za 2 °C, hkrati pa nam povprečne vrednosti za celo Slovenijo in na letni skali zelo malo povedo o porazdelitvi sprememb tekom leta. V večjo pomoč so nam sezonske vrednosti, še bolj primerne za kmetijstvo pa so analize mesečnih odstopanj. Analiza preteklih sprememb je pomembna z vidika poznavanja odzivov rastlin in živali, tako si namreč lažje predstavljamo, kaj bodo pomenile spremembe v prihodnosti, pri katerih lahko glede temperature zraka z veliko gotovostjo rečemo, da se bo dvig nadaljeval. Glede spremembe količine padavin je negotovost večja, vsekakor pa gre za spremenjene vzorce (ARSO, 2017).

Za prikaz povezanosti sistema rastlina-ozračje uporabljamo številne bioklimatske indekse, s katerimi lahko na osnovi različnih vremenskih spremenljivk (temperatura zraka in tal, padavine, sončno obsevanje, veter, zračna vlaga) ocenimo primernost določenega pridelovalnega območja za posamezne kmetijske rastline (Noce in sod., 2020; Rivas-Martinez in sod., 2011). Številne vrste kmetijskih rastlin uspevajo na širših geografskih in podnebnih območjih, nekatere pa lahko gojimo v razmeroma ozkih geografsko-podnebnih pasovih (Karoglan in sod., 2018). Ustrezni bioklimatski indeksi so na primer v vinogradništvu pomembna osnova za ocenjevanje splošne primernosti te panoge v določeni regiji (Rusjan in Koruza-Korošec, 2003). Bioklimatske indekse uporabljamo v kmetijstvu tudi za primerjavo med posameznimi regijami, za agroklimatske rajonizacije (Badr in sod., 2018; Rusjan in Koruza-Korošec, 2003) ter pomoč pri načrtovanju uvajanja primernih sort v sedanjih in napovedanih, spremenjenih podnebnih razmerah (Ceglar in sod., 2019). Bioklimatski indeksi navadno temeljijo le na povprečnih večmesečnih temperaturah zraka in njihovih razponih ter povprečnih količinah padavin: Ellenbergov kvocient je razmerje med temperaturo najtoplejšega meseca v letu in letno količino padavin (°C $mm^{-1}$), ombrotermični indeks je razmerje med povprečno količino padavin za mesece s pozitivnim povprečjem dnevne temperature in vsoto povprečne temperature za iste mesece (mm $°C^{-1}$) (Noce in sod., 2020) ipd. Biota dane regije je prilagojena, ne toliko skupnim padavinam ali skupnim akumuliranim temperaturam lokalnega podnebja, ampak bolj podnebnemu ritmu oziroma interakciji temperaturnih in padavinskih razmer (Tarman, 1992).

Grafični prikazi podnebnih razmer, podnebni diagrami, ki prikazujejo povezanost temperaturnih in padavinskih (vlažnostnih) razmer, imajo zato velik pomen pri prikazu podnebnih značilnosti z biološkega stališča, saj prikazujejo podnebni ritem in ne le skupne vrednosti meteoroloških spremenljivk (Rivas-Martinez in sod., 2011). Primer takega grafičnega prikaza je Griffith-Taylorjev podnebni diagram (Christie, 1993; O'Brien, 2015; Winlow, 2009). Taylorjeva uporaba izolinij (temperature, padavin/vlažnosti) je bila razširjena v prikazih povezave med podnebjem in razširjenostjo rastlinskih vrst, izdelani so bili podnebni diagrami, ki prikazujejo kritične vrednosti padavin in temperature za rast nekaterih pomembnih kmetijskih rastlin (za pšenico, riž, kavo in čaj) (Taylor Griffith, 2021).

V primeru Griffiths-Taylorjevega diagrama absciso (x os) predstavlja količina padavin, ordinato pa temperatura zraka. Za vsak mesec označimo točko, ki ustreza njegovi povprečni mesečni temperaturi zraka in mesečni količini padavin, ter točke medsebojno povežemo v zaporedju 1, 2, ..., 12, 1. Na ta način dobimo mnogokotnik z dvanajstimi stranicami. Čim bolj so Griffiths-Taylorjevi diagrami različnih krajev med seboj podobni, tem bolj je podobno njihovo podnebje. Nekaj informacije o podnebju posameznega kraja pa dobimo tudi iz oblike – podnebni diagrami krajev z bolj maritimnim podnebjem imajo bolj okroglo obliko kot kraji z bolj kontinentalnim, ki imajo bolj ozko (tudi prekrižano), pokončno obliko. Podnebni diagram pridobi ekološko vrednost, ko vanj vrišemo ugodne oziroma idealne toplotne in vlažnostne razmere za določeno vrsto (Tarman, 1992).

Za primer aplikativne uporabe Griffiths-Taylorjevih diagramov smo uporabili primer sadne muhe, ki spada med svetovno razširjene in gospodarsko pomembne škodljivce, na velikost in dinamiko populacije v posameznem letu pa zelo vplivajo vremenske razmere (Rot in sod., 2015), in sladkega krompirja, ki izvira iz toplej-

ših krajev (Paneque Ramirez, 2021). V Sloveniji poznamo več vrst gospodarsko pomembnih sadnih muh, med njimi breskovo muho (*Ceratitis capitata* (Wiedemann, 1824)), pri kateri je glavna gostiteljska rastlina vedno bolj razširjen kaki, ki ga sadimo tudi izven Primorske in s tem omogočamo breskovi muhi širjenje v druge regije (Žežlina, 2018). S spreminjanjem temperaturnih in vlažnostnih razmer zaradi podnebnih sprememb je torej pomembno spremljati, kako se spreminjajo ugodne razmere za razvoj toplotno zahtevnejših kultur in hkrati škodljivcev (Šlosár in sod., 2020). Namen prispevka je prikazati možnost uporabe Griffiths-Taylorjevih diagramov za analizo podnebnih sprememb na mesečni skali, primerjati diagrame za podnebno različne predele Slovenije in štiri različna obdobja ter hkrati preveriti, ali spremenjene podnebne razmere vplivajo na ugodnost/neugodnost rastnih razmer za izbrana primera (breskova muha, sladek krompir).

## 2 MATERIAL IN METODE DELA

Griffiths-Taylorjevi diagrami grafično predstavljajo dolgoletna mesečna povprečja temperature zraka in relativne vlage ali padavin. Obravnavali smo štiri 30-letna obdobja, in sicer 1961–1990, 1971–2000, 1981–2010 ter 1991–2020, po vrsti smo jih poimenovali kar prvo, drugo, tretje ter četrto obdobje. Diagrame smo pripravili za šest krajev v Sloveniji: Bilje (13°38', 45°54', 55 m n.m.v.), Kočevje (14°51', 45°39', 467 m n.m.v.), Ljubljano (14°31', 46°04', 299 m n.m.v.), Mursko Soboto (16°12', 46°39', 188 m n.m.v.), Rateče (13°43', 46°30', 864 m n.m.v.) in Šmartno pri Slovenj Gradcu (15°07', 46°29', 455 m n.m.v.). Za vsako postajo smo za vsako 30-letno obdobje izračunali povprečne mesečne vrednosti spremenljivk in njihove standardne napake, podatke za izračune pa smo pridobili z Agencije RS za okolje (ARSO, 2021). V besedilu so standardne napake prikazane ob vrednostih povprečij za znakom ± .

Za drugi del analize smo upoštevali pogoje za razvoj breskove muhe in rast sladkega krompirja . Po Tarmanu (1992) veljajo za breskovo muho naslednje mejne vrednosti povprečnih mesečnih temperatur in relativne vlažnosti:

- idealne razmere: 16-32 °C, 75-85 mm;
- zelo ugodne razmere: 10-35 °C, 60-90 mm;
- ugodne razmere: 3-38 °C, 40-100 mm.

Za sladki krompir (Valenzuela in sod., 2020; Paneque Ramirez, 2021) so mejne sledeče vrednosti povprečnih mesečnih temperatur in količin padavin:

- idealne razmere: 20-25 °C, letno* 900-1300 mm;
- ugodne razmere: 15-33 °C, letno* 500-1330 mm.

*Opomba: V virih je določena le letna količina padavin. Kot prvi približek za nadaljnje računanje smo za mesečno količino vzeli dvanajstino količine letnih padavin, kar sicer ne velja nujno po posameznih mesecih.

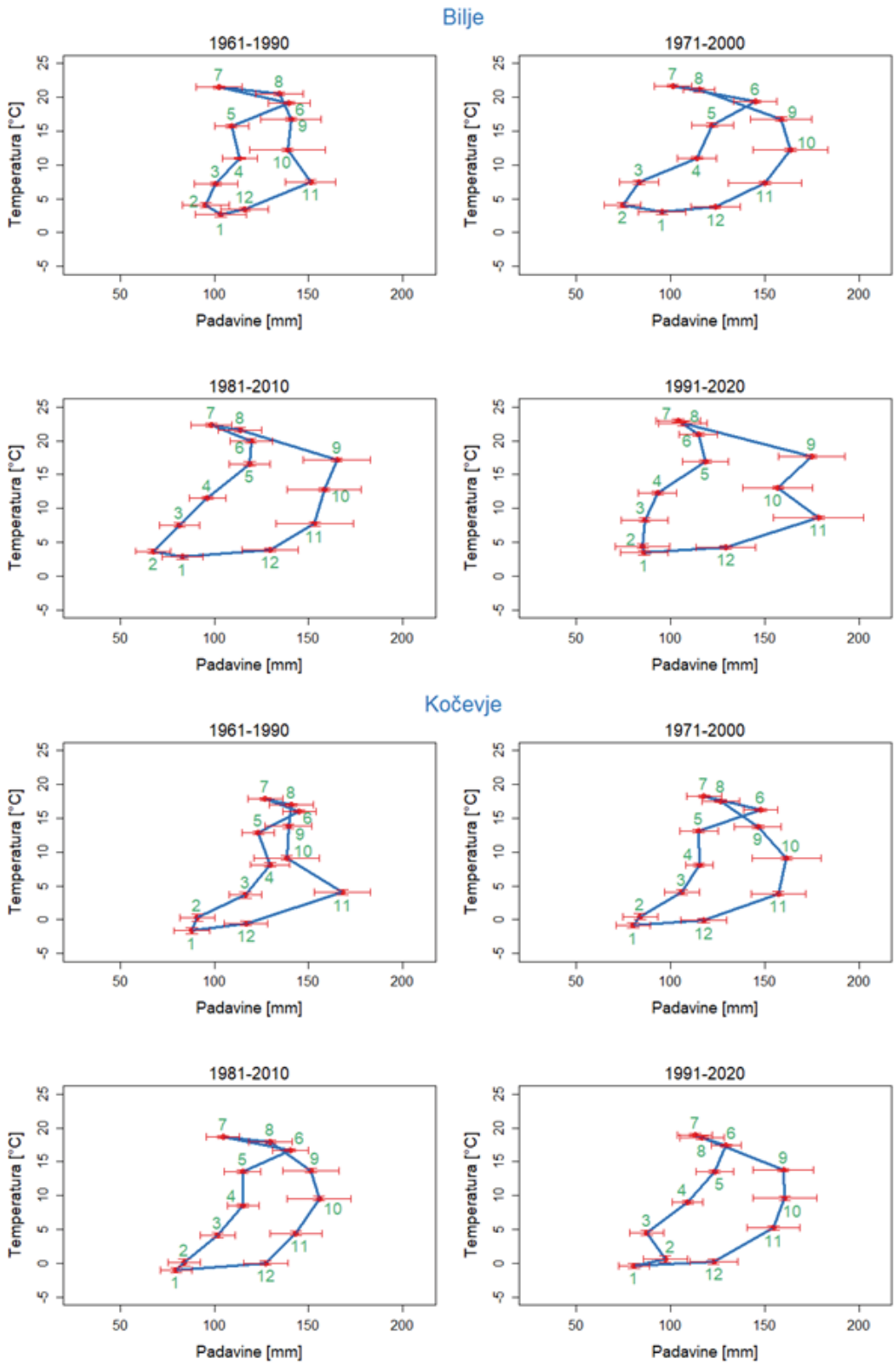
Higrotermična mesečna povprečja posameznih lokacij so pokazala, da razen Murske Sobote noben kraj ne izpolnjuje pogojev za doseganje ugodnih ali idealnih razmer za rast sladkega krompirja, zato smo graf pripravili le za Mursko Soboto.

## 3 REZULTATI IN DISKUSIJA

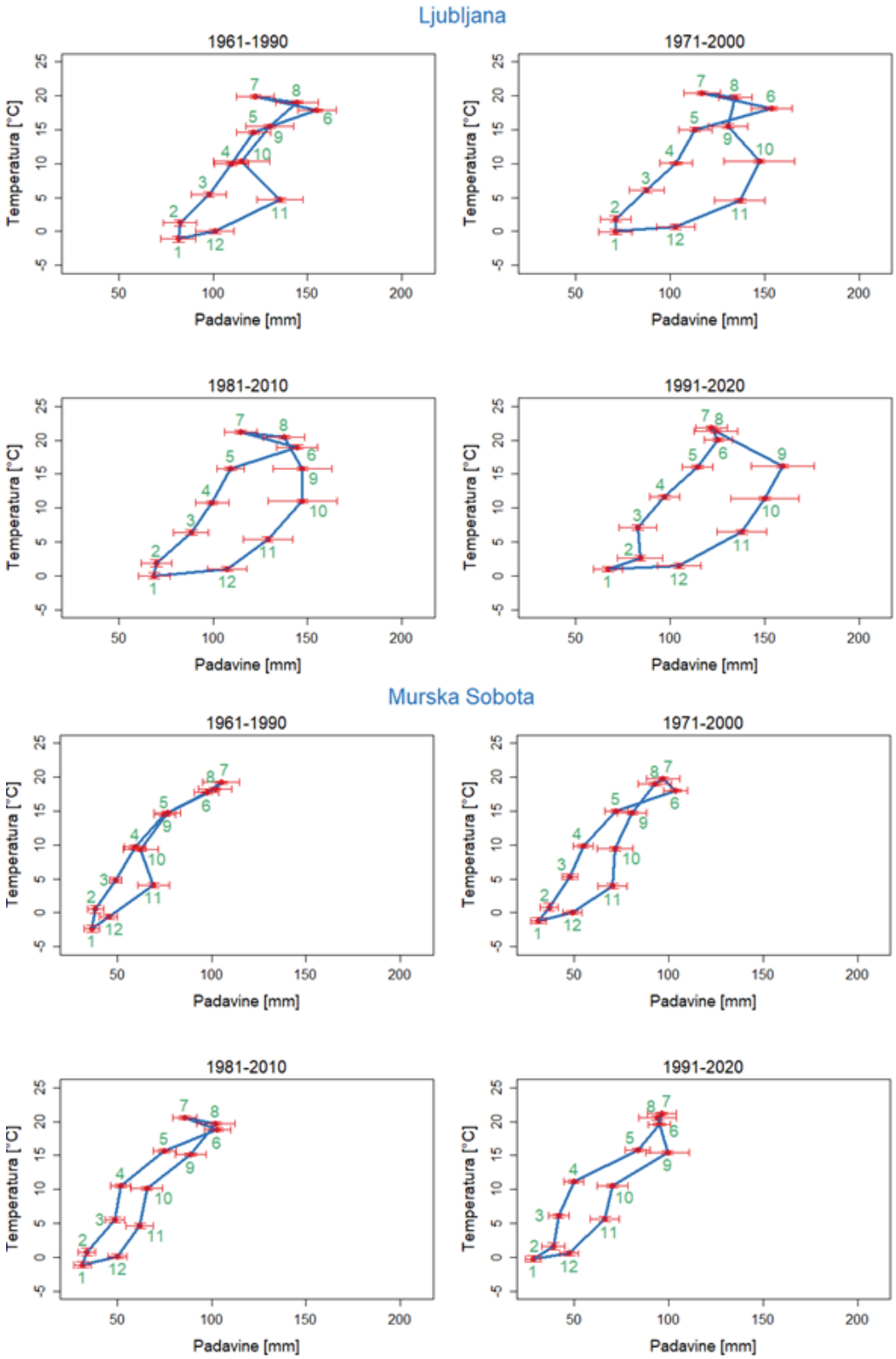
Meteorološka postaja v Biljah (Slika 1 zgoraj) se nahaja na 55 metrih nadmorske višine. Vse povprečne zimske temperature so pozitivne in z leti naraščajo – povprečna januarska temperatura v obdobju 1961–1990 je bila 2,7±0,3 °C, v obdobju 1991–2020 pa 3,5±0,3 °C. Sprememba povprečne temperature med obdobjema močno presega standardno napako povprečij. Zelo alarmantna za že tako sušno Goriško je povprečna količina padavin v juniju, ki se z leti občutno zmanjša: v prvem obdobju je povprečno padlo 140 ± 11 mm padavin, v četrtem obdobju pa le 115 ± 10 mm. Tudi pri padavinah je sprememba precej večja od standardne napake. September, oktober in november so v zaporednih obdobjih postali rahlo bolj namočeni, za dvakratnik standardne napake (34 mm) le september, ostala dva znotraj ranga napake.

Meteorološka postaja Kočevje (Slika 1 spodaj) se nahaja na nadmorski višini 467 metrov. V obravnavanem časovnem obdobju se je razporeditev količine padavin delno spremenila. Januar je postal nekoliko bolj suh v drugem obdobju glede na prvega, količina padavin v spomladanskih mesecih pa se je manjšala iz obdobja v obdobje (največ marca, za trikratnik standardne napake, 29 mm), z izjemo maja v četrtem obdobju. Manj je bilo tudi junijskih padavin, a je ta razlika precej manjša kot v ostalih krajih. Več padavin je padlo v septembru in oktobru: v obdobju 1961–1990 je bil najbolj namočen mesec november z 168 ± 15 mm padavin, v obdobju 1991–2020 pa oktober z 161 ± 17 mm. Novembrsko zmanjšanje količine padavin za 13 mm je znotraj standardne napake. Povprečna januarska temperatura je ostala negativna, a se je z -1,6 ± 0,5 °C zvišala na -0,4 ± 0,4 °C, julijska pa se je zvišala za 1,1 °C (s 17,9 ± 0,2 °C na 19 ± 0,2 °C).

Meteorološka postaja Ljubljana – Bežigrad (Slika 2 zgoraj) se nahaja na 299 metrih nadmorske višine. Podnebje se je iz prvega v četrto obdobje spremenilo: podnebne značilnosti aprila in oktobra ter maja in septembra so si bile v preteklosti precej podobne, sedaj pa so jesenski meseci precej bolj namočeni. Zelo opazna je zmanjšana junijska količina padavin, ki je bila v obdobju 1961–1990 155 ± 10 mm, v obdobju 1991–2020 pa le 125 ± 7 mm. V istem časovnem razponu se je zvišala




**Slika 1**: Griffiths-Taylorjev diagram povprečnih mesečnih temperatur in padavin s standardnimi napakami za štiri obdobja v Biljah (zgoraj) in Kočevju (spodaj)
**Figure 1**: The Griffiths-Taylor diagram of average monthly temperatures and precipitation with standard errors in four periods in Bilje (upper) and Kočevje (lower)




**Slika 2**: Griffiths-Taylorjev diagram povprečnih mesečnih temperatur in padavin s standardnimi napakami za štiri obdobja v Ljubljani (zgoraj) in Murski Soboti (spodaj)
**Figure 2**: The Griffiths-Taylor diagram of average monthly temperatures and precipitation with standard errors in four periods in Ljubljana (upper) and Murska Sobota (lower)

povprečna januarska temperatura, ki je bila sprva še negativna, in sicer -1,1 ± 0,4 °C, v zadnjem obdobju pa je dosegla vrednost 1 ± 0,4 °C.

V primerjavi z drugimi kraji po Sloveniji je Murska Sobota (Slika 2 spodaj) precej bolj suha, njen razpon dolgoletnih povprečnih mesečnih količin padavin znaša okoli 40-110 mm, medtem ko je razpon v Ljubljani okoli 70-160 mm. Najbolj suh mesec je bil in ostaja januar, najbolj moker pa je bil v prvem obdobju julij s 105 ± 10 mm, a se je v kasnejših obdobjih julijska količina padavin začela zmanjševati, tako da je v drugem in tretjem obdobju to značilnost prevzel junij s približno 103 ±7 mm padavin, v četrtem obdobju pa je bil najbolj namočen september, čeprav le z 99 ± 12 mm povprečnih padavin. Spremembe niso velike glede na standardno napako. Povprečna januarska temperatura je bila v obdobju 1961–1991 2,3 ± 0,5 °C, v obdobju 1991–2020 pa se je povišala na 0,2 ± 0,4 °C. Najtoplejši mesec ostaja julij, tudi ta se je ogrel, in sicer se je povprečna temperatura zvišala kar za 1,9 °C (z 19,3 ± 0,2 °C na 21,2 ± 0,2 °C).

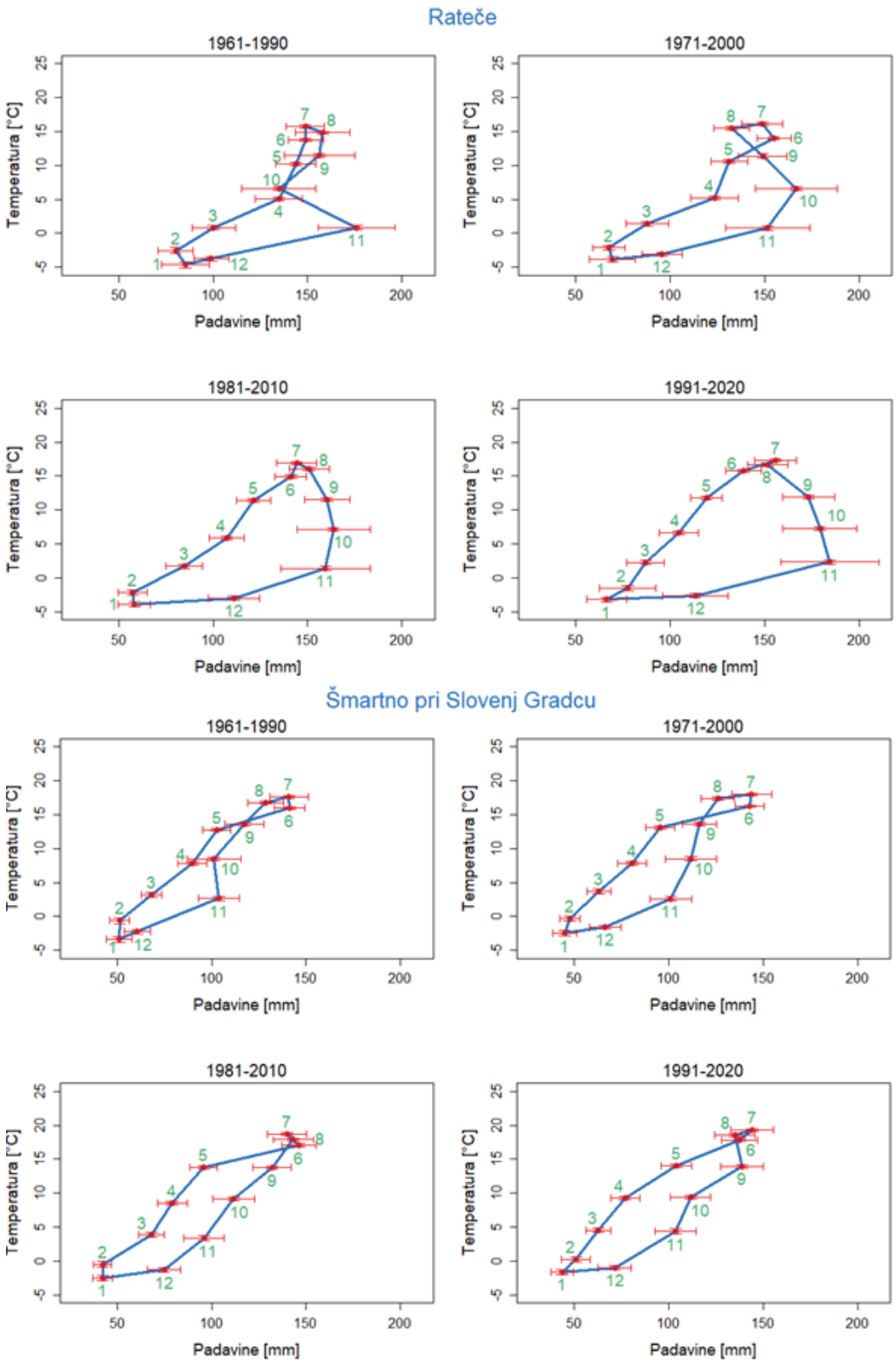
Meteorološka postaja Rateče (Slika 3 zgoraj) leži višje, na 864 m nadmorske višine. Zelo zanimivo je, da so si bile v prvem obdobju značilnosti aprila in oktobra zelo podobne. V oktobru je v povprečju zapadlo za Rateče malo padavin (135 ± 20 mm), kar pa se spremeni že v naslednjem obdobju, ko je oktober s 167 ± 22 mm postal najbolj namočen mesec in to tudi ostal do zadnjega obdobja, ko je največ padavin padlo novembra (184 ± 26 mm), a so spremembe večinoma znotraj standardne napake. Od obdobja 1961–1990 do 1991–2020 se je očitno zmanjšala povprečna količina padavin v oktobru (za 44 mm pri standardni napaki obeh povprečij okoli 20 mm), aprilu (za 30 mm pri standardni napaki okoli 11 mm) in maju (za 25 mm pri standardni napaki okoli 9 mm), v juniju pa le za 10 mm (kar je rang standardne napake). Količina padavin v juliju je ostala praktično nespremenjena. Ker se Rateče nahajajo na precej večji nadmorski višini kot ostali izbrani kraji, so povprečne mesečne temperature zraka precej nižje, povprečna mesečna temperatura pa se spusti pod ničlo za četrtino leta v vseh izbranih obdobjih. Povprečna januarska in julijska temperatura sta bili v obdobju 1961–1990 4,7 ± 0,4 °C oziroma 15,7 ± 0,2 °C, v obdobju 1991–2020 pa 3,2 ± 0,3 °C in 17,4 ± 0,2 °C. O spremembah temperature lahko govorimo bolj zanesljivo kot o spremembah padavin.

Meteorološka postaja Šmartno pri Slovenj Gradcu (Slika 3 spodaj) leži na nadmorski višini 455 m. V prvem obdobju so imeli negativno povprečno temperaturo zraka vsi zimski meseci, z najnižjim povprečjem v januarju (-3,4 ± 0,4 °C). V zadnjem obdobju sta imela negativno povprečno temperaturo le še januar in december, januarska temperatura pa se je zvišala na 1,6 ± 0,4 °C. Povprečna julijska temperatura se je zelo občutno zvišala s 17,6 ± 0,2 °C na 19,3 ± 0,2 °C. Količina padavin je podobno kot v Murski Soboti manjša kot v ostalih izbranih krajih. Najmanj padavin je v vseh obdobjih padlo v januarju, v obdobju 1961–1990 povprečno 51 ± 7 mm in v obdobju 1991–2020 povprečno 44 ± 6 mm, največ padavin pa je v prvem obdobju zapadlo v juniju (141 ± 10 mm), v zadnjem pa v juliju (144 ± 11 mm), a je pri obeh sprememba znotraj standardne napake. September je z leti postal opazno bolj namočen, količina padavin se je s 117 ± 10 mm povečala na 139 ± 11 mm.

Poleg sprememb v povprečni količini padavin v povezavi s temperaturo lahko opazujemo tudi povprečno relativno vlago, ki pomembno vpliva na (ne)ustreznost razmer za razvoj breskove muhe. V Biljah se je relativna vlažnost iz prvega obdobja v četrto v vseh mesecih zmanjšala najmanj za en odstotek, prihajalo pa je tudi do večjih relativnih razlik med relativno vlažnostjo pozimi in poleti: v obdobju 1961–1990 je znašala razlika med januarsko in julijsko relativno vlažnostjo 8,2 % (standardni napaki povprečij 0,9 in 0,5 %), v obdobju 1991–2020 pa kar 11,1 % (standardni napaki 1,1 in 1,0 %). V Kočevju se je povečala razlika med aprilsko in oktobrsko relativno vlažnostjo, ki je bila v prvem obdobju 6,5 % (standardni napaki 0,9 in 0,7 %), v zadnjem pa 9,5 % (standardni napaki 1,0 in 0,7 %). Najbolj vlažen mesec ostaja december, najmanj vlažen mesec april pa je zamenjal maj. Najmanjše razlike v relativni vlagi tekom obdobij so bile v Ljubljani.

V Murski Soboti se je najbolj zmanjšala povprečna relativna vlaga julija in sicer za 4,8 % pri standardnih napakah povprečij 0,9 %. V Ratečah so bile najbolj očitne spremembe v spomladanskih mesecih, povprečja so se zmanjšala tudi za več kot 3 % (standardne napake do 1,2 %). V Šmartnem pri Slovenj Gradcu je bil v prvih treh obdobjih maj zelo očitno najmanj vlažen mesec, v zadnjem obdobju pa so imeli april, maj in junij skoraj enako povprečno relativno vlažnost, in sicer približno 72 ± 0,8 %.

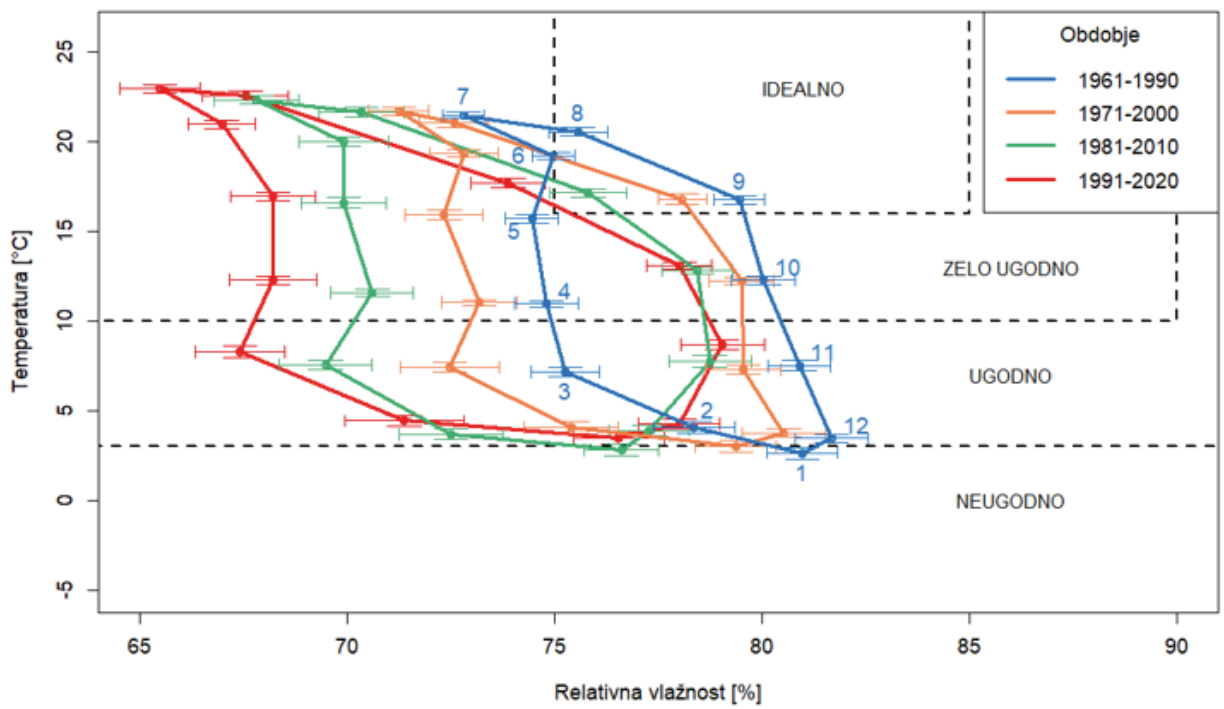
Glede na premike krivulje v Griffiths-Taylorjevem diagramu lahko grafično zelo dobro prikažemo spremembe ugodnih ali idealnih razmer za, na primer, breskovo muho. V Biljah (Slika 4) so bile povprečne razmere za razvoj breskove muhe v vseh mesecih z izjemo januarja 1961–1990 in 1981–2010 vsaj ugodne. Zelo ugodne razmere so trajale od aprila do oktobra. Prvi dve obdobji sta imeli idealne razmere tako avgusta kot septembra, tretje le septembra, zadnje pa ni imelo idealnih razmer v nobenem mesecu. Na spremembe glede ugodnih oziroma neugodnih razmer v Biljah vplivajo bolj kot višje poletne temperature spremembe relativne zračne vlage (Slika 4), ki se v zadnjih obdobjih zmanjšuje. Standardne napake temperaturnih povprečij so velikosti 0,2-0,3 °C, povprečne vrednosti so se med prvim in zadnjim obdob-



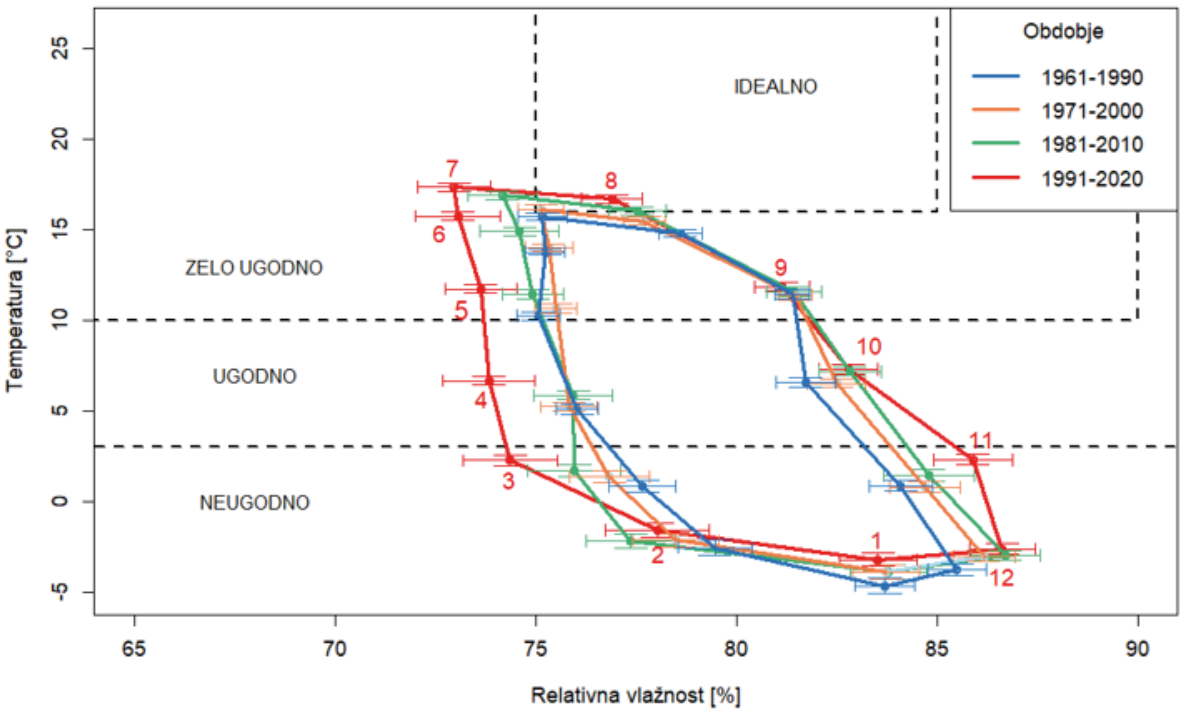

**Slika 3**: Griffiths-Taylorjev diagram povprečnih mesečnih temperatur in padavin s standardnimi napakami za štiri obdobja v Ratečah (zgoraj) in Šmartnem pri Slovenj Gradcu (spodaj)
**Figure 3**: The Griffiths-Taylor diagram of average monthly temperatures and precipitation with standard errors in four periods in Rateče (upper) and Šmartno pri Slovenj Gradcu (lower)

jem povišale za 0,4 do 2,0 °C. Pri relativni vlagi je razpon negativnih sprememb med 2 in 8 % pri standardnih napakah velikosti 0,5-1,4 %. Kljub temu, da v zadnjem obdobju zaradi tega ni več idealnih razmer za razvoj breskove muhe v Biljah, pa lahko iz diagrama vidimo, da je ploščina lika v območju zelo ugodno/ugodno večja, kar pomeni, da je možnost pojava tega škodljivca v povprečju v zadnjih letih večja. Zaradi višjih zimskih temperatur v Biljah v zadnjem obdobju ni več neugodnih razmer za razvoj breskove muhe, kar pomeni, kot so v svoji raziskavi že poudarili Rot in sod. (2015), da trend globalnega segrevanja ozračja in milejše zime lahko pripomorejo h krepitvi populacije breskove muhe na območju Istre, kot tudi k njeni širitvi na sever v Vipavsko dolino. Žežlina (2018) je preučeval pojavljanje breskove muhe na različnih lokacijah na Primorskem v letih 2016 in 2017. Ugotovil je, da so na pojav in številčnost muhe pomembno vplivali tudi temperatura, padavine in zračna vlaga, za začetek pojava je morala biti relativna zračna vlaga več kot 80 %. Očitni premiki diagramov po obdobjih (Slika 4) zaradi sprememb relativne zračne vlage kažejo na pomen spremljanja tako temperature kot tudi zračne vlage.

Ugodne razmere za razvoj breskove muhe so v Kočevju trajale v povprečju od marca do novembra, zelo ugodne pa od junija do septembra. Prvo obdobje je imelo idealne razmere od junija do avgusta, drugo le avgusta, ostali dve pa idealnih razmer nista imeli. V poletnih mesecih so se povprečne mesečne temperature od prvega do četrtega obdobja povišale za 0,7 do 1,4 °C (pri standardni napaki vseh povprečij 0,2 °C), relativna vlaga pa se je zmanjšala za 6,5 do 7,4 % (pri standardnih napakah med 0,7 in 1,2 %). V Ljubljani so bile povprečne ugodne razmere za razvoj breskove muhe v vseh obdobjih od marca do novembra, zelo ugodne pa od aprila, z izjemo obdobja 1961–1990 od maja, pa do oktobra. Idealne razmere sta imela le avgust 1961–1990 in september 1991–2020. Podobno je bilo v Murski Soboti - ugodno od marca do novembra, zelo ugodno v prvem in drugem obdobju od maja do septembra, v tretjem in četrtem pa od aprila do oktobra. Idealne razmere za breskovo muho sta imela le avgusta v obdobjih 1961–1990 in 1971–2000. Spremembe v teh dveh primerih niso tako izrazite. Ugodne razmere za razvoj breskove muhe trajajo v Ratečah (Slika 5) povprečno od aprila do oktobra, zelo ugodne pa od maja do septembra v vseh izbranih obdobjih. Idealne razmere so bile dosežene le v juliju 1971–2000 in v avgustu v obdobjih 1981–2010 ter 1991–2020. Višje poletne temperature v Ratečah pomenijo nekoliko daljše obdobje z idealnimi razmerami za breskovo muho. Avgustovska temperatura se je od prvega do četrtega obdobja povišala




**Slika 4**: Griffiths-Taylorjev diagram povprečnih mesečnih temperatur in relativne vlažnosti s standardnimi napakami za štiri obdobja v Biljah z označenimi idealnimi, zelo ugodnimi, ugodnimi in neugodnimi razmerami za breskovo muho
**Figure 4**: The Griffiths-Taylor diagram of average monthly temperatures and relative humidity with standard errors in four periods in Bilje with marked ideal, very favorable, favorable and unfavorable conditions for the Mediterranean fruit fly

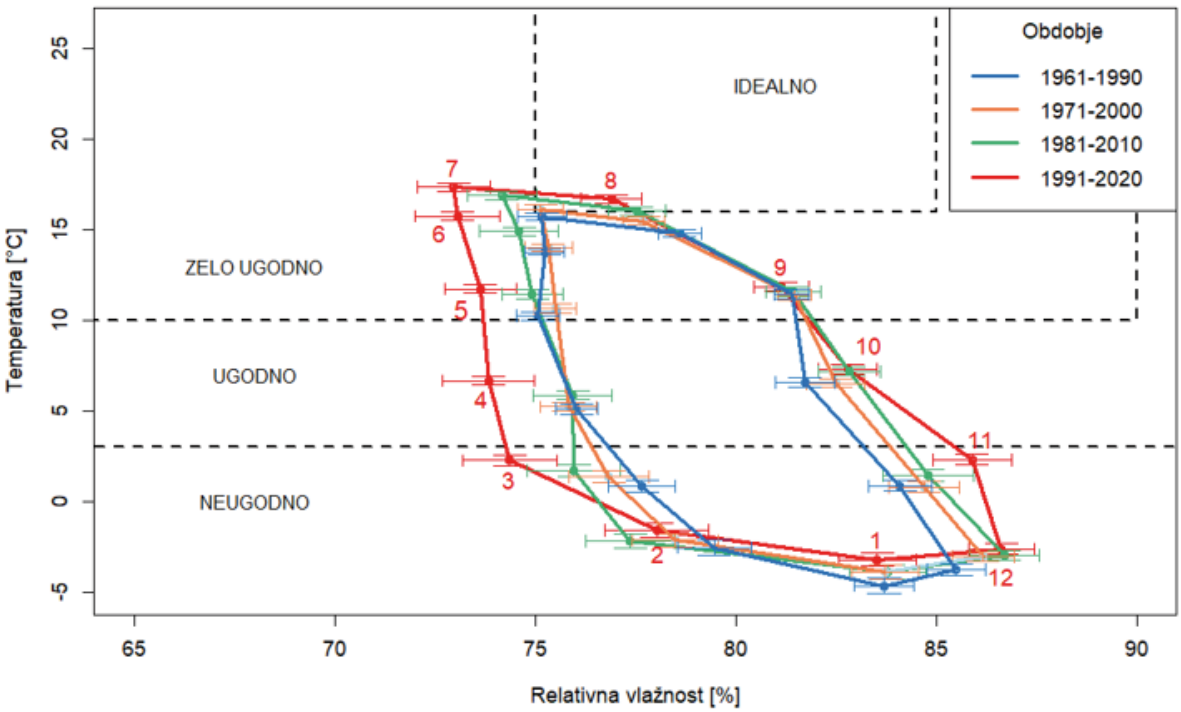



**Slika 5**: Griffiths-Taylorjev diagram povprečnih mesečnih temperatur in relativne vlažnosti s standardnimi napakami za štiri obdobja v Ratečah z označenimi idealnimi, zelo ugodnimi, ugodnimi in neugodnimi razmerami za breskovo muho
**Figure 5:** The Griffiths-Taylor diagram of average monthly temperatures and relative humidity with standard errors in four periods in Rateče with marked ideal, very favorable, favorable and unfavorable conditions for the Mediterranean fruit fly

za 1,9 °C (standardna napaka obeh povprečij je 0,2 °C), razlike v vlagi niso izrazite. Ugodne razmere za razvoj breskove muhe v Šmartnem pri Slovenj Gradcu so trajale povprečno od marca do oktobra oziroma novembra v obdobjih 1981–2010 in 1991–2020. Novembrska temperatura se je od prvega do četrtega obdobja povišala za 1,7 °C (standardna napaka povprečij 0,3 °C), relativna vlaga se ni spremenila. Zelo ugodne razmere so se začele maja in končale septembra. Idealne razmere so bile avgusta v prav vseh obdobjih, poleg tega pa še junija in julija 1961–1990 ter julija 1971–2000.

Ker se močni napadi in pomembne gospodarske škode zaradi breskove muhe pri nas pojavljajo na posameznih območjih le v posameznih letih (Rot in sod., 2015), bi bilo seveda smiselno v nadaljnjih raziskavah preučiti ne le povprečja, ampak tudi vremensko ekstremna leta. Na ta način bi lahko z diagrami za posamezno leto bolj natančno opredelili na primer vpliv ekstremno visokih temperatur, suše ali nadpovprečne količine padavin ter njihovih interakcij na rast in razvoj breskove muhe. Dolgoročno pa se pričakuje širitev vrste proti severu, na večje nadmorske višine in splošno povečanje številčnosti populacije na območjih, ki bi lahko postala primernejša v spreminjajočem se podnebju (Egartner in sod., 2018; Gilioli in sod., 2021).

Podobno kot za škodljivce lahko opazujemo tudi ustreznost rastnih razmer za poljščine. Ugodne razmere za rast sladkega krompirja so bile tako v Murski Soboti (Slika 6) v povprečju v prvem obdobju od junija do avgusta, v drugem od maja do avgusta, v tretjem in četrtem pa od maja do septembra. Idealne razmere so nastopile le v zadnjih dveh obdobjih, in sicer julija in avgusta. Julijska temperatura zraka se je med prvim in zadnjim obdobjem povišala za 1,9 °C in avgustovska za 2,2 °C (standardne napake povprečij so 0,2-0,3 °C), spremembe padavin pa so bile znotraj intervala standardnih napak, vendar spremembo lege krivulje na diagramu v tem primeru določa sprememba temperature. Zaradi podnebnih sprememb in višjih temperatur je v zadnjih letih precej raziskav namenjenih tudi modeliranju sprememb pri gojenju toplotno zahtevnejših rastlin v zmernih širinah, med drugim tudi sladkega krompirja (Gajanayake in sod., 2015; Raymundo in sod., 2014; Somasundaram in Mithra, 2008; Villordon in sod., 2009). Seveda je ob načrtovanju morebitnega uvajanja novih sort potrebno upoštevati še številne druge dejavnike, od vremenskih predvsem še količino in razporejenost padavin (Šlosár in sod., 2020), zračno vlago in vlago v tleh (Belehu in Hammes, 2004) ter temperaturo tal (Brandenberger in sod., 2014). Zelo pomembne so tudi dovolj velike razlike med dnevnimi




**Slika 6**: Griffiths-Taylorjev diagram povprečnih mesečnih temperatur in padavin s standardnimi napakami za štiri obdobja v Murski Soboti z označenimi ugodnimi in idealnimi razmerami za sladki krompir
**Figure 6**: The Griffiths-Taylor diagram of average monthly temperatures and precipitation with standard errors in four periods in Murska Sobota with marked favorable and ideal conditions for the sweet potato

in nočnimi temperaturami zraka (Loretan in sod., 1994; Gajanayake in sod., 2015), saj na tvorbo gomoljev ugodno vplivajo nižje nočne temperature (Kim, 1961; Lencha in sod., 2016). Kljub temu, da v Evropi zaenkrat pridelujejo sladki krompir le štiri države - Portugalska, Španija, Italija in Grčija (Mu in Li, 2019), pa možnosti pridelave te tržno zanimive poljščine proučujejo tudi v hladnejših predelih srednje Evrope (Šlosár in sod., 2020), kjer so klimatske razmere zelo podobne slovenskim. Daljša sezona rasti in višje temperature zraka ponujajo nove priložnosti v kmetijstvu, ki pa bodo močno odvisne od ostalih posledic naraščajočih izzivov pridelave (Arnell in Freeman, 2021).

## 4 SKLEPI

Kmetijstvo je dejavnost, ki se v veliki meri odvija na prostem in je kot tako močno odvisno kratkoročno od vremena in dolgoročno od podnebja. Zaradi sprememb podnebja, ki se že dogajajo, in projekcij, ki kažejo na stopnjevanje sprememb v prihodnosti, se bo kmetijstvo moralo prilagajati. Pri spremljanju podnebnih sprememb je za kmetijstvo letna skala veliko pregroba za nadaljnje ocene. Eden od načinov spremljanja podnebja je s podnebnimi diagrami, s katerimi grafično nazorno prikažemo mesečne vrednosti temperature zraka, padavin in zračne vlage.

Z Griffiths-Taylorjevimi diagrami smo prikazali časovne spremembe 30-letnih povprečij temperatur zraka, padavin in relativne vlage za šest podnebnih postaj. Že sama oblika diagrama, ki se pri kombinaciji temperatura-padavine na vseh šestih lokacijah spreminja, govori o podnebnih spremembah. Na vseh lokacijah so jasno vidne spremembe temperatur (povišanje), ponekod tudi relativne zračne vlage (zmanjšanje) ter delno spremenjeni sezonski vzorci padavin. Ozka oblika diagrama (večja razlika mesečnih vrednosti) nakazuje celinsko podnebje posamezne spremenljivke, bolj okrogla oblika (manjša razlika mesečnih vrednosti) pa mediteransko podnebje.

S prikazanimi diagrami so po obdobjih vidne spremenjene razmere za razvoj breskove muhe. Na splošno so se razmere za razvoj tega škodljivca izboljšale zaradi višjih temperatur, sploh na hladnejših lokacijah (Rateče). Na vseh postajah se je sicer zmanjšala relativna vlažnost, vendar v kombinaciji s temperaturo razmere v vseh mesecih ostajajo ugodne ali zelo ugodne za razvoj breskove muhe.

Diagrami z vrisanimi razmerami za rast sladkega

krompirja v Murski Soboti so pokazali, da se obdobje z ugodnimi razmerami v zadnjem obdobju podaljšuje (maj-september) glede na začetno obdobje (junij-avgust). Zaradi višjih temperatur zraka pa v zadnjih dveh obdobjih nastopajo v juliju in avgustu celo idealne razmere za rast sladkega krompirja.

V nadaljnjih študijah je poleg 30-letnih povprečij, ki nam prikazujejo dolgoročne spremembe, potrebno upoštevati tudi vremensko ekstremna leta (nadpovprečno vroča, suha ali namočena), saj na ta način pridobimo podatke o rastnih razmerah v posameznem letu in možnostih uvajanja novih sort glede na njihove meje razširjenosti zaradi podnebnih razmer. Prav tako pa bi grafi povedali več, če bi dodatno pridobili opis rastnih pogojev sort v posameznih fenofazah in jih ustrezno označili.

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# Resistance screening of white yam (*Dioscorea rotundata* Poir.) accessions against *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 using yam vines

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**Resistance screening of white yam (*Dioscorea rotundata* Poir.) accessions against *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 using yam vines**

**Abstract:** Root-knot nematode (*Meloidogyne incognita*) is an economically important phytoparasitic nematode species. In yam production, therefore, breeding for nematode resistance is an important environmentally friendly tool to manage root-knot nematodes damage. The aim of this study was to determine the reaction of 18 yam accessions to *M. incognita* inoculation under screen house conditions using single node vine cuttings. Vines of each accession were planted in sterilized soil and inoculated with 1000 infective juveniles of *M. incognita*. Resistance level of yam accessions were based on both galling index score and reproductive factor. There were a significant differences in final infective stage nematodes population, galling index, reproduction factor and yield of mini tuber among the accessions tested. Sixteen (89 %) of the accessions showed moderate resistance (GI ≥ 2, Rf ≤ 1) to the test pathogen with two accessions classified as susceptible. Accession TDr1515OP16/0030 recorded the highest mini tuber yield mass of 19.4 g, which was 74 % higher than accession 'TDr1515OP16/0108' which recorded the lowest yield of 10.4 g. The moderately resistant accessions identified in the study can be utilized to reduce nematodes reproduction and help manage root-knot nematode in yam production.

**Key words:** host plant resistance; host plant susceptibility; nematode suppression potential; white yams; southern root-knot nematodes

**Preučevanje odpornosti akcesij gvinejskega belega jama (*Dioscorea rotundata* Poir.) na ogorčico *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 z uporabo stebelnih izsečkov**

**Izvleček:** Ogorčica vozlanja korenin (*Meloidogyne incognita*) je ekonomsko pomembna fitoparazitska vrsta. Pri pridelavi jama je v njegovih žlahtniteljskih programih pomembno, okolju prijazno orodje vzgoja na ogorčice odpronih genotipov za uravnavanje škod, ki jo povzroča ta vrsta ogorčice. Namen raziskave je bil določiti odziv 18 akcesij jama na inokulacijo z ogorčico *M. incognita* v rastlinjaku z uporabo enonodijskih izsečkov. Stebelni izsečki jama so bili vsajeni v sterilizirana tla in inokulirani s 1000 kužnimi mladimi primerki *M. incognita*. Stopnja odpornosti akcesij jama je temeljila na indeksu okuženosti korenin z ogorčicami in njihovem reprodukcijskem faktorju. Med preizkuševanimi akcesijami jama je bila značilna razlika v končni stopnji okuženosti, indeksu vozlanja korenin, reporodukcijskem faktorju in v pridelku mini gomoljev jama. Šestnajst (89 %) od preučevanih akcesij je pokazalo zmerno odpornost (GI ≥ 2, Rf ≤ 1) na patogena. Dve akcesiji sta se izkazali kot občutljivi. Akcesija TDr1515OP16/0030 je imela največjo maso v pridelku mini gomoljev, 19,4 g, ki je bila za 74 % večja kot pri akcesiji TDr1515OP16/0108, pri kateri je bila masa najmanjša, 10,4 g. Zmerno odporne akcesije jama, identificirane v tej raziskavi, bi lahko uporabili za zmanjševanje razmnoževanja ogorčic in s tem zmanjšali okužbo z njimi pri pridelavi jama.

**Ključne besede:** odpornost gostiteljske rastline; potencial zatiranja ogorčic; beli gvinejski jam; južna ogorčica vozlanja korenin

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## 1 INTRODUCTION

White yams (*Dioscorea rotundata* Poir) play an important role in the lives and activities of several people including rural producers, processors and consumers in West Africa (Darkwa et al., 2019). It provides multiple opportunities for poverty reduction and nourishment for poor people in the West African sub-region (Sahore & Kamenan, 2007). Nutritionally, the crop provides substantial amounts of vitamins (thiamine and vitamin C), iron and potassium (Rudrappa, 2013) apart from being an important staple source of starch, sugars and fibers as well as proteins and trace amounts of lipids to consumers in the tropics and sub tropics. *Dioscorea* species also contain important secondary metabolites, steroidal saponins, diterpenoids and alkaloids, which have been exploited in the pharmaceutical industry (Das et al., 2014; Kumar et al., 2017).

Production of the crop is however, constraint by several factors, including low yield potential of local varieties, limited availability of planting materials as well as pests and diseases such as yam anthracnose, virus and nematodes. Plant parasitic nematodes have been implicated as important pest and limiting agent in yam production. Root-knot nematodes pest activities lead to galling and crazy roots syndrome of yam tubers thereby reducing quantity (yield) and quality of tubers. Also, wounds created by the stylets of pest during feeding serves as entry point for other microorganisms which leads to establishment of disease complexes on tubers. This reduces shelf life of infected yam tubers, market value and subsequently increases food insecurity. Phytoparasitc nematodes management in white yams production have depended on the use of synthetic chemicals, application of soil amendment such as neem products prior to planting and crop rotation. Employing most of these management options are limited in use due to high monetary costs, bulkiness, time consumption, feasibility and adverse effects on the environment as well as mammalian toxicity (Plowright & Kwoseh, 2000).

Attempts to develop improved white yam varieties with pests and diseases resistance, wide adaptability and good organoleptic characteristics are being explored by crop protectionist and yam breeders. Identifying resistant white yam cultivars are safe to manage root-knot nematode stress in yam production to reduce the negative impact associated with application of synthetic chemicals on non-targeted soil borne microorganisms and the environment. Plant host resistance management is environmentally friendly, sustainable and at little cost to smallholder farmers. Again, identifying nematodes resistance in white yams would improve breeding activities by the introgression of resistant genes into adapted varieties with desired traits. In the current study, 18 white yam accessions were evaluated for their reactions to *M. incognita* using single node cuttings.

## 2 MATERIALS AND METHODS

### 2.1 SOURCES OF WHITE YAM ACCESSIONS

Eighteen white yam accessions (Table 1) were obtained from the International Institute of Tropical Agriculture (IITA) and Yam Improvement Programme of the CSIR-Crops Research Institute, Kumasi, Ghana.

### 2.2 SOIL PREPARATION AND STERILIZATION

Soil was prepared by mixing top soil and river sand in a ratio of 3:1 and sterilized in an autoclave at 121 °C for 20 min. The sterilized soil was air dried for a week before use. This was to ensure dissipation of trapped gases. It was also to avoid possible effect of heat on the vine cuttings. The air dried sterilized soil was measured and distributed into one liter plastic screen house pots and placed on concrete benches.

**Table 1**: List of white yam accessions and source of collection

| Accessions | Source |
|---|---|
| TDr 1515 OP16/0108 | CSIR-Crops Research Institute |
| TDr 95/18544 | IITA |
| TDr 1515 OP16/0059 | CSIR-Crops Research Institute |
| TDr 95/19158 | IITA |
| TDr 1515 OP16/0105 | CSIR-Crops Research Institute |
| TDr 1515 OP16/0043 | CSIR-Crops Research Institute |
| TDr 95/19177 | IITA |
| TDr 1515 OP16/0081 | CSIR-Crops Research Institute |
| TDr 00/00362 | IITA |
| TDr 98/01067 | IITA |
| TDr 1515 OP16/0042 | CSIR-Crops Research Institute |
| TDr 98/00604 | IITA |
| TDr 1515 OP16/0092 | CSIR-Crops Research Institute |
| TDr 1515 OP16/0102 | CSIR-Crops Research Institute |
| TDr 1515 OP16/0046 | CSIR-Crops Research Institute |
| TDr 1515 OP16/084 | CSIR-Crops Research Institute |
| TDr 1515 OP16/0176 | CSIR-Crops Research Institute |
| TDr 1515 OP16/0030 | CSIR-Crops Research Institute |

### 2.3 EXTRACTION AND MAINTENANCE OF *Meloidogyne incognita* EGGS/JUVENILES

A population of *M. incognita* isolated from tomato was maintained and multiplied on susceptible tomato variety 'Pectomech'. Seedlings of the tomato were grown in plastic pots filled with the sterilized soil. Two weeks after planting, the tomato seedlings were inoculated with the eggs of the nematode pest. Eight weeks after inoculation, galled tomato plants were uprooted, washed under running tap water to get rid of all soil and galled roots cut into pieces (ca 2 cm). Nematode eggs were extracted following Hussey and Barker (1973) sodium hypochlorite (NaOCl) method. The extracted eggs were washed into a graduated beaker, and the volume adjusted to 100 ml with sterile distilled water. The nematode egg-water suspension was placed on laboratory benches for 24 hours at 24 ± 2 °C. This was to allow eggs hatching into second stage juveniles. Hatched juveniles were harvested and counted using a counting tray with the aid of a compound microscope.

### 2.4 RESISTANCE SCREENING OF WHITE YAM ACCESSIONS

Single node vines of 2 months old plants of each accession growing on the field was cut and washed under running water to remove debris. The excised vines were planted in sterilized sandy loam soil and placed in the screen house (Fig.1). Two months after planting yam vines which allowed initial rooting to occur, 1000 *M. incognita* infective stage juveniles were introduced approximately 2 cm deep into the soil surrounding roots of each white yam plant. Inoculated plants were arranged in completely randomized design with 3 replications on screen house benches and maintained in the screen house at 28 ± 2 °C. Eighty days after inoculation, white yam mini tubers were harvested, counted and weighed to determine yield. Each mini tuber harvested was examined and the extent of damage due to nematodes were scored on a scale of 1-5 (1 = no symptoms on tuber surface, 2 = slight damage (1-25 % of symptoms on tuber surface), 3 = mild damage (26-50 % symptoms on tuber surface), 4 = heavy damage (51-75 % symptoms on tuber surface), and 5 = severe damage (> 75 % symptoms on tuber surface). Soil samples were collected from each pot and final nematodes population in 200 cubic centimeter (cc) soil extracted and counted. The experiment was carried out in the 2018 and 2019 cropping season, using the same set of cultivars to determine the consistency of differences in nematode resistance.

**Fig. 1**: White yam accessions establishment in pots under screen house conditions

### 2.5 STATISTICAL ANALYSIS

Data collected for the two years were pooled together for analysis. Data on final nematode numbers were log (x + 1) transformed to comply with assumption of normal distribution. Statistical analysis was performed using analysis of variance (ANOVA) with Genstat and differences between significant means separated using Tukey's HSD ($p < 0.05$). The level of resistance or susceptibility of each yam accession was based on both galling and reproduction index (proportion of final nematodes recovered to initial nematodes applied) as described by Afolami et al. (2004) (Table 2). Linear regression analysis was performed to determine the relationship between final nematodes count and galling index using Microsoft Excel.

## 3 RESULTS

### 3.1 PATHOGENICTY AND REPRODUCTION EFFICIENCY OF *M. INCOGNITA* ON YAM ACCESSIONS

White yam plants established successfully from the single node vines cuttings (Fig.1). At harvest, it was observed that mini tubers harvested from uninoculated pots were healthy/clean with no symptoms of *M. incognita* damage (Fig. 2A). It was however not the same for the inoculated pots as they showed varied symptoms of root-knot nematodes infestation. Symptoms of root-knot nematode infestation included appearance of galls on mini tubers and roots as well as crazy rooting syndrome (Fig. 2B).

Results of the study revealed that the various yam

**Table 2**: Resistance rating scale for root-knot nematodes

| [a]Plant damage (gall index) | [b]Reproduction Index | Degree of resistance (DR) |
|---|---|---|
| ≤ 2 | ≤ 1 | Resistant |
| ≤ 2 | ≥ 1 | Tolerant |
| ≥ 2 | ≤ 1 | Moderately resistant |
| ≥ 2 | ≥ 1 | Susceptible |

[a]Gall index: 0 = no gall formation; 5 = heavy gall formation
[b]Reproductive factor: Rf = Pi/Pf where Pi = initial population density, and Pf = final population density

accessions reacted differently to *M. incognita* infestation. The nematodes incited galling not only on the yam roots but also on the tubers (Fig 2B). Ability of the nematode to reproduce varied significantly ($p < 0.05$) under the different white yam accessions. It was observed that nematode reproduction was highest in accession TDr 98/01067 compared to other accessions. Whilst TDr 98/01067 recorded 1040 juveniles (J2)/200 cc soil, both TDr1515OP16/0105 and TDr 98/00604 recorded 670 J2/200 cc (Table 3). Similarly, galling index significantly ($p < 0.05$) varied between the accessions. The highest galling index of 2.7 was recorded in two accessions namely TDr 00/00362 and TDr 98/01067. Majority (50 %) of the accessions recorded gall indices of 2.0 compared to 16.6, 22.0, and 11.1 % of the accessions recording gall indices of 2.3, 2.5 and 2.7 respectively. The highest reproductive index of 1.4 was recorded in accession TDr 98/01067 which was not significantly different ($p > 0.05$) from TDr 00/00362, which recorded 1.3. However, the lowest reproduction index of 0.7 was recorded in four accessions, namely TDr1515 OP16/0105, TDr 95/19158, TDr 98/00604 and TDr95/18544 (Table 3). Based on galling reproduction indices, 16 accessions were classified as moderately resistant whilst two namely TDr 00/00362 and TDr 98/01067 were classified as susceptible to the pest.

### 3.2 YIELD OF WHITE YAM MINI TUBERS AND RELATIONSHIP BETWEEN FINAL NEMATODES POPULATION AND MINI TUBER HEALTH

Mini tuber yields were significantly different ($p < 0.05$) with TDr1515OP16/0030 recording the highest mini tuber mass of 40.20 g. This was 74.0 % more than that of TDr1515OP16/0108 which recorded the least (10.4 g) (Table 4). It was also observed that *M. incognita* soil population at harvest had effect on the severity of mini tuber galling. There was a positive relationship between final number of second stage *M. incognita* recovered and tuber damage recorded as galling index (Fig.3). It was observed that increase in the final second stage juvenile population, corresponded significantly with yam mini tuber galling severity.

## 4 DISCUSSION

Nematode-resistant genotypes of crop plants are generally unaffected or little affected by nematodes attack and greatly contribute to reducing nematode infestations. Eighteen white yam accessions evaluated in the present study is critical in the effort of identifying genetic sources to manage root-knot nematode, which is

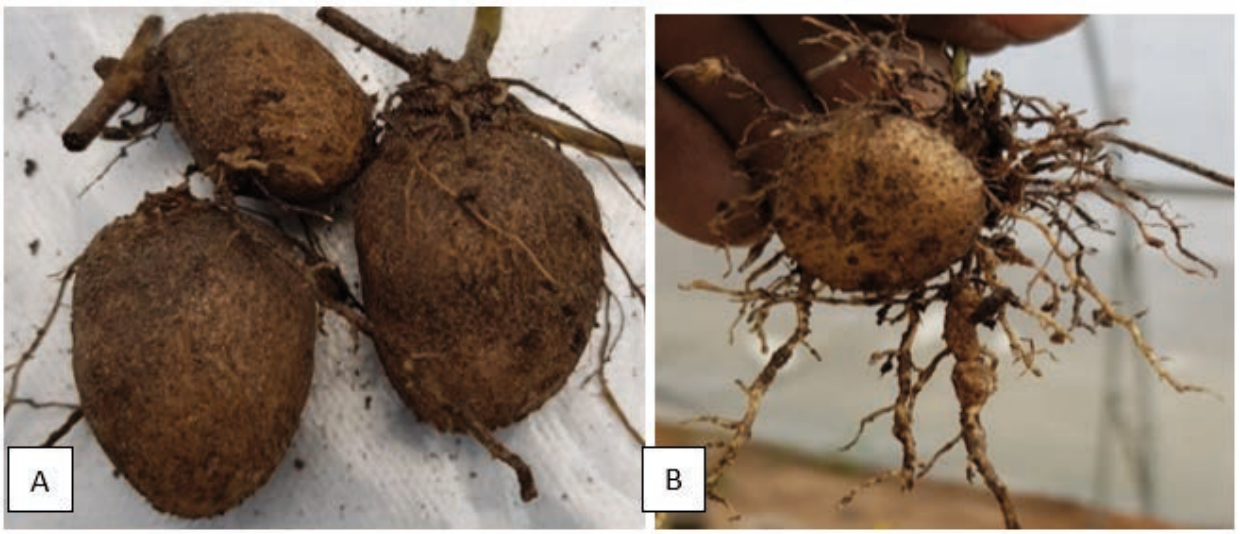


**Fig. 2**: Healthy (A) and *M. incognita* infested (B) mini tubers from inoculated and uninoculated pots respectively

**Table 3**: Reproduction of *Meloidogyne incognita*, galling index, reproduction index (RI) and resistance levels of white yam accessions

| Accession | [a]Nematodes count/200cc soil | [b]GI | [c]RI | [d]Resistance Level |
|---|---|---|---|---|
| TDr1515 OP16/0105 | 670.0 (2.83) | 2.0 | 0.7 | MR |
| TDr 95/19158 | 671.7 (2.83) | 2.0 | 0.7 | MR |
| TDr 98/00604 | 670.0 (2.83) | 2.0 | 0.7 | MR |
| TDr95/18544 | 673.3 (2.83) | 2.0 | 0.7 | MR |
| TDr 1515 OP16/0030 | 680.0 (2.83) | 2.0 | 0.7 | MR |
| TDr 1515 OP16/0042 | 682.3 (2.83) | 2.0 | 0.7 | MR |
| TDr 1515 OP16/0059 | 680.0 (2.83) | 2.0 | 0.7 | MR |
| TDr95/19177 | 682.7 (2.83) | 2.0 | 0.7 | MR |
| TDr 1515 OP16/0043 | 686.7 (2.84) | 2.0 | 0.7 | MR |
| TDr 1515 OP16/0108 | 776.7 (2.89) | 2.3 | 0.8 | MR |
| TDr 1515 OP16/084 | 780.0 (2.89) | 2.3 | 0.8 | MR |
| TDr 1515 OP16/0176 | 786.7 (2.89) | 2.3 | 0.8 | MR |
| TDr 1515 OP16/0102 | 846.7 (2.92) | 2.5 | 0.9 | MR |
| TDr 1515 OP16/0046 | 850.0 (2.92) | 2.5 | 0.9 | MR |
| TDr 1515 OP16/0092 | 850.0 (2.93) | 2.5 | 0.9 | MR |
| TDr 1515 OP16/0081 | 863.3 (2.93) | 2.5 | 0.9 | MR |
| TDr 00/00362 | 1030.0 (3.01) | 2.7 | 1.3 | S |
| TDr 98/01067 | 1040.0 (3.02) | 2.7 | 1.4 | S |
| HSD ($p < 5$ %) | (0.01) | 0.08 | 0.01 | |
| CV | (1.7) | 4.5 | 1.7 | |

[a]Final *M. incognita* extracted from 200 $cm^3$ soil, [b]Gall index: 0 = no gall formation; 5 = heavy gall formation, [c]Reproduction index: RI = Pi/Pf where Pi = initial population density, and Pf = final population density, [d]Resistance level based on the RI and GI where MR-Moderately Resistant and S-Susceptible

currently not controlled in yam production. There was a varied response of the white yam accessions to *M. incognita* infestation. Differential responses of plant genotypes to nematodes infection were reported in previous studies (Kagoda et al., 2004; Osei et al., 2015; Kankam et al., 2019). Accessions TDr 00/00362 and TDr 98/01067 found to be susceptible to the test pest allowed higher nematodes reproduction with increased population densities and a higher disease severity compared to other accessions screened. Susceptibility of plants to nematodes according to Cervantes-Flores et al. (2008) may be due to the presence of unfavorable alleles that reduce their level of resistance. Sixteen of the yam accessions screened in this study were identified to be moderately resistant with none being categorized as highly resistant or immune to the test pathogen. Clearly, results obtained showed a reduced root-knot nematode reproduction and galling severity (Rf < 1, GI < 2) in moderately resistant accessions compared to those rated to be susceptible. Moderately resistant accessions according to Roberts (2002) and Zwart et al. (2019), supports low or intermediate reproduction compared to susceptible genotypes. Identification of moderately resistant accessions in this study agrees with previous screening studies. Karuri et al. (2017) and Aydinli et al. (2019) identified accessions of *Cucurbita maxima* Duchesne, *Cucurbita moschata* Duchesne ex Poir. and sweet potato that were moderately resistant to root-knot nematode. Moderately resistant plants according to Singh et al. (2012) provides durable resistance against pathogens since it is controlled by multiple resistant genes that reduce multiplication of nematodes within their host (Cervantes-Flores et al., 2008; Lee et al., 2021). High reproduction of nematodes in their host increases extent of damage caused. The positive relationship between nematodes population and galling index scores as observed in the present study agrees with findings of El-Sherif et al. (2007) and Charegani et al. (2012). This may explain why TDr 00/00362 and TDr 98/01067 rated as susceptible in the current study and supported higher *M. incognita* reproduction recorded higher galling index scores.

Root-knot nematode infestation in accessions TDr

**Table 4**: Yield (g) of white yam accessions at 4 months after planting

| Yam accessions | Mini tuber mass (g) |
|---|---|
| TDr 1515 OP16/0108 | 10.4 |
| TDr95/18544 | 11.1 |
| TDr 1515 OP16/0059 | 11.7 |
| TDr 95/19158 | 12.2 |
| TDr 1515 OP16/0105 | 13.0 |
| TDr 1515 OP16/0043 | 13.2 |
| TDr95/19177 | 14.0 |
| TDr 1515 OP16/0081 | 14.1 |
| TDr 00/00362 | 15.0 |
| TDr 98/01067 | 15.5 |
| TDr 1515 OP16/0042 | 15.6 |
| TDr 98/00604 | 15.6 |
| TDr 1515 OP16/0092 | 19.4 |
| TDr 1515 OP16/0102 | 19.90 |
| TDr 1515 OP16/0046 | 31.20 |
| TDr 1515 OP16/084 | 31.20 |
| TDr 1515 OP16/0176 | 32.40 |
| TDr 1515 OP16/0030 | 40.20 |
| HSD (P<5 %) | 1.1 |
| CV | 1.7 |

00/00362 and TDr 98/01067 affected their appearance due to galling and crazy rooting on symptoms tubers. However, mass of these two were in some instances higher than moderately resistant accessions. This observation confirms the assertion of Bridge et al. (2005) that *Meloidogyne* spp., do not necessary decrease tuber mass but marketability. Earlier studies reporting on variations in yield of crops have attributed differences in yield performance to genotypic characteristics (Ene et al., 2016; Usman et al., 2017). The moderately resistant white yam accessions identified in this study will help reduce *Meloidogyne incognita* population build up and contribute to the management of root-knot nematode menace in yam production.

## 5 FUNDING

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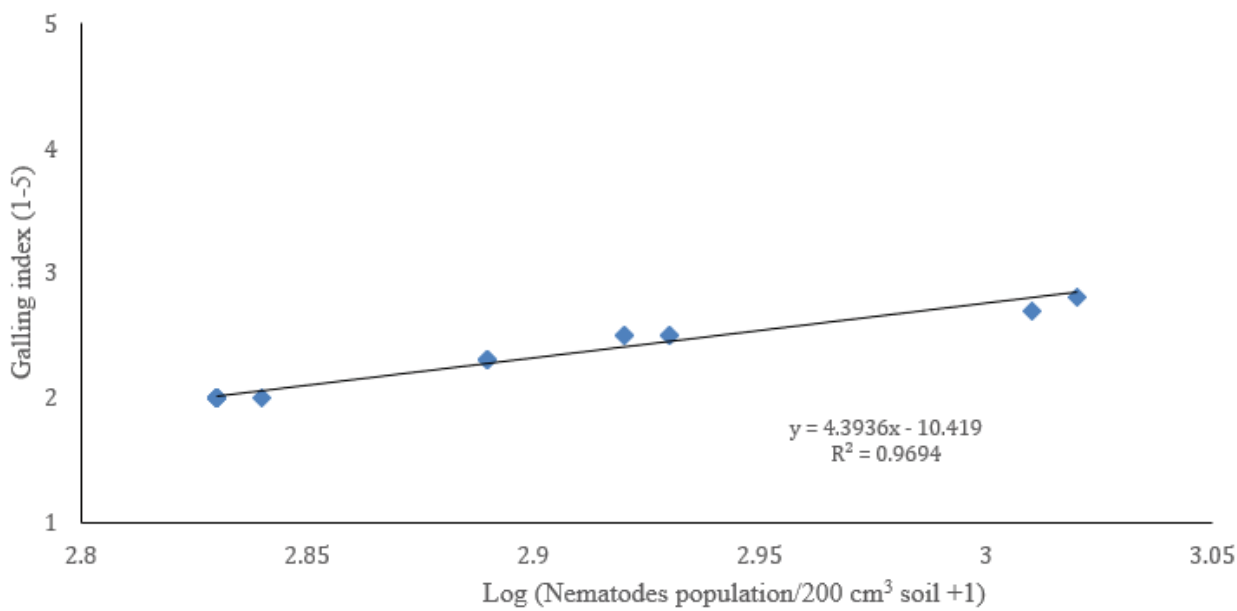


**Fig. 3**: Relationship between *M. incognita* population and galling index

Breeding sections of the CSIR-Crops Research Institute, Kumasi, Ghana.

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# Effect of different grain spawn materials on *Pleurotus ostreatus* (Jacq.) P. Kumm. mushroom cultivation under unregulated and regulated fruiting conditions

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**Effect of different grain spawn materials on *Pleurotus ostreatus* (Jacq.) P. Kumm. mushroom cultivation under unregulated and regulated fruiting conditions**

**Abstract:** Quality spawn, which is also dependent on grain composition, is a critical factor that must be optimized to achieve successful and profitable mushroom farming. The characteristics of grain spawn composition (Factor A) and two microclimatic fruiting conditions (Factor B) were studied in the cultivation of *Pleurotus ostreatus*. Eight different grain material combinations (GMC1-8) made from wheat, barley, oat, and millet were used to prepare spawn and tested for mushroom cultivation under unregulated and regulated fruiting conditions. The physicochemical characteristics of the different grain spawn, substrate, time to attain the first flush, and BE (biological efficiency) in the different GMCs under the two fruiting conditions were determined. The differences in nutrient compositions of the GMCs tested did not result in a significant difference in the nutrient composition of the cultivation substrate. GMCs containing barley and oat gave BE values that were not significantly different under the two microclimatic conditions tested. GMCs containing 100 % wheat and millet resulted in the poorest BE recorded. However, equal combination of wheat, barley, and oat (GMC8) gave the best results among GMCs tested. Furthermore, it is more cost-efficient to use the GMC8 combinations since wheat is cheaper than all other grains tested.

**Key words:** biological efficiency; grain materials characteristics; microclimate in fruiting house; substrate colonization

**Učinek različnih gojilnih substratov iz žitnih zrn na gojenje ostrigarja (*Pleurotus ostreatus* (Jacq.) P. Kumm.) v razmerah uravnavane in neuravnavane tvorbe trosnjakov**

**Izvleček:** Kakovosten inokulacijski micelij in gojišče, ki je odvisno tudi od sestave žitnih zrn sta kritična dejavnika, ki morata biti optimizirana za doseganje uspešnega in donosnega gojenja gob. Preučevane so bile lastnosti in sestava žitnih zrn inokulacijskega micelija (Factor A) in dva režima mikroklimatskih razmer (Factor B) za tvorbo trosnjakov pri gojenju ostrigarja. Za pripravo inokulacijskega micelija in njegovega gojišča je bilo uporabljeno osem različnih kombinacij (GMC1-8) žitnih zrn, in sicer pšenice, ječmena, ovsa in prosa v razmerah uravnavane in neuravnavane tvorbe trosnjakov. Določene so bile fizikalno-kemijske lastnosti različnih žitnih zrn pri pripravi inokulacijskega micelija, njegovega gojišča, časa za doseganje prve tvorbe trosnjakov v dveh mikroklimatskih razmerah in različnih kombinacijah GMC. Preiskušene razlike v mineralni sestavi GMC niso dale značilnih razlik v sestavi mineralih hranil gojišča. GMC, ki so vsebovala ječmen in oves so imela biološko učinkovitost (BE), ki ni bila značilno različna v obeh preiskušenih mikroklimatskih razmerah. GMC, ki so vsebovala 100 % pšenico in proso so imela najslabšo biološko učinkovitost. Kombinacija enakih odmerkov pšenice, ječmena in ovsa (GMC8) je imela najboljši rezultat med preiskušenimi gojišči (GMCs). Cenovno je kombinacija GMC8 najboljša, ker je pšenica cenejša kot vsa ostala preiskušena žita.

**Ključne besede:** biološka učinkovitost; lastnosti žitnih zrn; mikroklima v prostoru tvorbe trosnjakov; kolonizacija substrata

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## 1 INTRODUCTION

The rapid development of industrial mushroom production around the world especially exotic mushrooms, is due to the growing interest in healthy eating. Today, more than 2,000 species of edible and / or medicinal mushrooms are known, many of them are widely used as a source of bioactive compounds (Friedman, 2016). Mushrooms of the genus *Pleurotus* (Fr.) P. Kumm., popularly called oyster mushrooms, are the second most consumed mushroom in the world (Sánchez, 2010). In Ukraine, the three industrially cultivated species are *P. ostreatus* (Jacq.) P. Kumm. (oyster mushroom), *P. pulmonarius* (Fr.) Quél (lung oyster), and *Pleurotus eryngii* (DC.) Quél. (popularly called king oyster), and each of them has valuable medicinal and nutritional properties (Bukhalo et al., 2011).

Spawn type and quality is a critical factor in the process of mushrooms cultivation. It is composed of a base, carrying the vegetative mycelia of the target fungi to be cultivated, which is used to inoculate the cultivation substrate. Various types of materials and technology for spawn production have been reported (Green, 1977). The most common technology, patented by James W. Sinden (Sinden, 1932), utilizes grain to make the spawn. Spawn quality is critical to successful mushrooms cultivation, and many factors must be considered and optimized to produce quality spawn. The main factors are:

1. Microbiological purity of the culture, i.e. the absence of foreign microbiota (bacteria, yeast, molds) in the mushroom culture (Dudka et al., 1978; Petrova, 2010);

2. Suitability of spawn base material (grains, sawdust, wood chips, etc.) for rapid colonization by target mushroom mycelia (Jhune et al., 2000; Sainos et al., 2006);

3. Grain-carrying potential, which is the quantity and quality of nutrients in grains that supports actively growing fungal cells during the transition to a cultivation substrate or during long-term storage (Mamiro & Royse, 2008; Zhang et al., 2014)

4. The number of inoculum units within a given quantity/mass of ready to-use spawn.

It is generally believed that the number of grains per unit of spawn quantity determines the number of inoculation points and rate of substrate colonization; hence the more the number of grains, the better and quicker colonization is expected (Subramanian et al., 2014; Sofi et al., 2014; Khonga et al., 2013). The importance of spawn material quality in successful mushrooms production was recognized as far back as 1905, by B. M. Duggar in one of the first books on industrial cultivation of mushrooms "The principles of mushroom growing and mushroom spawn making" (Duggar, B. M., 1905). The large number of scientific publications dedicated to improving the quality and methods of spawn production, and its application in different countries, indicate its importance in the mushroom industry (Alekseyenko et al., 2010; Bhatti et al., 2007; Hoa & Wang, 2015; Royse & Chalupa, 2009). The search for the best cereals grain for use in spawn production is complicated by factors related to the types of available local grains, as well as their moisture and nutritional contents, including the cultivation conditions i.e. soil and climate (Stanley & Awi-Waadu, 2010; Jiskani et al., 2007; Rosado et al., 2002; Ivanova & Kovalyshyna, 2018). In countries where there is a shortage of cereals, the use of wheat results in expensive spawn, hence they use sorghum (*Sorghum bicolor* (L.) Moench)) grains, cotton (*Gossypium hirsutum* L.) waste, and in some cases the leafy part of aquatic plants (Mahmoud, 2006).

The industrial production of mushroom spawn on cereal grains in developed countries is highly a technical process which continues to improve over the years. Quality spawn for commercial mushroom production is expensive. Hence, research to find affordable and effective ingredients, as well as low-cost technology, to lower the cost of commercial spawn is intense (Gregori et al., 2008). The mixture of different materials that should increase the nitrogen content in the ready spawn, and thus improve its colonization and speed of adaptation to cultivation substrates, is an area of active research (Hoa & Wang, 2015; Ivanova & Kovalyshyna, 2018). Kananen and McDaniel (2000) patented the formula of seed mycelium with mineral components (perlite and vermiculite), which increased the number of inoculation points in 100 grams of spawn to 20 thousand pieces. It is known that the properties of spawn materials used for inoculation have a decisive influence on the duration of the incubation period, biological efficiency (BE), and even the morphological features of the mushrooms cultivated (Soko et al., 2019)

The most available grain raw material useful in spawn production in Ukraine and most parts of Europe is wheat, and the least are oat and millet. Millet, which experienced significant reduction in production since 2017 (Petrenko, 2019). Also, the export and domestic demands on a particular grain crop can lead to significant price fluctuations. The nutrient content of grain depends on many physicochemical factors and cultivation conditions: the soil composition and climatic conditions under which they were grown, as well as inherent genetic characteristics and nutrient availability, can affect their performance as materials for spawn production (Shapovalenko et al., , 2017). These factors have made grains other than wheat expensive materials for making spawn. We believe that the use of local resources (grains) to produce quality spawn will reduce spawn cost and

boost mushrooms production and supply to the market at any particular location.

The biochemical composition of grain spawn material and microclimatic factors (temperature, humidity, air composition, and light) play significant roles in the vegetative mycelia adaptation to cultivation substrate after spawning, and these factors are readily optimized in large-scale industrial production situations (Bisko & Dudka, 1987; Zaikina et al., 2007; Vdovenko, 2015) However, in small-scale production, it is difficult to optimize factors like room temperature; sharp changes in outdoor temperature in winter and summer significantly affect the microclimate of the growing chambers. Such fluctuations could significantly reduce the productivity of cultivated mushrooms.

This study was done to assess the influence of grain-carrier composition on the productivity of *P. ostreatus* under two microclimatic conditions. The hypothesis is that different grains and their mixtures will have significant effects on fruit body yield during cultivation and that grain spawn material compositions can affect BE in oyster mushrooms differently under regulated and unregulated microclimatic conditions in *P. ostreatus*.

## 2 MATERIALS AND METHODS

### 2.1 GRAINS

The cereal grains used as spawn materials were wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), millet (*Panicum miliaceum* L.), and oat (*Avena sativa* L.). The general physical dimensions of the grains used in this experiment is reposted by Pilipyuk (2010) and shown in Table 1 below.

### 2.2 FUNGAL CULTURE

*Pleurotus ostreatus*, strain 2301 IBK (*P.o.* 2301), which is widely cultivated in commercial production during the winter in Ukraine, was selected and used for this study due to its performance reported in previous studies (Bisko et al., 2016; Myronycheva et al., 2017). The culture was obtained from the culture collection at the M. G. Kholodny Institute of Botany, National Academy of Sciences (NAS), Ukraine. Actively growing mycelia was inoculated onto 2 % malt extract agar and, incubated at 26 ± 1 °C for 8 days, and thereafter stored at 3 ± 1 °C until use. To prepare grain inoculum, stored culture from above was used to inoculate Petri dishes (90 mm) containing about 35 ml of 2 % PDA and incubated at 24 ± 1 °C for 8 days. Colonized medium in a plate was added to 250 ml sterile water in a blender and blend for 20 s to obtain mycelia suspension.

### 2.3 MOTHER SPAWN

A mixture of millet grain (95 %), rape seed (4 %), flax seed (0.5 %) and $CaCO_3$ (0.5 %) was used to prepare the mother spawn. First, the rape seed was soaked in water overnight (8-10 h) and drained before use. The millet was boiled in water for 35 ± 5 minutes, after which heating was discontinued, and the grains were allowed to remain in the hot water for additional 20 ± 3 minutes, and then drained for 15 ± 3 minutes. That treatment brought the millet grain moisture content to 44 ± 2 %.

The millet grain was then poured into a bath, and the pre-soaked rape seed, flax and $CaCO_3$ were added and mixed properly, before loading (5500 ± 53 g each) into heat-resistant polypropylene bags (580 x 490 mm), PP75/BEU6/X47-57 (from Sac02, Veldeken 29, 9850 Deinze, Belgium). The bags were sterilized at 129-132 °C, 1.1 bar (16 PSI) for three hours. After cooling to 26 ± 2 °C, the grain mixture was inoculated under aseptic conditions with the culture suspension from above, at the rate of 75 ml per bag, sealed and shaken to evenly distribute the suspension in the grain. The inoculated bags were placed on shelves in a clean room at a temperature of 22 ± 2 °C and relative humidity of 65 ± 5 %, to incubate for 10 days to achieve full colonization and matured mother spawn. The mother spawn was cooled to 11 ± 1 °C and stored at 0-2 °C until it was used (within 7 days).

**Table 1**: Physical characteristics and dimension of common grains

| Type of grain | Length, mm | Width, mm | Thickness, mm | Weight/10 g |
|---|---|---|---|---|
| Wheat | 4.0 – 11.2 | 1.6 – 4.0 | 1.6 – 3.4 | 2.5 – 3.5 |
| Barley | 7.0 – 14.6 | 2.0 – 5.0 | 1.4 – 4.5 | 3.0 – 4.6 |
| Oat | 8.0 – 18.0 | 1.4 – 4.0 | 1.2 – 3.5 | 2.2 – 4.2 |
| Millet | 1.9 – 3.2 | 1.5 – 2.9 | 1.3 – 2.0 | 0.4 – 0.7 |

Source: (Pilipyuk, 2010)

## 2.4 DIFFERENT TEST SPAWN

Wheat, millet, barley and oat combined in various proportions were used to prepare the various test spawn with different grain material compositions (GMCs). The combinations were: 1) wheat (1:0), control; 2) wheat-millet (2:1); 3) wheat-millet (1:2); 4) barley (1:0); 5) barley-millet (2:1); 6) oat (1:0); 7) oat-millet (2:1); and 8) millet-wheat-oat (1:1:1). From empirical observations, the various grains used require different cooking times to achieve desired moisture content, which is usually determined visually by the presence of a dry endosperm residue in the grain with a thickness of not more than 1 mm. Therefore, it was necessary to implement different cooking times for the grains used in the experiment.

To make GMC1, wheat (120 kg) was boiled in 150 l of water for 16 ± 2 minutes and allowed to remain in the hot water for additional 20 ± 2 minutes. For GMC2 or 3, millet (40 or 80 kg) was first boiled in 150 l of water for 20 ± 3 minutes, then wheat (80 or 40 kg), respectively was added and cooking continued for another 16 ± 2 minutes, and thereafter left in the hot water for additional 20± 1 minute. For GMC4, 120 kg barley was boiled in 150 l of water for 25 ±5 minutes and left in the hot water for additional 10 ± 2 minutes. For GMC5, millet (40 kg) was poured into 150 l of boiling water and cooked for 20 ±3 minutes, then 80 kg barley was added and cooked for additional 25 ±5 minutes and left in the hot water for additional 10 ± 3 minutes. For GMC6, oat grains (120 kg) was boiled in 150 l of water for 25 ± 5 minutes and allowed to stay an additional 10 ± 2 minutes in hot water before use. In GMC7, millet (40 kg) was boiled in 150 l water for 20 ± 2 minutes; 80 kg of oat grains was added and boiled further for another 25 ± 2 minutes and left in the hot water for 10± 5 minutes. GMC8 had three grains components. In 150 l of water, millet (40 kg) was boiled for 20 ± 5 minutes, oat (40 kg) was added and continued to cook for another 10 ± 3 minutes, and wheat (40 kg) was added to the rest grain in the pot and boiled for another 16 ± 2 minutes and allowed to stay in the hot water for 10 ± 1 minute.

The grains and mixtures made were drained of water and 1 % chalk (dry mass of grains) was added. The prepared mixture was packed (6095 ± 48 g each) into polypropylene bags (PP75/BEU6/X47-57) and sterilized as above. Upon cooling to room temperature, the bags were inoculated with mother spawn at the rate of 1.3 ± 0.2 % per wet mass of spawn material in the bag, sealed and mixed properly to distribute the inoculum evenly in the grains within each bag. Shaking was done on the 5$^{th}$ day after inoculation to aid quick and uniform colonization throughout the spawn bags. After 10 days, the spawn was cooled and stored in the same manner as mother spawn before use.

## 2.5 SUBSTRATES

Substrate for mushrooms cultivation was made from sunflower husk and barley straw at a ratio of 1:3 and pasteurized as previously reported (Holub et al., 2010). The characteristics of the substrate used were humidity 71.8 ± 4.1 %; pH 8.02 ± 0.31; the ratio C/N = 69.3 ± 6.4. After cooling, substrates were inoculated with spawn made from different GMCs above. The amount of spawn per bag was 4.8 ± 0.1 % (wet mass) and thoroughly mixed into the substrate in the common mechanical process for substrate inoculation in commercial production in Ukraine (Bisko & Dudka, 1987; Bandura et al., 2017). Each cylindrical substrate bag was made with plastic bags (size + 33×90 cm). A total of 55 ± 5 bags each was prepared for each of the 8 different spawn types (GMCs), with the following physical characteristics (average): diameter–22 cm, height–75 cm, mass–12430 ± 230 g, density 440 kg m$^{-3}$. 50 bags of each treatment were randomly distributed in growing chambers and 10-12 slit of 5-7 cm long at a distance of 10-15 cm from each other in a checkerboard pattern were punched onto each bag.

## 2.6 MICROCLIMATIC CONDITIONS

The influence of microclimatic conditions was studied in two different growing rooms. The cultivation rooms or grow room GC1 is the unregulated growing condition, which did not have equipment for regulating air mixture and recirculation system. Heated and humidified air was supplied to the chamber through a ventilation opening with an area of 0.45 m² in a volume that provided three times air changes per hour during the fruiting period. Air excess was removed through 3 windows under the roof (the total area of the opening was 1.3 m²). The microclimatic conditions of $CO_2$ and light in the grow room at the factory were unstable and depended on the parameters of the outside climate conditions.

Humidity and air composition ($CO_2$/$O_2$ ratio) in GC 2 were maintained by distributing humidified air mixture in the chamber through an air duct system.

The chamber where incubation was done for two weeks prior to fruiting was semi-dark, because light was turned on only during visual inspection (10-15 min per day) and the growing chambers windows allowed limited natural light with intensity from 50 to 70 lm m$^{-2}$ into the chamber. The induction of fructification was initiated on the 14$^{th}$ day after inoculation by lighting from 150 to

200 lm $m^{-2}$ up to 8 hours per day. The temperature in the chamber was gradually reduced from 20-22 °C to 16-18 °C over a period of 48 hours by using active ventilation with fresh air.

## 2.7 SAMPLES ANALYSES

Samples from substrates for analysis were taken before inoculation with spawn and on the 2nd day after inoculation and placement in the growing chambers. In the test spawn, samples were taken before inoculation with mother spawn and after incubation and the spawn was ready for use. Samples for spawn material analysis were taken prior to use for spawn preparation and after. Analysis for substrate bags and grain spawn materials were done in triplicate. Water content of spawn and substrates was determined by thermogravimetry at a temperature

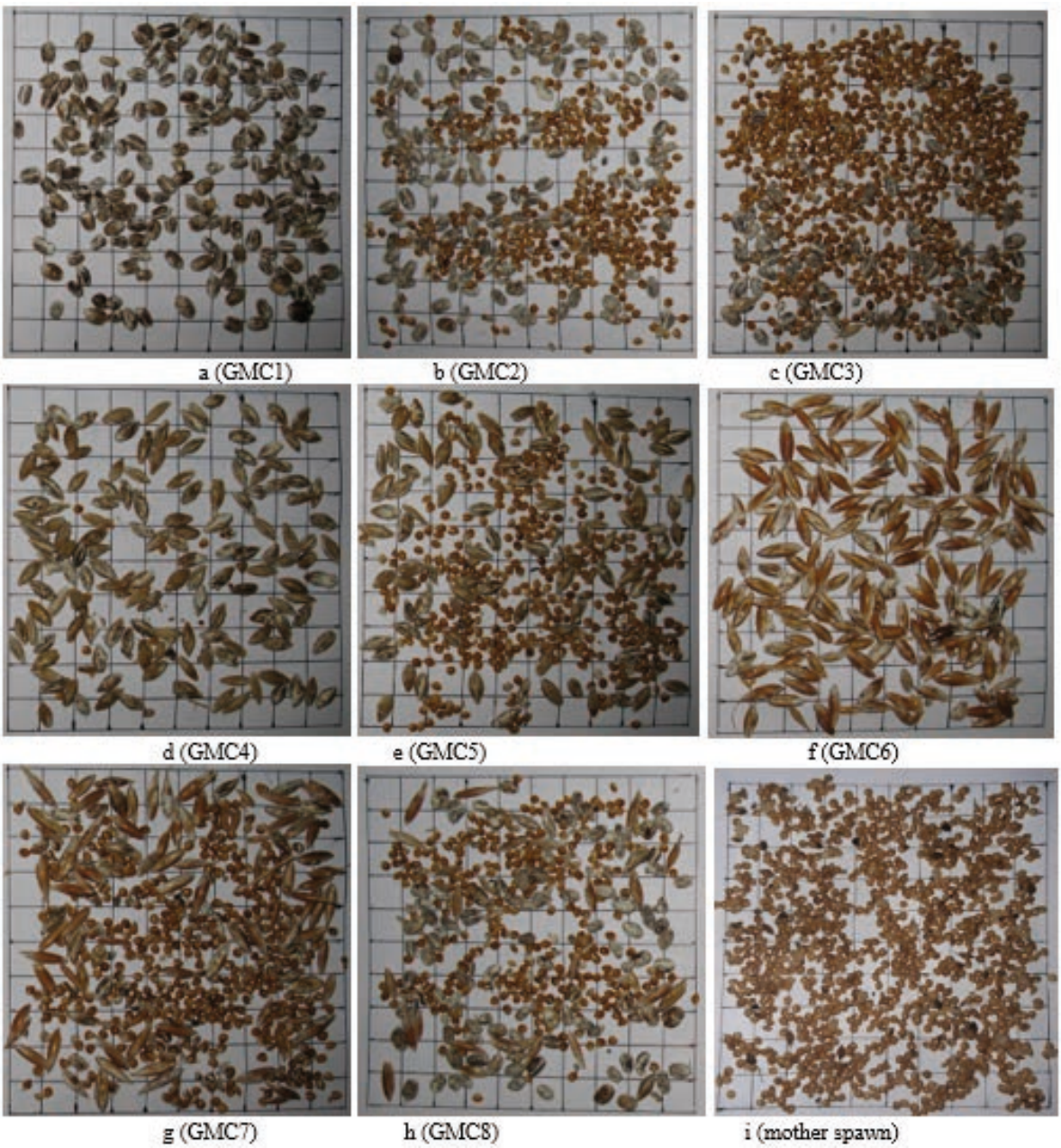


**Figure 1**: Appearance of the grains (10 g) in the eight different compositions of spawn materials spread on 100 $cm^2$ area: a) Wheat (1:0); b) Wheat-Millet (2:1); c) Wheat-Millet (1:2); d) Barley (1:0); e) Barley-Millet (2:1); f) Oat (1:0); g) Oat-Millet (2:1); h) Millet-Wheat-Oat (1:1:1); i) Millet-Rape-Flax (95:4:1)

of 102 ± 1 °C. The ash content was determined by the method of dry ashing at a temperature of 600 °C for 3 hours (Melent'eva et al., 2005).

To determine pH, 10 g of sample grain, spawn and substrate was added to 50 ml distilled and deionized water in a flask containing a magnetic rod. The flask was placed on a magnetic stirrer for 15 minutes, after which it was allowed to settle for 10 min and the supernatant was filtered into a new tube. The solution obtained was used for pH determination with pH meter (150 ME, LTD «Izmeritel`naya tekhnika», Russia) following the manufacturer's instructions.

The total nitrogen content was determined by the Kjeldahl method using the chloramine Pochynok's method. The C/N ratio was determined by the formula C/N = 0.52 × (100-a) / N, where *a* is the ash content (%); 0.52 is a coefficient of carbohydrate content, adjusted for biochemical characteristics of raw materials; N is the content of total nitrogen in the substrate (Zenova et al., 2002). The theoretical amount of total nitrogen in the substrate (*Ns*) after application of the spawn was calculated by the formula:

*Ns = amount of nitrogen in the substrate × (1 - spawn application coefficient) + amount of nitrogen in spawn × spawn application coefficient (1).*

The date of pinhead formation and the date of harvest at fruit bodies' maturity for each bags of substrate were recorded. The mass of mushrooms harvested from individual substrate bags was recorded. Biological efficiency (BE) was calculated by the ratio of the fruiting bodies' mass, collected during the first fruiting flush to the mass of dry solids in the substrate.

*BE (%) = mass FB /dry mass of substrate × 100% (2).*

Statistical analysis of data was performed using Microsoft Office Excel 2016 (license № HXV8M-8YJJ4-BCGR3-MRYX-8747Q) with QI Macros 2020 software and information complex "Agrostat New" (2013) (Ushkarenko et al, 2013).

## 3 RESULTS AND DISCUSSION

### 3.1 SPAWN

The analysis of mother spawn made from millet, rape and flax seed gave values of humidity 44,4 ± 0,83 %; total nitrogen 1.46 ± 0.08 %, ash 2.04 ± 0.12 %, C/N ratio 34.9 ± 0.75 / 1; the number of grains in 10 g was 980 ± 7 pieces, visual appearance is shown in Fig. 1-i. The physical appearance of the eight test (a-h) and mother (i) spawn compositions are shown in Figure 1.

Samples from each of the eight spawn compositions (GMC1-8) were taken twice: the first time after sterilization and the second, from matured spawn. They were analyzed for humidity, pH and the number of grains, indicated significant differences in the parameters measured. Data obtained from the analysis are shown in Table 2.

Statistical analyses indicate significant differences in moisture content, pH and the number of grains in 10 g of the spawn compositions tested. (Table 1). The lowest humidity was registered in GMC3 (40 %) and the highest in GMC6 (about 57 %). The pH in GMC1 was significantly different from the rest, despite the numerical differences in values recorded. The GMC3 gave the highest number

**Table 2**: Characteristics of grain spawn for growing *P. ostreatus* (average ± standard error $s_e$)

| | Grain material composition (GMC) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Indicators | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | $LSD_{05}$ |
| Moisture content (%) | $48.4^{b} \pm 0.05$ | $43.4^{bc} \pm 0.29$ | $40.0^{c} \pm 0.26$ | $45.7^{b} \pm 0.21$ | $45.2^{b} \pm 0.63$ | $57.2^{a} \pm 0.35$ | $51.2^{b} \pm 0.19$ | $43.8^{bc} \pm 1.05$ | 1.47 |
| pH | $6.75^{a} \pm 0.02$ | $6.03^{c} \pm 0.01$ | $6.35^{bc} \pm 0.01$ | $6.10^{c} \pm 0.01$ | $6.41^{b} \pm 0.02$ | $5.89^{c} \pm 0.03$ | $6.02^{c} \pm 0.02$ | $6.15^{c} \pm 0.01$ | 0.10 |
| Total N content (%) | 1.76 ± 0.02 | 1.77 ± 0.02 | 1.53 ± 0.03 | 1.98 ± 0.23 | 1.81 ± 0.02 | 1.87 ± 0.03 | 1.83 ± 0.02 | 1.69 ± 0.05 | 0.27 |
| Ash content (%) | 2.64 ± 0.02 | 3.26 ± 0.07 | 3.38 ± 0.18 | 4.04 ± 0.15 | 3.60 ± 0.26 | 4.96 ± 0.20 | 4.30 ± 0.07 | 3.99 ± 0.08 | 0.48 |
| *C/N ratio | 28.8 | 28.4 | 32.9 | 25.8 | 27.7 | 26.4 | 27.2 | 29.6 | 3.29 |
| #Grains in 10 g GMC | $189 \pm 3^{c}$ | $464 \pm 16^{b}$ | $712 \pm 33^{a}$ | $159 \pm 2^{c}$ | $395 \pm 13^{b}$ | $192 \pm 4^{c}$ | $481 \pm 10^{b}$ | $441 \pm 20^{b}$ | 56 |

*C/N = carbon : nitrogen ratio; N = Nitrogen; C = Carbon, GMC = Grain material composition

and was significantly different from the rest due to the size and abundance of millet. In fact, the results show that the GMCs can be grouped into three categories: Group a is represented by GMC3 (millet:wheat = 2:1), which had 712 ±33 grains. Group b is represented by GMC 2, 5, 7 and 8 where the number of grains ranged between 395 ±-13 and 481±10. Group ***c*** is represented by GMC1 and 6 where the number of grains ranged between 189 ±3 and 192 ± 4.

The highest total N was registered in GMC 4, the least was in GMC 3; apart from GMC3, the total N content in the rest of the GMCs were not significantly different. There was no statistical difference in the ash content and C/N ratio for all GMCs tested.

## 3.2 SUBSTRATE

The substrate used was produced by aerobic fermentation and the pasteurization method that is standard in oyster mushroom cultivation in Ukraine. [30]. After fermentation and pasteurization, the substrates during each repeated test were thoroughly mixed, bagged and analyzed for consistency before use in the cultivation. Analysis of the substrates gave results of humidity ranging from 68.5 ± 0.34 to 73.9 ± 0.12; pH from 7.95 ± 0.05 to 8.14 ± 0.07; ash content from 5.55 ± 0.23 to 7.06 ± 0.18 and C/N ratio from 59.0 ± 1.06 to 68.2 ± 1.84. A *t*-test comparison of total nitrogen (%) in the substrate indicated some differences, but did not result in significant difference in the N content among the substrates inoculated with the different GMCs and tested after two days of incubation (Table 3).

## 3.3 CLIMATIC CONDITIONS IN FRUITING ROOM

There were no statistical differences in the temperature, humidity and carbon dioxide parameters in both GC1 and GC2. However, the daily fluctuations in temperature (± 5.5 °C), humidity (± 7.7 %;) and carbon dioxide (± 155 ppm) in GC1 during fruiting were very high compared to GC2. The details of values obtained from three growing cycles for the parameters are shown in Table 4

## 3.4 EFFECT DIFFERENT GMCS ON TIME TO ATTAIN TOTAL SUBSTRATE COLONIZATION AND FIRST FLUSH

There was no significant difference in the number of days (8 ± 1 day) to achieve total substrate colonization in the different GMCs (spawn) tested. Among all the spawn types tested, GMC 7 significantly prolonged the time to

**Table 3**: Analysis of total nitrogen content in grains, spawn and substrates for the cultivation of *P. ostreatus* (average ± standard error $s_e$)

| Grain material composition (GMC) | Total nitrogen, % | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mycelium (means with SE) | | | Substrate (means with SE) | | | Theoretical calculated with average data |
| | after sterilization (A) | In the ready spawn* (B) | Reach (B–A)* | before inoculation (C) | after inoculation (D) | Reach (D – C)** | |
| 1 | $1.15^{c} \pm 0.03$ | $1.76^{abc} \pm 0.02$ | 0.61 | $0.71^{c} \pm 0.02$ | 0.8 ± 0.03 | 0.09 | 0.76 |
| 2 | $1.14^{c} \pm 0.04$ | $1.77^{abc} \pm 0.02$ | 0.63 | $0.79^{ab} \pm 0.02$ | 0.87 ± 0.04 | 0.08 | 0.83 |
| 3 | $1.13^{c} \pm 0.05$ | $1.53^{c} \pm 0.03$ | 0.40 | $0.83^{a} \pm 0.01$ | 0.9 ± 0.02 | 0.07 | 0.86 |
| 4 | $1.58^{a} \pm 0.03$ | $1.98^{a} \pm 0.23$ | 0.40 | $0.75^{bc} \pm 0.03$ | 0.85 ± 0.01 | 0.10 | 0.81 |
| 5 | $1.43^{b} \pm 0.04$ | $1.81^{ab} \pm 0.02$ | 0.38 | $0.72^{bc} \pm 0,01$ | 0.82 ± 0.01 | 0.10 | 0.77 |
| 6 | $1.48^{ab} \pm 0.04$ | $1.87^{ab} \pm 0.03$ | 0.39 | $0.74^{bc} \pm 0.01$ | 0.83 ± 0.04 | 0.09 | 0.79 |
| 7 | $1.36^{b} \pm 0.03$ | $1.83^{ab} \pm 0.05$ | 0.47 | $0.78^{ab} \pm 0.02$ | 0.85 ± 0.01 | 0.07 | 0.83 |
| 8 | $1.25^{bc} \pm 0.03$ | $1.69^{bc} \pm 0.05$ | 0.44 | $0.77^{abc} \pm 0.05$ | 0.85 ± 0.02 | 0.08 | 0.81 |
| LSD | 0.12 | 0.26 | | 0.07 | 0.15 | | |
| p | 0.000 | 0.069 | | 0.051 | 0.52 | | |

*(B-A) = Change GMCs N content before and after incubation
**(D-C) = Change substrate N content before and after incubation

**Table 4**: Technical parameters of microclimatic growing conditions (GC) (average ± standard error $s_e$)

| Microclimatic conditions | Cultivation stage | Growing chamber | |
|---|---|---|---|
| | | GC 1 | GC 2 |
| Temperature (°C) | Incubation | 19.1 ± 6.0 | 22.0 ± 1.3 |
| | Fruiting | 18.7 ± 5.5 | 18.2 ± 0.3 |
| Humidity (%) | Incubation | 74.3 ± 7.1 | 78.7 ± 2.5 |
| | Fruiting | 92.1 ± 7.7 | 85.2 ± 3.4 |
| $CO_2$ content (ppm) | Incubation | 2039 ± 65 | 1245 ± 34 |
| | Fruiting | 961 ± 155 | 1222 ± 19 |

first flush harvest, 45 in GC1 and 34 days in GC2. There was no significate difference in the time to first harvest in all GMC´s tests under GC1 condition. It was the same observation under GC2 except GMC7. GMC5 and GMC6 were the fastest to attain total substrate colonization and first harvest. GMC7 inoculated substrate took the longest time to reach the harvesting stage, a similar trend was observed for time to attain total colonization in GC1. Duncan's test for comparison of averages indicated no significant difference ($p > 0.05$) in the number of days to reach first flush for a substrate inoculated with the same test GMC under GC1 or GC2, except in two treatments (GMC 6 and 7). Statistical analysis indicated 46 % interaction effect between spawn type (factor A) and microclimatic conditions (factor B) on the time to first harvest. However, the individual factor A and B had 10.5 % and 31.1 %, respectively, effect on time to first harvest. A statistical *t*-test comparison of means from cultivation under the two microclimatic conditions (GC1 and 2) indicated that the longest period to attain first flush was recorded in substrates inoculated with spawn made from a mixture of millet and oat, GMC7 (1:2) (Fig. 2), where it exceeded 45 days in GC1 and 34 days in GC2.

### 3.5 EFFECT OF GMCS AND MICROCLIMATIC CONDITIONS ON BIOLOGICAL EFFICIENCY

Under the controlled microclimate condition GC2, there was higher fruit body yield and BE compared to GC1 regardless of the GMC composition used (Fig. 3).

The highest BE (57 %) was obtained under GC2, when GMC6 was the spawn used, which was not significantly different from yield obtained in GMC5 and 8. The least was GMC7 (20 %). There was a slight difference in the GC1, because the highest BE was in GMC8 (41.1 %) and the least was in GMC7 (20 %). It should be noted that the grain mixtures used in GMC5 and 8 also gave higher yields even in unregulated microclimate conditions (GC1). The BE indicators for GMC1 (control), GMC3 (wheat-millet (1:2), and 7 (oat-millet (2:1) were lower and did not differ significantly under GC1 and 2 microclimate conditions.

The current global expansion of small-scale production of edible and medicinal mushrooms has necessitated the need to study, and optimize, all the factors that in-

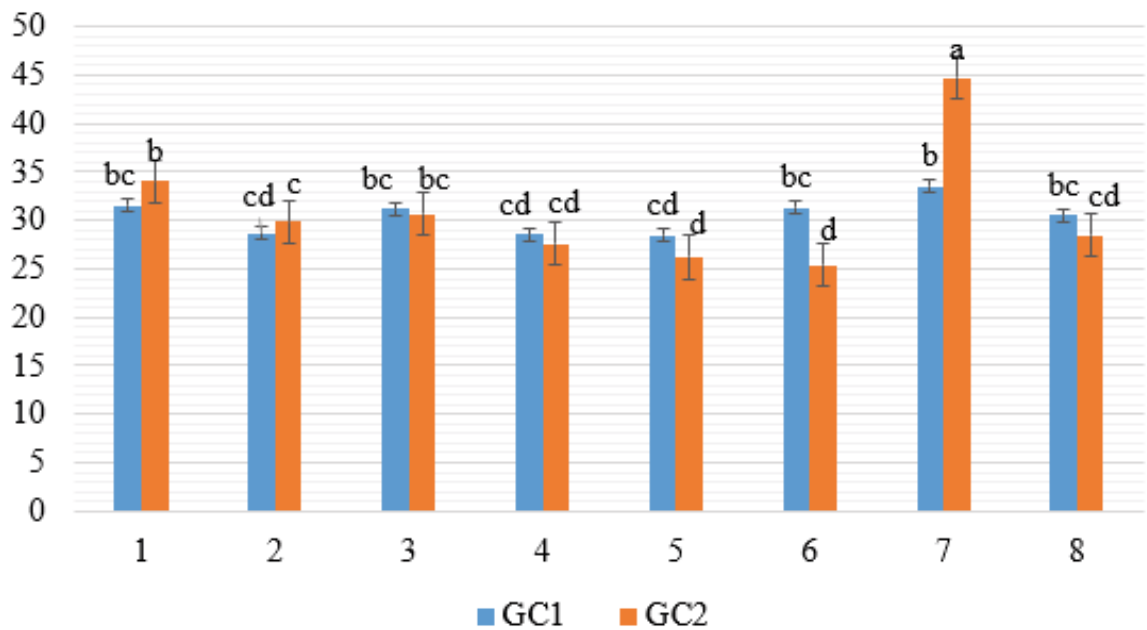


**Fig. 2**: Number of days to first harvest *P. ostreatus* in different GMCs under cultivation in unregulated (GC1) and regulated (GC2) microclimatic conditions

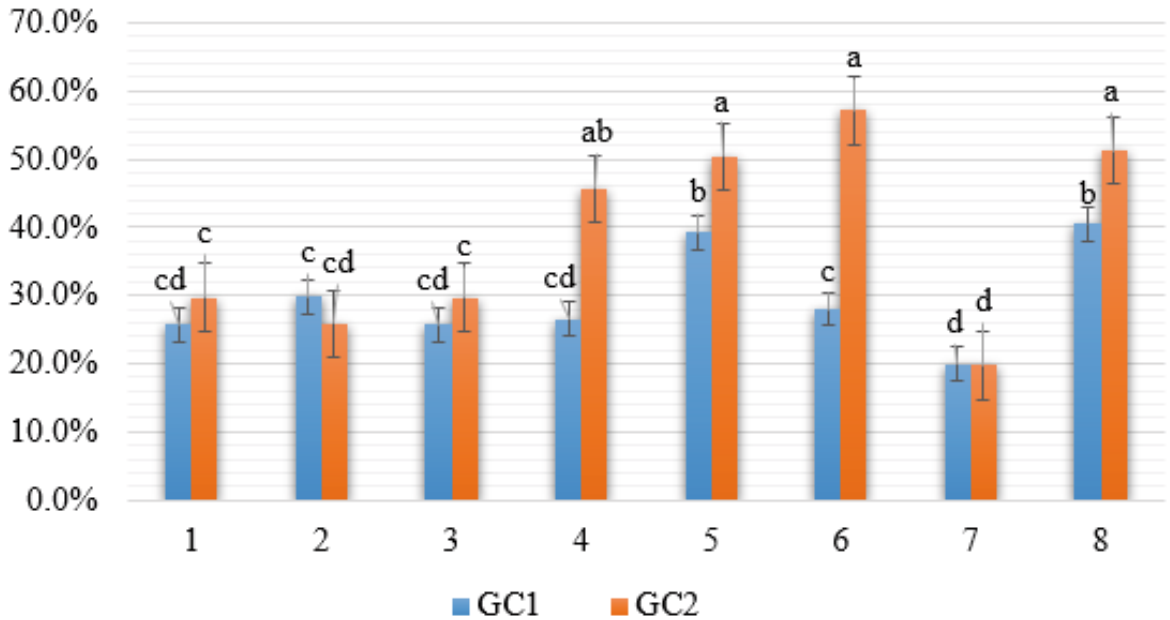


**Fig. 3**: Biological efficiency (%) in *P. ostreatus* with test spawn GMC1-8 during cultivation under unregulated (GC1) and regulated (GC2) microclimatic conditions

fluence efficient and profitable mushroom production, including the influence of spawn composition on yield outputs (Evdokimova et al., 2002). Although cereals are commonly used as spawn materials, their availability, nutritional content, and prices can affect the spawn quality and cost. In one report, grains were replaced with wood waste and obtained results that indicated significant reduction in the cost of spawn, although the incubation period was prolonged (Sofi et al., 2014). Other researchers used sorghum grains and found them to be suitable and cost effective for mushroom spawn (Jiskani et al., 2007; Stanley & Awi-Waadu, 2010; Willis et al., 2012). In Europe and America, highly effective additives (cereals bran, beer pellets, organic metals salts, etc.) have been added to wheat and barley to improve spawn quality (Jhune et al., 2000; Rosado et al., 2002; Mamiro & Royse, 2008; Gregori et al., 2008; Krupodorova & Barshteyn, 2015). Subramanian et al. (2014) tested different grains as spawn material and found oat and barley gave the best BE results during the cultivation of *P.ostreatus.*

It is also known that the type of soil and climatic conditions under which grains are grown can affect their physicochemical properties (Dubovik, 2007; Petrova, 2010; Gy`rka et al., 2015). However, the cooking process is critical for obtaining optimum water content in different grain materials, which allow rapid mycelia colonization and individual grain penetration during incubation. The sequence of cooking times during GMCs preparation was carefully calculated and followed in this experiment to achieve what was the best water content of grains in the test-spawn materials (wheat, millet, barley and oat). The significant differences in water content, pH and the number of grains in 10 g of the spawn compositions tested (Table 2) is due to the inherent nature of the grain materials, especially their water-holding capacity. Only the pH in GMC1 was significantly different from the rest, despite the numerical differences in values recorded for the others; we believe that this is entirely due to the inherent properties of wheat compared to the properties of the other grains tested. The grain material composition (GMC3) gave the highest number (712 ± 33) of inoculation points and was significantly different from the rest. It is obvious that this composition has the highest amount of millet, which had the least size and mass among the grains used. Millet's physical dimension (Table 1) vis-à-vis its presence and abundance in the GMCs is responsible for the group a, b and c of the GMCs tested. However, its presence did not seem to improve spawn quality.

The highest total N was registered in GMC4 (1.98 %), the least was in GMC3 (1.53 %); apart from GMC3, the total N content in the rest of the GMCs was not significantly different ($p = 0.051$). There were significant differences ($p > 0.001$) in the moisture content of grains used due to the grain material, prevailing climatic and soil conditions where the grain was cultivated, and the grain's postharvest processing (Horshchar V. & Horshchar O., 2011; Kaminskyi & Hliieva, 2015; Petrenko, 2017). However, the differences recorded did not seem to be critical

in the quality of the spawn produced because they did not affect yield. This is not surprising because prior to use, the grains were cooked and became hydrated to optimum grain water content for mycelia colonization and penetration.

There were significant differences in the pH of the grain materials, but that factor also did not affect the quality of the spawn. It is known that basidiomycetes, especially white rot fungi, are capable of adjusting the pH of its growth medium to its optimum growth condition (Bisko & Dudka, 1987; Bukhalo et al., 2011; Truhonovec et al., 2013). Therefore, the differences in the pH could be readily adjusted to optimum condition by *P. ostreatus* used in spawn preparation. The number of grains presented very interesting results and can be grouped into three significant categories, a, b and c. It appears that there was no correlation between the number of grains and the yield, especially in GC2, where the correlation coefficient of the number of grains with the BE from the substrates inoculated with different GMCs ranged from 0.37 – 0.42. This was a rather surprising finding, because it does not align with reports in literature. Many data reported indicate that the number of grains in spawn can affect their abundance and distribution in substrate and colonization rate, earliness to fruit body initiation and mushroom yield (Smetanina, 2013; Khonga et al., 2013, Subramanian et al., 2014). Though it is correct that the number of inoculation points are different, in this study as high as 712 in GMC3 and as low as 159 in GMC4, we did not observe that it was a critical factor that affected colonization rate and BE.

The different GMC tested gave different times to reach first harvest and BE from the cultivation studies (Figures 2 and 3). It was interesting to find that wheat, used universally for spawn production, appeared to not perform as well as other grains (except millet, in some cases). The nutritional content of wheat is not significantly different from that of other grains. However, it is possible the cover coat on millet, barley and oat may be responsible. During the inoculation of substrate in industrial production of oyster mushrooms, the mechanical process used to disentangle the compact spawn, for even distribution in the substrate, results in a grinding effect, which removes mycelia from the surfaces of the individual wheat grain in the spawn. This causes a delay in mycelia regeneration and initiation of substrate colonization. The seed coats that other grains carry protect the mycelia against that grinding effect, which could be responsible for the better performance observed in the colonization rate in the GMCs containing wheat and other grains.

Millet performed best when it was in combination with barley. It was not able to reverse the lag in the time of colonization when in combination with wheat (GMC1, 2 and 3) and it even performed worse when in combination with oat, as in the case of GMC7. However, when used in equal combinations, millet, wheat and oat produced colonization results that were not significantly different from the other spawn tested in this experiment.

The BE data (Fig. 3) indicate that the spawn made from grain mixtures of barley and millet (2:1) and millet, wheat and oat (1:1:1) are promising for improving the efficiency of industrial production of *P. ostreatus*. Oat's use as spawn carrier may be appropriate only for cultivation done in controlled microclimatic conditions, as in GC2.

The substrates inoculated with GMC1-3 had BE that were inferior to those without wheat, except for GMC8. It is possible the delayed effect on colonization is transient and resulted in the observed depressed BE, which were statistically significant in some cases (Figure 3). The substrates that contained oat, barley or millet (except GMC7) had better results, which were significantly different from others, especially under GC2 conditions. The BE in the other grains' composition was not significantly different from all the GMC containing wheat under GC1. The combination of millet and oat (GMC7) performed the worst among all spawn combinations tested; at this time, we have no explanation for why this is the case.

GMC8, which is a 1 : 1 : 1 ratio combination of millet, wheat and oat, showed BE results comparable to GMC 4-6, which were the best spawn and did not contain wheat. It appears that the combination of grains in GMC8 overcame the limiting effect that other wheat-containing GMCs had on colonization and BE. It was reported that different types of grains have different chemical contents that could affect their colonization by mycelia (Sainos et al., 2006). The combination of the millet, wheat and oat may have presented a suite of biochemical compounds that elicit different substrate degradation enzymes in *P. ostreatus* mycelia and made GMC8 to become the best among those tested. Furthermore, it is possible that the presence of millet and oat, which have seed coats, was sufficient to eliminate the mechanical damages of mycelia on wheat grains in GMC8 spawn composition. However, further research is needed to understand the biochemical composition and the interplay between the enzymes they induce for degradation and utilization. Microclimatic conditions are known to play key roles in determining mushroom production and biological efficiency (Dudka et al., 1978; Chang & Hayes, 2013; OECD, 2015; Bellettini, et al., 2019). Therefore, the influence of temperature, humidity, air composition and lighting is a constant topic of research, because as the range of cultivated mushrooms expands, the number of strains requiring different optimal microclimatic conditions increases, too. In our study, the microclimatic con-

ditions in GC1 and GC2 were similar (Table 4) but the fluctuations in the parameters of temperature, humidity and CO2 were enough to cause significant changes in the yield and BE. Furthermore, it is also evident that the GMC that was optimum for the two climatic conditions were different: GMC5 and 8 For GC1 and GMC6 and 8 for GC2. The fact that GMC8 the optimum spawn for GC1 and 2 makes it the prime combination for the mass production of spawn that could be used under unregulated (GC1) and regulated (GC2) cultivation conditions. This is important because there are currently many small mushroom production facilities across Europe and in many developing countries that do not have sophisticated growing chambers.

## 4 CONCLUSIONS

These experiments tested the suitability of different grain materials for spawn production. Results indicated that the use of wheat alone, or in combination with millet, is not the best among the tested grains in terms of time to first harvest and BE. Barley, oat and their combinations performed equally well as spawn materials for BE. It was obvious that the grow room condition, whether regulated or unregulated, had significant effect on BE and preference for spawn composition. Higher BE were obtained under regulated cultivation conditions for most spawn types tested. However, the combination that stood out the best was that of wheat, millet and oat (GMC8) because it out-performed all other substrate combinations under regulated and unregulated conditions. Furthermore, it will be more cost efficient to use GMC8 because it could represent significant savings on spawn materials and thereby lower the price of spawn in the mushrooms industry.

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# Assessing the impact of varietal resistance and planting dates on pest spectrum in chickpea

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**Assessing the impact of varietal resistance and planting dates on pest spectrum in chickpea**

**Abstract:** The cotton bollworm *Helicoverpa armigera* [Hübner (1808)] is one of the most widely spread pest which limits the chickpea production, while the beet armyworm, *Spodoptera exigua* (Hübner, 1808) has emerged as a serious pest in recent years, in southern India and parasitic wasp *Campoletis chlorideae* Uchida, 1968 is an important larval parasitoid which naturally manages both pests under field condition. Insecticides adoption leads to development of resistance in pod borer. In view of climate change scenario, the focus of the present studies was the identification of climate resilient cultivars of chickpea for pod borers and the results reveled, that there were significant variations in the level of eggs and larval population among the genotypes. Across seasons, the crop sown in October recorded the maximum number of eggs. 'ICC 3137' had the highest number of *H. armigera* eggs (11.6) across seasons. 'JG 11', (6.3) in 2012 and' ICCV 10' (3.6) in 2013 recorded the lowest number of *H. armigera* eggs. During 2014-15, the maximum(80.7) *H. armigera* larval incidence was observed in October sown crop and the lowest (21.1) in January crop. The number of *S. exigua* larvae were substantially higher in the December crop. For all seasons, the highest number of *C. chlorideae* were found in October crop. Across seasons, multiple regression analysis for both pest had a strong interaction with weather patterns.

**Key words:** chickpea; pod borer; *Helicoverpa armigera*; *Spodoptera exigua*; *Campoletis chlorideae*

**Ocenjevanje vpliva odpornosti sorte in datumov setve na pojav škodljivcev na čičeriki**

**Izvleček:** Južna plodovrtka (Helicoverpa armigera [Hübner (1808]) je škodljivec, ki že dolgo najbolj omejuje pridelek čičerike, medtem, ko sovka Spodoptera exigua (Hübner [1808]) postaja pomemben škpodljivec v južni Indiji v zadnjih letih. Parazitska osica Campole-tis chlorideae Uchida 1968 je pomemben parazitoid gosenic obeh vrst za uravnavanje njunih populacij v poljskih razmerah, pred--vsem zato, ker uporaba insekticidov vodi k odpornosti škodljivcev. Glede na scenarij bodočih podnebnih sprememb je prepozna-vanje odpornih sort čičerike na škodljivca zelo pomembno in je predmet te raziskave. Ugotovljene so bile značilne razlike v številu jajčec in gosenic med genotipi. Glede na rastno dobo je imel posevek, sejan oktobra, največ jajčec, z največjim številom (11,6) na genotipu ICC 3137. Genotip JG 11 (6,3) v letu 2012 in ICCV 10 (3,6) v letu 2013 sta imela najmanjše število jajčec južne plodovrtke. V obdobju 2014-15 je bilo največ gosenic (80,7) pri oktobrski setvi in najmanjše (21,1) pri setvi januarja. Gosenic vrste S. exigua je bilo znatno več pri setvi v decembru. V vseh obdobjih opazovanja je bilo največje število parazitoidov C. chlori--deae pri setvi v oktobru. V vseh preučevanih obdobjih je analiza multiple regresije za oba škodljivca pokazala močan vpliv vre-mena.

**Ključne besede:** čičerka; plodovrtka; *Helicoverpa armigera; Spodoptera exigua; Campoletis chlorideae*

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## 1 INTRODUCTION

The increasing human population and food demands are placing unprecedented pressure on agriculture and natural resources. Safeguarding crop productivity by protecting crops from damage by insect pests, pathogens and weeds is a major pre-requisite to ensure food and nutritional security and conserve the natural resources (Bohinc et al.,. 2019). Chickpea (*Cicer arietinum* L.) is one of the most important grain legume crops in Asia and parts of East and North America, Mediterranean Europe, Australia, Canada and USA (Kelly et al., 2000). Chickpea is the most predominant crop in India, accounting for 40 % share of the total pulse production, followed by pigeon pea *Cajanus cajan* (L.) Millsp. (18-20 %), mungbean, *Vigna radiata* (L.) Wilczek (11 %), urdbean, *Vigna mungo* (L.) Hepper (10-12 %), lentils, *Lens culinaris* Medik. (8-9 %) and other legumes (20 %) (Anonymous, 2011, Jaba et al., 2021). Currently chickpea is grown around the globe on over 17.81 million hectares with a production of 17.19 million tonnes of which Asia accounts for 77 % of the total world production (FAOSTAT, 2018). In India, the area under chickpea production during 2017-18 was about 10.6 million ha with a production of 11.1 million tonnes (Anonymous, 2018). There is a steady decline in the area, production, and productivity of chickpea (Babu et al., 2018). More than 200 species of insects live and feed on chickpea. Most of the pests have a sporadic or restricted distribution or are seldom present at high densities to cause economic losses. On the other hand, some of them can be devastating to these crops. The cotton bollworm (*Helicoverpa armigera* [Hübner, 1808] is one of the most dominant insect pests in agriculture, accounting for half of the total insecticides usage in India for protection of crops. The beet armyworm (*Spodoptera exigua* (Hübner, 1808)) is an emerging serious pest of chickpea, especially in southern India. The young larvae of *S. exigua* initially feed gregariously on chickpea foliage. As the larvae mature, they become solitary and continue to eat, producing large, irregular holes on the foliage (Ahmed et al., 1990; Sharma et al., 2007). Being leaf feeder, the beet armyworm consumes much more chickpea tissues than the cotton bollworm, *H. armigera*, but it has not been reported as being serious pest on pods. In view of their economic importance in agriculture, strategies for integrated management of these pests have been suggested (Lal et al., 1986; Pimbert, 1990; Wightman et al., 1995). However, development of an effective management programme depends much on the reliable estimate of field population densities which can be achieved through developing suitable sampling plans based on the distribution pattern of the pest within a field (Southwood, 1978; Taylor, 1984). The pod borer could be managed to some extent naturally under field conditions by larval parasitoid *Campoletis chlorideae* Uchida, 1957 (Hymenoptera: Ichneumonidae) in chickpea ecosystem. It causes up to 78 % parasitisation of early instars under natural conditions (Agnihotri et al., 2011). However, activity of the parasitoid occurs only during November to March, coinciding with the vegetative stage of the crop and winter season.

The indiscriminate use of chemical insecticides to control these insect pests leads to resistance in insect, secondary pest outbreaks, threat to their natural enemies and residual effect on environment. To overcome above threats some workers have advocated adopting the agronomical practices like altering the date of sowing, which might be a possible resort to protect chickpea crop from this pest (Summerfield, 1990; Singh et al., 2002). Several researchers have studied the effect of different dates of sowing and the seasonal abundance of cotton bollworm with the corresponding yield of chickpea in different parts of India. It is learnt from the past studies that the sowing date has a great impact on the incidence of the pest which may be attributed to the difference in weather conditions (Deka et al., 1989; Yadava et al., 1991; Cumming and Jenkins, 2011). Early planted crops harbored less pest population corresponding to high yield than the late sown crops (Chaudhary and Sachan, 1995; Ambulkar et al., 2011; Prasad et al., 2012). Limited work was carried out on this subject and the information available at present is very scanty. Therefore, the present study was carried out to evaluate the effect of different dates of sowing and weather parameters on the incidence of *H. armigera, S. exigua* and *C. chlorideae* populations in chickpea under field conditions.

## 2 MATERIALS AND METHODS

The experiments were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India (latitude 17°27'N, longitude 78°28'E, and altitude 545 m above mean sea level), during the post-rainy seasons of 2012-15 (October to January). The test entries were planted in deep black soils (Vertisols) during the post rainy/ *Rabi* season at monthly intervals.

We monitored the incidence of legume pod borer/ cotton bollworm, *H. armigera,* beet armyworm, *S. exigua* and parasitic wasp, *C. chlorideae* on five chickpea genotypes (ICCL 86111 and ICCV 10 – resistant, and JG 11 and KAK 2 – commercial checks, and ICC 3137 – susceptible check) sown at monthly intervals between October to January during *Rabi* season for three years. These genotypes were categorized as resistant and sus-

*Helicoverpa armigera* eggs

*Helicoverpa armigera* larva

*Spodoptera exigua* eggs

*Spodoptera exigua* larvae

Cocoon of larval parasitoid, *Campoletis chloridaeae*

**Plate 1:** Insect pests complex in chickpea ecosystem @Source: ICRISAT

ceptible based on the number of *H. armigera* larvae, eggs, leaf damage rating and the number of *C. chlorideae* cocoons (Shankar et al., 2014). In each sowing window, the experiment was laid out in randomized block design (RBD) with three replications for each genotype, in a plot of four rows with a spacing of 30 cm between rows and 10 cm between plants within a row. The plots were separated by an alley of 1 m. The seeds were sown with a 4-cone planter at a depth of 5 cm below the soil surface at optimum soil moisture conditions. The seedlings were thinned to a spacing of 30 cm between the plants within a row after 15 days of seedling emergence. Basal fertilizer (N : P : K : = 100 : 60 : 40) was applied in rows before sowing. Top dressing with urea (80 kg $ha^{-1}$) was done at one month after crop emergence. Intercultural/weeding operations were carried out as and when needed. There was no insecticide application in the experimental plot.

The observations were recorded at 15 days after germination (DAG) for each sowing, on number of eggs/egg masses of *H. armigera* and *S. exigua* respectively, larvae of both pests and larval parasitoid *C. chlorideae* cocoons on five randomly selected plants at fortnightly intervals (Plate 1). Weather data during the experimental period was obtained from the agro meteorology station at ICRISAT farm. The correlation analysis of the weather parameters viz., maximum, and minimum temperature, morning and evening relative humidity and rainfall with the eggs and larval population of *H. armigera*, *S. exigua* and *C. chlorideae* cocoons across sowings was carried out using GenStat 14$^{th}$ edition. The data on insect population (eggs and larvae) was analyzed using square root transformation ($\sqrt{}$ x+0.5) in RBD as described by Panse & Shukhatme (1985), while yield data were recorded from the all plots after harvest and converted to grain yield (kg $ha^{-1}$).

## 3 RESULTS

### 3.1 OVIPOSITION PREFERENCE OF *H. ARMIGERA* FEMALES ON DIFFERENT GENOTYPES OF CHICKPEA ACROSS SOWINGS

There were huge contrasts in the numbers of *H. armigera* eggs across various dates of planting as over the seasons as appeared in Table 1. The egg laying diminished with planting dates till December (26.3–2.7 in 2012-13; 17.0–1.0 in 2013-14; 36.33–2.33 in 2014-2015 and 26.5–3.8 across three seasons), with a slight increase in January (8.0 in 2012 13; 7.3 in 2013-2014; 6.3 in 2014-2015 and 6.2 across three seasons). Higher numbers of eggs were recorded in 2012-13 contrasted with 2013-14 and 2014-15. Most noteworthy numbers of eggs were seen in the crop sown in October, across seasons.

There were no significant differences in number of *H. armigera* eggs during 2012-13 in all the chickpea genotypes, yet critical significant differences were observed in 2013-14 and 2014-15. Among the genotypes tested, 'ICC 3137' had the maximum number of eggs (11.63) across all seasons followed by '8.03' in 'KAK 2'. The lowest number of eggs were recorded on 'JG 11 (6.3)' in 2012-13, 'ICCV 10 (3.6)' in 2013-14 and 5.66 on 'ICCV 10' and 'ICCL 86111' during 2014-15. Across seasons, 'ICC 3137' was generally favored for egg laying (11.64) followed by 'KAK 2 (8.03)', 'ICCV 10' and 'JG 11 (5.8 and 6.0)' were relatively non-preferred for egg laying.

### 3.2 POPULATION OF *H. ARMIGERA LARVAE* ON DIFFERENT GENOTYPES OF CHICKPEA ACROSS SOWINGS

Significant differences were observed in *H. armigera* larval incidence across sowing dates across seasons (Table 2). It was highest in October sown crop (80.7) while lowest in the December sown crop (20.1) during 2012-13. During 2013-14, the incidence of *H. armigera* was higher in the crop sown during November (40.7) and it was maximum in October sown crop (56.86). But lower incidence of *H. armigera* larvae was recorded in January sown crop (21.1) during 2014-15. Across seasons, the occurrence of *H. armigera* declined from October (58.9) to December (22.4) and increased (38.0) in the January sown crop.

There were significant differences in the incidence of *H. armigera* larvae in all genotypes across all seasons. The highest number of *H. armigera* larvae were recorded on 'ICC 3137' (55.2) which was on par with 'KAK 2' (39.9). The lowest number of *H. armigera* larvae were recorded on 'ICCV 10' (28.2) followed by 'ICCL 86111' (29.5).

### 3.3 EGG LAYING BY *S. EXIGUA* ON DIFFERENT GENOTYPES OF CHICKPEA ACROSS SOWING DATES

There were no significant differences in the number of *S. exigua* egg masses across sowings in 2012-13 cropping season (Table 3). No egg masses were seen in the October sown crop across all the seasons except in 'KAK 2' during 2013-14 (5.0). The highest egg laying was recorded in December sown crop during 2013-14 (3.00) and 2014-15 (1.33) on 'ICCL 86111'. The number of egg

**Table 1**: Evaluation of different chickpea genotypes for resistance to *H. armigera* egg laying at different sowing dates

| | *Helicoverpa armigera* eggs (2012-2013) | | | | | *Helicoverpa armigera* eggs (2013-2014) | | | | | *Helicoverpa armigera* eggs (2014-15) | | | | | *Helicoverpa armigera* eggs (Pooled) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | |
| Genotype | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean |
| ICC 3137 | 26.3 | 7.0 | 5.7 | 6.0 | 11.3 | 17.0 | 4.7 | 4.3 | 7.3 | 8.3 | 36.33 | 15.0 | 4.66 | 5.3 | 15.33 | 26.57 | 8.9 | 4.88 | 6.2 | 11.63 |
| | (10.0) | (5.6) | (5.3) | (5.9) | (6.7) | (8.1) | (5.1) | (5.3) | (5.8) | (6.1) | (5.97) | (3.63) | (2.27) | (2.41) | (3.97) | (7.93) | (4.77) | (4.2) | (4.70) | (5.42) |
| ICCL 86111 | 22.7 | 6.0 | 3.3 | 8.0 | 10.0 | 7.3 | 2.7 | 7.0 | 1.0 | 4.5 | 8.97 | 8.0 | 2.33 | 3.33 | 5.66 | 12.9 | 5.56 | 4.21 | 4.1 | 6.71 |
| | (8.4) | (5.5) | (4.8) | (6.2) | (6.2) | (5.4) | (4.6) | (5.3) | (3.9) | (4.8) | (2.47) | (2.25) | (1.68) | (1.95) | (2.48) | (5.42) | (4.11) | (3.9) | (4.01) | (4.3) |
| ICCV 10 | 16.0 | 4.7 | 8.0 | 4.3 | 8.3 | 6.3 | 3.3 | 1.0 | 3.7 | 3.6 | 9.0 | 4.0 | 3.33 | 6.3 | 5.66 | 10.43 | 4.0 | 4.11 | 4.76 | 5.8 |
| | (7.8) | (5.0) | (5.7) | (5.0) | (5.9) | (5.7) | (4.9) | (3.8) | (4.8) | (4.8) | (2.77) | (1.54) | (1.95) | (2.61) | (2.48) | (5.42) | (3.81) | (3.8) | (3.617) | (4.16) |
| JG 11 | 14.0 | 3.7 | 2.7 | 5.0 | 6.3 | 9.8 | 5.7 | 3.7 | 4.0 | 5.8 | 9.33 | 6.66 | 5.0 | 2.66 | 5.91 | 11.04 | 5.35 | 3.8 | 3.88 | 6.0 |
| | (7.2) | (4.8) | (4.6) | (5.1) | (5.4) | (6.1) | (5.5) | (5.0) | (4.7) | (5.3) | (2.63) | (2.18) | (2.34) | (1.77) | (2.53) | (5.31) | (4.16) | (3.98) | (3.857) | (24.3) |
| KAK 2 | 20.7 | 5.0 | 6.3 | 6.0 | 9.5 | 5.3 | 3.3 | 2.7 | 5.3 | 4.2 | 23.46 | 11.33 | 2.33 | 4.66 | 10.45 | 16.48 | 6.25 | 3.80 | 5.32 | 8.03 |
| | (8.7) | (4.9) | (5.4) | (6.3) | (6.4) | (5.3) | (5.0) | (4.7) | (5.2) | (5.0) | (4.72) | (3.11 | (1.68) | (2.27) | (3.30) | (6.24) | (4.33) | (3.92) | (4.59) | (4.77) |
| Mean | 19.9 | 5.3 | 5.2 | 5.9 | 9.1 | 9.2 | 3.9 | 3.7 | 4.3 | 5.3 | 17.41 | 8.98 | 3.53 | 4.46 | 8.60 | 15.48 | 6.07 | 7.64 | 7.64 | 4.61 |
| | (8.4) | (5.2) | (5.2) | (5.7) | (6.1) | (6.1) | (5.0) | (4.8) | (4.9) | (5.2) | (4.23) | (3.07) | (2.0) | (2.22) | (3.01) | (15.4) | (4.24) | (4.15) | (4.15) | (7.64) |
| | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) |
| Genotype (G) | 0.169 | 1.71 | 0.37 | NS | | 0.02 | 3.3 | 0.29 | 0.83 | | <.001 | 10.13 | 0.063 | 0.181 | | <.001 | 6.15 | 0.2041 | 0.5843 | |
| Sowing (S) | <.001 | 22.3 | 0.33 | 0.95 | 21 | 0.002 | 5.71 | 0.26 | 0.74 | 19.2 | <.001 | 40.77 | 0.057 | 0.162 | 17.4 | <.001 | 28.49 | 0.1826 | 0.5226 | 15.3 |
| G x S | 0.852 | 0.57 | 0.74 | NS | | 0.34 | 1.17 | 0.58 | NS | | <.001 | 4.32 | 0.126 | 0.362 | | 0.341 | 1.17 | 0.4082 | 1.1687 | |

**Table 2**: Evaluation of different chickpea genotypes for resistance to *H. armigera* larvae at different sowing dates

| | *Helicoverpa armigera* larvae (2012-2013) | | | | | *Helicoverpa armigera* larvae (2013-2014) | | | | | *Helicoverpa armigera* larvae (2014-15) | | | | | *Helicoverpa armigera* larvae (Pooled) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | |
| Genotype | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean |
| ICC 3137 | 113.2 | 43.0 | 22.0 | 29.3 | 51.9 | 56.0 | 69.3 | 33.7 | 74.3 | 58.3 | 94.66 | 57 | 34.33 | 36.0 | 55.5 | 87.95 | 56.43 | 30.01 | 46.53 | 55.23 |
| | (23.3) | (13.9) | (9.2) | (11.6) | (14.5) | (15.6) | (17.8) | (11.6) | (16.9) | (15.5) | (11.46) | (8.65) | (5.90) | (6.04) | (7.48) | (16.79) | (13.45) | (8.9) | (9.7) | (12.21) |
| ICCL 86111 | 69.7 | 46.7 | 22.3 | 28.3 | 41.8 | 31.0 | 30.7 | 18.3 | 18.7 | 24.7 | 46.66 | 31.66 | 26.33 | 15.33 | 30.0 | 49.12 | 36.33 | 22.31 | 20.78 | 32.14 |
| | (18.3) | (14.4) | (9.4) | (11.2) | (13.3) | (12.1) | (12.1) | (8.7) | (8.1) | (10.2) | (7.35) | (6.07) | (5.18) | (3.97) | (5.52) | (12.58) | (10.86) | (7.76) | (7.76) | (9.74) |
| ICCV 10 | 49.7 | 21.0 | 11.7 | 31.0 | 28.2 | 32.3 | 29.7 | 20.0 | 44.7 | 31.7 | 31.33 | 23.33 | 23.66 | 20.66 | 24.75 | 37.77 | 24.68 | 18.75 | 32.12 | 28.26 |
| | (15.3) | (9.9) | (7.0) | (12.1) | (11.1) | (12.2) | (12.2) | (8.6) | (13.2) | (11.5) | (6.17) | (5.19) | (4.91) | (4.60) | (5.02) | (11.22) | (9.1) | (6.84) | (9.97) | (9.28) |
| JG 11 | 74.3 | 34.3 | 21.7 | 23.0 | 38.3 | 34.7 | 32.3 | 17.2 | 36.3 | 30.1 | 49.33 | 31.66 | 16.0 | 20.33 | 29.33 | 52.77 | 32.75 | 18.3 | 26.54 | 32.59 |
| | (18.4) | (12.3) | (9.5) | (10.4) | (12.7) | (13.2) | (12.5) | (8.6) | (12) | (11.6) | (7.90) | (6.19) | (4.06) | (4.56) | (5.46) | (13.17) | (10.33) | (7.39) | (8.99) | (9.97) |
| KAK 2 | 96.7 | 42.0 | 23.3 | 24.3 | 46.6 | 42.3 | 41.7 | 29.8 | 37.7 | 37.9 | 62.33 | 49.66 | 16.33 | 13.3 | 35.41 | 67.11 | 44.45 | 8.0 | 25.0 | 39.93 |
| | (20.8) | (13.9) | (9.4) | (10.5) | (13.6) | (14.4) | (13.7) | (10.5) | (12.3) | (12.7) | (9.07) | (8.00) | (4.10) | (3.71) | (5.99) | (14.76) | (11.87) | (23.14) | (8.84) | (10.8) |
| Mean | 80.7 | 37.4 | 20.1 | 27.2 | 41.3 | 39.3 | 40.7 | 23.8 | 38.3 | 36.5 | 56.86 | 38.66 | 23.33 | 21.1 | 35.0 | 58.95 | 39.0 | 22.0 | 30.2 | 38.0 |
| | (19.2) | (12.9) | (8.9) | (11.1) | (13) | (13.5) | (13.7) | (9.6) | (12.5) | (12.3) | (7.57) | (6.25) | (4.88) | (4.65) | (5.95) | (13.7) | (11.12) | (7.77) | (9.04) | (10.41) |
| | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) |
| Genotype (G) | <.001 | 7.03 | 0.49 | 1.39 | | <.001 | 20.95 | 0.43 | 1.24 | | 0.002 | 4.98 | 0.149 | 0.427 | | 0.004 | 4.55 | 0.543 | 1.555 | |
| Sowing (S) | <.001 | 104.9 | 0.43 | 1.24 | 12.9 | <.001 | 23.41 | 0.39 | 1.11 | 12.2 | <.001 | 27.8 | 0.133 | 0.382 | 20.9 | <.001 | 28.44 | 0.486 | 1.391 | 18.1 |
| G x S | 0.012 | 2.62 | 0.97 | NS | | 0.071 | 1.87 | 0.87 | NS | | 0.309 | 1.21 | 0.298 | 0.854 | | 0.541 | 0.92 | 1.086 | 3.11 | |

**Table 3**: Evaluation of different chickpea genotypes for resistance to *Spodoptera exigua* egg laying at different sowing dates

| | *Spodoptera exigua* eggs (2012-2013) | | | | | *Spodoptera exigua* eggs (2013-2014) | | | | | *Spodoptera exigua* eggs (2014-15) | | | | | *Spodoptera exigua* eggs (Pooled) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | |
| Genotype | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean |
| ICC 3137 | 0.0 | 0.7 | 0.3 | 0.7 | 0.42 | 0.0 | 0.0 | 1.0 | 0.3 | 0.32 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.23 | 0.33 | 0.56 | 0.28 |
| | (0.71) | (1.09) | (0.89) | (1.09) | (0.95) | (0.71) | (0.71) | (1.22) | (0.89) | (0.88) | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.83) | (.94) | (0.89) | (0.84) |
| ICCL 86111 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 3.0 | 0.0 | 0.82 | 0.0 | 0.0 | 1.33 | 0.0 | 0.33 | 0.0 | 0.1 | 1.44 | 0.0 | 0.38 |
| | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.89) | (1.87) | (0.71) | (1.04) | (0.71) | (0.71) | (1.35) | (0.71) | (0.91) | (0.71) | (0.77) | (1.31) | (0.71) | (0.87) |
| ICCV 10 | 0.0 | 0.0 | 0.0 | 0.3 | 0.075 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.33 | 0.0 | 0.08 | 0.0 | 0.0 | 0.11 | 0.1 | 0.053 |
| | (0.71) | (0.71) | (0.71) | (0.89) | (0.60) | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.91) | (0.71) | (0.76) | (0.71) | (0.71) | (.77) | (.77) | (0.74) |
| JG 11 | 0.0 | 0.3 | 0.0 | 0.3 | 0.15 | 0.0 | 0.0 | 0.7 | 0.0 | 0.19 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.18 |
| | (0.71) | (0.89) | (0.71) | (0.89) | (0.80) | (0.71) | (0.71) | (1.09) | (0.71) | (0.80) | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.71) | (0.77) | (0.83 | (.77) | (0.77) |
| KAK 2 | 0.0 | 0.3 | 0.7 | 0.7 | 0.42 | 5.0 | 0.0 | 1.7 | 0.0 | 1.78 | 0.0 | 0.0 | 0.66 | 0.0 | 0.16 | 1.66 | 0.0 | 1.04 | 0.23 | 0.73 |
| | (0.71) | (0.89) | (1.09) | (1.09) | (0.94) | (4.2) | (0.71) | (1.48) | (0.71) | (1.85) | (0.71) | (0.71) | (1.08) | (0.71) | (0.81) | (1.87) | (0.71) | (1.21) | (0.83) | (1.17) |
| Mean | 0.0 | 0.26 | 0.12 | 0.42 | 0.0 | 1.0 | 1.06 | 1.29 | 0.06 | 0.84 | 0.0 | 0.0 | 0.46 | 0.0 | 0.11 | 0.33 | 0.08 | 0.63 | 0.2 | 0.13 |
| | (0.71) | (0.85) | (0.82) | (0.93) | (0.82) | (1.40) | (0.60) | (1.27) | (0.74) | (1.00) | (0.71) | (0.71) | (0.98) | (0.71) | (0.78) | (0.94) | (0.77) | (1.04) | (0.79) | (0.88) |
| | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) |
| Genotype (G) | 0.151 | 1.79 | 0.07 | 0.2 | | 0.09 | 2.17 | 0.09 | 0.27 | | 0.876 | 0.3 | 0.013 | 0.036 | | 0.185 | 1.64 | 0.0457 | 0.1308 | |
| Sowing (S) | 0.176 | 1.74 | 0.06 | 0.18 | 6.6 | 0.002 | 5.83 | 0.08 | 0.24 | 8.7 | 0.018 | 3.79 | 0.011 | 0.032 | 6.1 | <.001 | 8.83 | 0.0409 | 0.117 | 5.9 |
| G x S | 0.952 | 0.41 | 0.14 | 0.4 | | 0.305 | 1.22 | 0.19 | 0.53 | | 0.986 | 0.3 | 0.025 | 0.072 | | 0.163 | 1.51 | 0.0914 | 0.2616 | |

masses differed significantly across sowing dates in all cropping seasons. Comparative pattern was observed across seasons, and the highest numbers of egg masses were recorded in December sown crop (0.63). Comparatively higher number of egg masses were recorded in 2013- 14 than in 2012-13 and 2014-15.

There were no significant differences in egg laying across genotypes in 2012-13. The least number of egg masses were seen on 'KAK 2' (0.7) followed by 'ICCL 86111' (0.38) across seasons. The number of egg masses deposited on different genotypes differed during 2013-14 cropping season. The highest numbers of egg masses (1.7) were recorded on 'KAK 2', while no egg masses were recorded on 'ICCV 10'. Across seasons, the highest number of *S. exigua* egg masses (0.73) were recorded on 'KAK 2', followed by 'ICCL 86111' (0.38) and 'ICC 3137' (0.28). The interaction effects were critical over the seasons. No egg masses were recorded in the October sown crop in all the crop growing seasons, besides 0.80 on 'KAK 2' during 2013-14.

### 3.4 POPULATION OF *S. EXIGUA* LARVAE ON DIFFERENT CHICKPEA GENOTYPES ACROSS SOWINGS

There were significant differences in *S. exigua* larval incidence across sowing dates. The number of *S. exigua* larvae were highest in the crop sown during January (16.1; 15.5), followed by the December (11.6) during 2012-13 and 2013-14 respectively. But during 2014-15, the number of *S. exigua* larvae were significantly higher in the crop sown during December (15.8), followed by November (9.46). Across the seasons, *S. exigua* larval incidence was significantly higher in December sown crop (12.9), than the crop sown in October, November and January. However, minimum *S. exigua* larvae were recorded in January sown crop of 2014-15 due to the drought conditions. The December sown crop was most affected by *S. exigua* larvae in all the cropping seasons (2012-2015). The larval incidence was comparatively higher in 2012-13 than in 2013-14 and 2014-15 (Table 4).

### 3.5 VARIATION IN PARASITIZATION OF *H. ARMIGERA* BY THE LARVAL PARASITOID *C. CHLORIDEAE*

Significant differences were observed in the number of *C. chlorideae* cocoons in different sowing dates across seasons (Table 5). During 2012-13 cropping season, higher number of cocoons were recorded in the December sown crop (3.4), followed by October sown crop (2.4) while in other crop growing seasons maximum number of cocoons were recorded during October 2013-14 and November 2014-15. There were no significant differences in the number of *C. chlorideae* cocoons on different genotypes in all the seasons. However, the highest number of cocoons were recorded on 'ICC 3137' (2.5) and lowest on 'KAK 2' (1.6) and 'JG 11' (1.7).

### 3.6 INFLUENCE OF CLIMATIC CONDITIONS ON PEST INCIDENCE IN CHICKPEA ACROSS SOWING PATTERNS

In the October sown crop (Table 6), the maximum temperature exhibited a negative correlation with *H. armigera* larval population. The *S. exigua* egg masses were decidedly corresponded with RH, while other weather parameters were non-significant with the insect pest population in all the crop growing seasons. In the November sown crop (Table 7), only *H. armigera* larval population showed a significant positive correlation with minimum temperature and RH. While in December sown crop (Table 8) the *H. armigera* eggs population was significantly positively correlated with maximum temperature and negatively correlated RH. While significant negative correlation was observed between the *S. exigua* larvae and minimum temperature. In the case of January sown crop (Table 9), the *H. armigera* larval population was essentially decidedly associated with most extreme and least temperature, and contrarily related with RH across seasons.

Multiple regression analysis of the *H. armigera, S. exigua* eggs and larval population showed a significant interaction with weather parameters during all cropping seasons (Table 10). The coefficients of multiple determinations ($R^2$) were 0.795, 0.844, 0.793 for *H. armigera* eggs, *S. exigua* egg masses and *S. exigua* larval populations respectively, during October sown crop. Whereas, in November sown crops the $R^2$ for *H. armigera* larvae was 0.821. The $R^2$ for *H. armigera* eggs and *S. exigua* larvae were 0.979 and 0.866 respectively during December sown crop. In January sown crop, the $R^2$ value for *H. armigera* larvae was 0.866.

## 4 DISCUSSION

In the chickpea ecosystem, the insect pest range varies with different plantings on different genotypes. In the current study the maximum number of *H. armigera* eggs, larvae, and *C. chlorideae* cocoons were recorded in 2012-13, owing to good meteorological scenarios, such as rain followed by optimum temperature, which result-

**Table 4**: Evaluation of different chickpea genotypes for resistance to *Spodoptera exigua* larvae at different sowing dates

| | *Spodoptera exigua* larvae (2012-2013) | | | | | *Spodoptera exigua* larvae (2013-2014) | | | | | *Spodoptera exigua* larvae (2014-15) | | | | | *Spodoptera exigua* larvae (Pooled) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | | 30th | 30th | 30th | 30th | |
| Genotype | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean | Oct | Nov | Dec | Jan | Mean |
| | 3.7 | 8.3 | 7.7 | 15.7 | 8.8 | 3.0 | 0.3 | 2.7 | 14.3 | 5.1 | 6.66 | 5.0 | 17.33 | 1.0 | 7.5 | 4.43 | 4.53 | 9.24 | 10.33 | 7.14 |
| ICC 3137 | (5.0) | (5.5) | (5.7) | (7.7) | (5.9) | (4.3) | (3.7) | (4.6) | (6.4) | (4.8) | (2.02) | (1.65) | (4.22) | (1.22) | (2.82) | (5.11) | (3.773) | (3.62) | (4.84) | (4.33) |
| ICCL 86111 | 6.3 | 13.3 | 6.3 | 20.3 | 11.6 | 0.0 | 1.0 | 11.0 | 8.3 | 5.1 | 4.66 | 11.66 | 19.33 | 0.33 | 9.0 | 3.65 | 8.65 | 12.21 | 15.97 | 10.1 |
| | (5.9) | (7.1) | (5.6) | (7.9) | (6.6) | (3.5) | (3.9) | (5.8) | (5.7) | (4.7) | (1.80) | (2.17) | (4.45) | (0.91) | (3.08) | (4.84) | (3.73) | (4.39) | (5.28) | (4.56) |
| ICCV 10 | 4.0 | 2.7 | 16.7 | 7.7 | 7.8 | 25.0 | 2.3 | 10.3 | 5.7 | 10.8 | 2.66 | 13.6 | 10.6 | 0.0 | 6.75 | 10.53 | 6.2 | 12.53 | 4.47 | 8.44 |
| | (5.2) | (4.6) | (6.9) | (6.10 | (5.7) | (5.3) | (4.5) | (5.9) | (5.2) | (5.2) | (1.35) | (2.35) | (3.34) | (0.71) | (2.69) | (4.00) | (3.95) | (3.82) | (5.38) | (4.28) |
| JG 11 | 4.7 (5.4) | 12.7 (6.6) | 8.0 (6.1) | 11.7 (7.1) | 9.3 (6.3) | 1.0 (3.7) | 0.0 (3.5) | 27.7 (7.6) | 19.7 (8.4) | 12.1 (5.8) | 5.33 (1.89) | 5.0 (1.59) | 16.6 (4.14) | 0.0 (0.71) | 6.75 (2.69) | 3.67 (5.40) | 8.9 (3.66) | 17.43 (3.89) | 10.47 (5.95) | 9.37 (4.73) |
| KAK 2 | 4.7 (5.4) | 13.3 (6.7) | 19.3 (7.7) | 25.0 (9.5) | 15.6 (7.3) | 1.0 (3.8) | 3.0 (4.6) | 6.3 (4.9) | 29.3 (9.7) | 10.2 (5.8) | 4.33 (1.71) | 12.0 (2.33) | 15.0 (3.93) | 0.0 (0.71) | 7.83 (2.88) | 3.34 (6.64) | 9.43 (3.637) | 13.53 (4.54) | 18.1 (5.51) | 11.1 (5.08) |
| Mean | 4.7 (5.4) | 10.1 (6.1) | 11.6 (6.4) | 16.1 (7.7) | 10.6 (6.4) | 2.0 (4.1) | 1.3 (4.1) | 11.6 (5.7) | 15.5 (7.1) | 8.6 (5.3) | 4.73 (2.28) | 9.46 (3.15) | 15.8 (4.03) | 0.26 (0.87) | 7.56 (2.84) | 5.13 (3.75) | 6.94 (4.05) | 12.99 (5.3) | 11.86 (5.19) | 9.24 (4.59) |
| | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) |
| Genotype (G) | 0.112 | 2.01 | 0.44 | NS | | 0.469 | 0.91 | 0.54 | NS | | 0.202 | 1.57 | 0.05 | 0.143 | | 0.58 | 0.72 | 0.38 | 1.087 | |
| Sowing (S) | 0.002 | 5.79 | 0.39 | 1.13 | 23.9 | <.001 | 9.06 | 0.48 | 1.38 | 35.5 | <.001 | 44.65 | 0.045 | 0.128 | 15.6 | 0.002 | 5.79 | 0.34 | 0.972 | 28.6 |
| G x S | 0.633 | 0.82 | 0.88 | NS | | 0.263 | 1.29 | 1.08 | NS | | 0.018 | 2.43 | 0.1 | 0.287 | | 0.913 | 0.48 | 0.759 | 2.174 | |

**Table 5:** Evaluation of different chickpea genotypes for resistance to *Campoletis* cocoon at different sowing dates

| Genotype | *Campoletis* cocoons (2012-2013) | | | | | *Campoletis* cocoons (2013-2014) | | | | | *Campoletis* cocoons (2014-15) | | | | | *Campoletis* cocoons (Pooled) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30th Oct | 30th Nov | 30th Dec | 30th Jan | Mean | 30th Oct | 30th Nov | 30th Dec | 30th Jan | Mean | 30th Oct | 30th Nov | 30th Dec | 30th Jan | Mean | 30th Oct | 30th Nov | 30th Dec | 30th Jan | Mean |
| ICC 3137 | 1.3 | 0.3 | 3.3 | 0.0 | 1.22 | 7.5 | 7.7 | 0.0 | 0.3 | 3.87 | 1.66 | 5.33 | 0.33 | 2.33 | 2.41 | 3.48 | 4.44 | 1.21 | 0.87 | 2.50 |
| | (1.34) | (0.89) | (5.0) | (0.71) | (1.98) | (5.5) | (6.4) | (0.71) | (0.89) | (3.37) | (1.07) | (1.94) | (0.91) | (1.68) | (1.70) | (2.63) | (3.07) | (2.0) | (1.09) | (2.25) |
| ICCL 86111 | 1.7 | 0.0 | 4.0 | 0.0 | 1.42 | 5.5 | 3.7 | 2.5 | 0.3 | 3.0 | 0.66 | 5.33 | 0.33 | 1.33 | 1.91 | 2.62 | 3.01 | 2.27 | 0.54 | 2.11 |
| | (1.48) | (0.71) | (5.0) | (0.71) | (1.97) | (4.9) | (5.1) | (4.2) | (0.89) | (3.78) | (0.83) | (1.99) | (0.91) | (1.35) | (1.54) | (2.40) | (2.6) | (3.37) | (0.98) | (2.33) |
| ICCV 10 | 3.7 | 0.3 | 6.7 | 0.3 | 2.75 | 4.5 | 3.0 | 0.0 | 0.7 | 2.06 | 2.66 | 4.0 | 0.33 | 0.66 | 1.91 | 3.62 | 2.43 | 2.34 | 0.44 | 2.22 |
| | (5.0) | (0.89) | (5.8) | (0.89) | (3.12) | (4.7) | (4.8) | (0.71) | (0.89) | (2.07) | (1.34) | (1.59) | (0.91) | (1.08) | (1.55) | (3.68) | (2.42) | (3.4) | (0.95) | (2.61) |
| JG 11 | 2.7 | 0.0 | 2.3 | 0.0 | 1.25 | 5.8 | 3.0 | 2.0 | 0.3 | 2.77 | 2.0 | 2.0 | 0.33 | 1.66 | 1.5 | 3.5 | 1.66 | 1.54 | 0.21 | 1.76 |
| | (4.7) | (0.71) | (4.4) | (0.71) | (2.63) | (5) | (4.8) | (4.1) | (0.89) | (3.06) | (1.18) | (1.18) | (0.91) | (1.47) | (1.41) | (3.62) | (2.23) | (3.13) | (1.02) | (2.50) |
| KAK 2 | 2.7 | 1.0 | 0.77 | 0.0 | 1.11 | 5.0 | 4.0 | 2.0 | 0.3 | 2.82 | 1.0 | 2.33 | 0.33 | 0.0 | 0.91 | 2.9 | 2.44 | 1.01 | 0.21 | 1.64 |
| | (4.6) | (1.22) | (0.89) | (0.71) | (1.83) | (5.5) | (5.3) | (3.8) | (0.89) | (3.42) | (0.93) | (1.27) | (0.91) | (0.71) | (1.19) | (3.67) | (2.59) | (1.86) | (0.77) | (2.22) |
| Mean | 2.42 | 0.32 | 3.41 | 0.06 | 1.54 | 5.7 | 4.3 | 1.3 | 0.4 | 2.92 | 1.6 | 3.8 | 0.33 | 1.2 | 1.73 | 3.22 | 2.79 | 1.67 | 0.45 | 2.04 |
| | (3.42) | (0.88) | (4.21) | (0.74) | (2.30) | (5.1) | (5.3) | (3.8) | (3.7) | (3.10) | (1.44) | (2.07) | (0.91) | (1.30) | (1.49) | (3.20) | (2.58) | (2.79) | (0.96) | (2.28) |
| | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) | Fp | Vr | SE ± | LSD (P 0.05) | CV (%) |
| Genotype (G) | 0.279 | 1.32 | 0.21 | 0.6 | | 0.36 | 1.12 | 0.2 | 0.57 | | 0.155 | 1.77 | 0.035 | 0.1 | | 0.885 | 0.29 | 0.1961 | 0.5614 | |
| Sowing (S) | <.001 | 10.36 | 0.19 | 0.54 | 17.4 | <.001 | 20.58 | 0.18 | 0.51 | 15.5 | <.001 | 15.48 | 0.031 | 0.09 | 14.1 | 0.024 | 3.52 | 0.1754 | 0.5021 | 20.7 |
| G x S | 0.611 | 0.84 | 0.42 | 1.2 | | 0.398 | 1.09 | 0.4 | 1.15 | | 0.319 | 1.2 | 0.07 | 0.201 | | 0.984 | 0.31 | 0.3922 | 1.1228 | |

**Table 6**: Correlation between pest incidence and different weather parameters during 2013-2015 in chickpea in October sown crop

| | | Temperature (°C) | | | |
|---|---|---|---|---|---|
| | Rain (mm) | Maximum | Minimum | Relative Humidity morning (%) | Relative Humidity evening (%) |
| *H. armigera* eggs | -0.098 | 0.409 | -0.419 | 0.309 | -0.343 |
| *H. armigera* larvae | -0.609 | -0.892* | -0.462 | -0.632 | -0.168 |
| *S. exigua* egg mass | 0.847 | 0.386 | 0.577 | 0.919** | 0.613 |
| *S. exigua* larvae | 0.720 | 0.570 | 0.561 | 0.891* | 0.488 |
| *Campoletis* cocoon | 0.307 | 0.718 | -0.073 | 0.415 | -0.188 |

*, ** Significant at $p \leq 0.05$ and 0.01

**Table 7**: Correlation between pest incidence and different weather parameters during 2013-2015 in chickpea in November sown crop

| | | Temperature (°C) | | | |
|---|---|---|---|---|---|
| | Rain (mm) | Maximum | Minimum | Relative Humidity morning (%) | Relative Humidity evening (%) |
| *H. armigera* eggs | -0.335 | -0.218 | -0.821 | 0.644 | 0.178 |
| *H. armigera* larvae | 0.327 | 0.698 | 0.82 | -0.905* | -0.609 |
| *S. exigua* egg mass | -0.578 | -0.725 | 0.2 | 0.203 | 0.619 |
| *S. exigua* larvae | -0.455 | -0.08 | -0.755 | 0.505 | 0.097 |
| *Campoletis* cocoon | 0.708 | 0.516 | 0.68 | -0.619 | -0.606 |

*, ** Significant at $p \leq 0.05$ and 0.01

**Table 8:** Correlation between pest incidence and different weather parameters during 2013-2015 in chickpea in December sown crop

| | | Temperature (°C) | | | |
|---|---|---|---|---|---|
| | Rain (mm) | Maximum | Minimum | Relative Humidity morning (%) | Relative Humidity evening (%) |
| *H. armigera* eggs | 0.818 | 0.881* | 0.956** | -0.921** | -0.427 |
| *H. armigera* larvae | 0.445 | 0.722 | 0.683 | -0.846 | -0.805 |
| *S. exigua* egg mass | -0.52 | -0.419 | -0.6221 | 0.425 | -0.113 |
| *S. exigua* larvae | -0.8 | -0.805 | -0.916* | 0.813 | 0.237 |
| *Campoletis* cocoon | -0.45 | -0.077 | -0.163 | -0.117 | -0.72 |

*, ** Significant at $p \leq 0.05$ and 0.01

**Table 9**: Correlation between pest incidence and different weather parameters during 2013-2015 in chickpea in January sown crop

| | | Temperature (°C) | | | |
|---|---|---|---|---|---|
| | Rain (mm) | Maximum | Minimum | Relative Humidity morning (%) | Relative Humidity evening (%) |
| *H. armigera* eggs | -0.291 | 0.594 | 0.453 | -0.55 | -0.318 |
| *H. armigera* larvae | 0.538 | 0.975** | 0.99** | -0.994** | -0.325 |
| *S. exigua* egg mass | 0.233 | -0.117 | 0.04 | -0.077 | 0.565 |
| *S. exigua* larvae | -0.381 | -0.275 | -0.255 | 0.143 | 0.37 |
| *Campoletis* cocoon | -0.015 | 0.301 | 0.338 | -0.44 | 0.17 |

*, ** Significant at $p \leq 0.05$ and 0.01

**Table 10:** Regression between weather parameters and insect pest population in chickpea across seasons

| Season | Insect-pests | Regression equation | $R^2$ Value |
|---|---|---|---|
| October | *H. armigera* eggs | Y = 309.36 - 2.19 (Rain) -10.24 (Max.Temp) -8.94 (Min.temp)- 6.70 (RH1) + 2.70 (RH2) | 0.7959 |
| | *S. exigua* egg mass | Y =-7.98 + 0.080 (Rain)+ 0.0 (Max.Temp) + 0.15 (Min.temp) + 0.0875 (RH1) + 0.011 (RH2) | 0.844 |
| | *S. exigua* larvae | Y =-59.33 + 0.577 (Rain) + 0.0 (Max.Temp) + 1.26 (Min.temp) + 0.65 (RH1) -0.28 (RH2) | 0.793 |
| November | *H. armigera* larvae | Y = 99.06 + 6.04 (Rain) + 0.0 (Max.Temp) + 0.22 (Min.temp)- 1.05 (RH1) + 1.09 (RH2) | 0.821 |
| December | *H. armigera* eggs | Y = 19.46 + 0.80 (Rain) -0.39 (Max.Temp) + 0.27 (Min.temp)- 0.12 (RH1) -0.361 (RH2) | 0.979 |
| | *S. exigua* larvae | Y = 6.86 + 8.81(Rain) +0.628 (Max.Temp) -1.50 (Min.temp)+ 1.38 (RH1) -6.02 (RH2) | 0.866 |
| January | *H. armigera* larvae | Y = 6.86 + 8.81(Rain) +0.628 (Max.Temp)-1.50 (Min.temp)+ 1.38 (RH1) -6.02 (RH2) | 0.866 |

ed in increased pod borer activity under field conditions. There were considerable differences in *H. armigera* larval incidence across the test genotypes in the early plantings, while the differences were less noticeable in the late plantings. Though the number of *H. armigera* and *S. exigua* larvae decreased as planting dates progressed, the extent of *H. armigera* damage increased across all cropping seasons. The current studies are in corroboration with Shankar et al., (2014) who reported that the number of *S. exigua* and *H. armigera* larvae were maximum in October planting compared to late planting. The present studies additionally link with the work of Shah & Shahzad (2005) who observed that the oviposition by *H. armigera* was low from December to Mid- February due to cold conditions, whereas Ali et al., (2003) reported that the numbers of eggs laid by *H. armigera* differed considerably across sowings and genotypes of cotton. Similarly, Ali et al., (2009) ascertained that there were no significant variations in larval population and damage across genotypes and different sowing dates. Hossain et al., (2008) found that the *H. armigera* larval population was high in early sown crops (October 15$^{th}$ to November 1$^{st}$) and delayed sowings (November 1$^{st}$ to 30$^{th}$) resulted in lower population of *H. armigera*. Accessions ICC 506EB, ICC 12476, ICC 12477, ICC 12478 and ICC 12479 showed oviposition non-preference and suffered low leaf damage (Narayanamma et al., 2007).

The cocoons of the parasitoid *C. chlorideae* also attenuated with the planting dates, that ultimately resulted in an enormous decrease in biological control of *H. armigera* larvae. The inflated temperature across the planting dates, resulted in increased damage by *H. armigera* and also a reduction in the dry matter and grain yield. The current findings were consistent with Pavani et al., 2019, who reported the highest levels of parasitoid activity in the October planted crop, and lowest in the January planted crop. The parasitoid was more active at temperatures ranging from 15 to 28 degrees Celsius (Jaba & Agnihotri 2018; Jaba et al., 2016). The parasatization came down after January (5$^{th}$ SW) in chickpea sole crop and there was negative correlation ascertained with minimum temperature and morning RH. In case of intercropping system, the result elucidated that a significant positive correlation was observed with evening RH and rainfall in consecutive years.

The results of the correlation analysis in the present study are in corroboration with earlier reports by Patnaik & Senapati (1996), who observed a negative correlation between mean temperature ranges and larval incidence. However, a positive association was observed between *H. armigera* and *S. exigua* larvae, and similar results were earlier reported by Sharma (2012). The positive correlation has also been reported earlier between *H. armigera* larval incidence and the maximum and the minimum temperatures by (Sharma et al., 2005; Shah and Shahzad, 2005; Upadhyay et al., 1989; Pandey 2012). Ugale et al., (2011) reported that moth emergence was negatively correlated with the maximum (r = -0.62) and minimum temperatures (r = -0.75), but there was no association with relative humidity. Prasad et al., (1989); Jaba & Agnihotri, 2015 confounded that minimum temperature and rainfall exerted a negative influence on pheromone trap catches of *H. armigera*. The population of *H. armigera* and *S. exigua* larvae was negatively correlated with relative humidity across the genotypes.

## 5 CONCLUSION

The present studies were carried out to identify climate resilient cultivars and best sowing window with least pest incidence under climate change scenarios. Our results, concluded that the egg laying by *H. armigera* diminished across sowing dates until December, while a small increase was recorded in the January sown crop. In the early plantings there were significant differences among the genotypes, but such differences were less apparent in the late plantings. 'ICC 3137' was most preferred for egg laying, followed by 'KAK 2', The genotypes 'ICCV 10' and 'JG 11' were relatively not preferred for egg laying. There were no significant differences in egg laying by *S. exigua* in the crops sown in October, November, and January. The highest numbers of *S. exigua* egg masses were recorded on 'KAK 2', followed by 'ICC 3137' in the December sown crop. The *S. exigua* larval incidence was greater in the January sown crop than in the crops sown in October, November, and December. Though the number of *H. armigera* larvae decreased with the planting dates, the extent of damage by *H. armigera* increased across the planting dates across seasons. The cocoons of the parasitoid *C. chlorideae* decreased with the planting dates, which ultimately resulted in decreased biological control of *H. armigera*. As the temperature exaggerated across the planting dates, there was an increase in damage by *H. armigera* under field conditions.

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## 7 CONFLICTING INTEREST

The authors declare no conflict of interest.

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# *Pleurotus* cultivation: a sustainable way to utilize agrowaste

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***Pleurotus* cultivation: a sustainable way to utilize agrowaste**

**Abstract:** In the present study two species of *Pleurotus* namely *Pleurotus florida* (Mont.) Singer and *Pleurotus ostreatus* (Jacq.) P.Kumm. were cultivated using three different agro waste substrates such as paddy straw, sugarcane bagasse, banana leaves and its mixture in equal proportion. The fastest colonization and maximum numbers of heads were produced on paddy straw substrate. Banana leaves and paddy straw substrates reported the highest yield of mushroom fruitbodies, biological efficiency and biomass loss in *P. florida* and *P. ostreatus*. It was noticed that the growth and development of fruitbodies on sugarcane bagasse was minimum and development of competitor moulds was observed on it. In the selected substrates banana leaves posses the highest percentage of nitrogen, carbon and cellulose. The results showed the possibility of utilizing different agrowaste for cultivation of oyster mushroom, which will boost the income of farmers.

**Key words:** growth parameters; mushrooms cultivation; yield; different substrates; oyster mushroom

**Gojenje ostrigarjev (*Pleurotus*): trajnosten način uporabe odpadkov iz kmetijstva**

**Izvleček:** V raziskavi sta bili gojeni dve vrsti ostrigarja (*Pleurotus*), *Pleurotus florida* (Mont.) Singer in *Pleurotus ostreatus* (Jacq.) P.Kumm. na treh različnih gojiščih iz kmetijskih odpadkov in sicer na riževi slami, odpadkih predelave sladkornega trsa, listih bananovca in njihovih mešanicah v enakih deležih. Najhitrejša kolonizacija in največje število trosnjakov sta bila dosežena, ko je bil substrat riževa slama. Mešanica listov bananovca in riževe slame sta dali največji pridelek trosnjakov, največjo biološko učinkovitost in največjo izgubo biomase pri gojenju obeh vrst. Opaženo je bilo, da sta bila rast in razvoj trosnjakov najslabša na substratu iz ostankov predelave sladkornega trsa zaradi kompeticije s plesnimi. Izbrani listi bananovca so imeli največji odstotek dušika, ogljika in celuloze. Rezultati so pokazali možnost uporabe različnih odpadkov v kmetijstvu za gojenje ostrigarjev, kar bi povečalo prihodke kmetov.

**Ključne besede:** rastni parametri; gojenje gob; pridelek; različni substrati; ostrigarji

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## 1 INTRODUCTION

India has diverse agro-climatic zones which are suitable for cultivating wide range of plants including food crops, cash crops and horticultural products. Burgeoning demand for food throughout the world has led to an exponential increase in food production as anywhere in the world all over India. The advancement of agricultural production has undoubtedly resulted in increased amount of agricultural waste and agro-industrial waste. A significant growth of agricultural waste is prone to occur globally if developing countries continue to step up farming systems. This organic waste should be handled carefully in a sustainable way to avoid unwanted environmental side effects. Agricultural waste also termed as crop residue which includes field residues as well as processed residues. In most of the states of India, crop residues are mainly utilized for animal feed.

Many countries leverage crop residues produced by farming practices in different directions. It has been used in a processed or unprocessed form depending on the desired application. Potential alternatives include livestock feed, composting, bio-energy production and deployment in other extended farming activities like cultivation of mushrooms. Several nations including Japan, China, Nepal, Malaysia, Nigeria, Indonesia, Thailand and Philippines are using their agricultural waste to develop bio-energy and fertilizers (Lohan et al., 2018).

Mushroom cultivation has become popular throughout the world. Edible fungi production can greatly enhance sustainability, economic strength of the farmers. Diversification in agriculture sector is inevitable because of some key factors like population growth, food scarcity, poverty and malnutrition among developing and underdeveloped countries. Mushroom cultivation is an ideal method which posses unique advantages than other waste management technologies. It helps the farmers to increase their income effectively by utilizing their own agricultural land waste. This kind of management has drawn more and more attention because of the nutritious output from waste. Mushroom cultivation can help to mitigate hunger and improve livelihoods by providing a fast-growing nutritious food supply and a stable source of employment and wealth (Rachna et al., 2013).

Nearly 355 million tones of crop residues are produced every year. In this, about 170 million is left out for burning and manure preparation. If India utilizes one percent of these waste, it will become a top mushroom producing country in the world (Tewari and Pandey, 2002). Among the entire cultivated mushroom, oyster mushroom posses many advantages due to rapid mycelial growth, great colonization potential, easy and economical cultivation techniques and suitable for cultivating under different climatic conditions.

The present study was carried out to find out the possibilities in utilization of agrowaste for the cultivation of edible mushroom and its effect on growth performance and morphological parameters.

## 2 MATERIALS AND METHODS

### 2.1 COLLECTION OF AGRICULTURAL WASTE MATERIAL

Agro waste materials such as paddy straw, sugarcane bagasse and banana leaves were used for the present study. Paddy straw and banana leaves were collected from farmers and sugarcane bagasse was purchased from sugarcane vendors. The water content of the substrates were completely removed by drying in dried sun light. It was stored in airtight bags to used for the cultivation.

### 2.2 PURCHASE OF SPAWN

Sorghum grain based spawn of *Pleurotus florida* and *Pleurotus ostreatus* were procured from certified cultivation centre, MSM Mushroom Corner, Mushroom Cultivation Training and Seed Sale, Rediyarpatti, Tirunenlveli and used for the present study.

### 2.3 CULTIVATION OF MUSHROOM

The selected agro-wastes were cut into small pieces and soaked in water for 12 – 14 hours and sterilized at 121 °C for 20 -30 minutes by using pressure cooker. After sterilization, it was cooled down in a clean room by shade drying to remove the excess water content present in the sterilized substrate. The sterilized substrates were filled in polypropylene bags to a height of 8 cm approximately. A handful of grain based spawns were sprinkled over the layer. Likewise, few layers were placed on the bag. Holes were made on the bags to facilitate ventilation. The spawned bags were kept under 22 °C–25 °C temperature and required humidity of 85 % was maintained in the cropping room.

### 2.4 EXPERIMENTAL DESIGN

Selected species were cultivated by bag method using three different agrowaste as substrates.

Substrates were collected from the farmers and mar-

ket place. Substrates were dried in the sun light and then cooked in the pressure cooker for 20 minutes to sterilize.

Substrate A: Paddy straw (PS)

Substrate B: Sugarcane bagasse (SB)

Substrate C: Banana leaf (BL)

Substrate D: 1:1:1 ratio of paddy straw, sugarcane bagasse, banana leaf (MIX)

## 2.5 GROWTH PARAMETER ANALYSIS

### 2.5.1 Colonizing period (spawn run)

Spawn run refers to the period during which mycelia spread and colonies the substrate so that it is completely covered. It is a vegetative stage in the development of the fungus which requires specific conditions to be successful (Oei, 1991). Number of days required for the colonization of fugal mycelium in the substrate is counted from the day of inoculation.

### 2.5.2 Pinheads and fruit bodies developed

Pinheads and fruiting bodies developed on the substrate were counted manually.

### 2.5.3 Yield

After the maturation the fruit bodies were hand-picked and immediately weighed using electronic balance (in gram unit).

### 2.5.4 Biological efficiency (Carvalho et al., 2012)

Biological efficiency is a term frequently used in the mushroom industry to describe the potential of the macro fungus to yield fruiting body (mushroom) from a known mass of substrate.

$$\textit{Biological efficiency (\%)} = \frac{\textit{Fresh mass of mushroom}}{\textit{Dry mass of the substrate}} \times 100$$

### 2.5.5 Organic mass loss (Carvalho et al., 2012)

Organic mass loss of the substrate was calculated by using the following formula:

$$\textit{Organic mass loss (\%)} = \frac{(\textit{Initial substrate dry mass} - \textit{residual})}{\textit{Initial substrate dry mass}} \times 100$$

(residual – final mass of the substrate present in the mushroom cultivation bag after last yield)

### 2.5.6 Morphological parameters

Length and width of stipe and pileus were measured immediately after harvesting with the help of thread and measuring scale.

# 3 RESULTS AND DISCUSSION

## 3.1 COLONIZING PERIOD (SPAWN RUN)

Spawn run duration differs depending on species type and substrate used. The substrates used for the present study directly affect the time to attain the maximum mycelial growth and also take part in the yield attribute. Time required for completion of spawn running in *P. florida* and *P. ostreatus* varied on different substrates ranged from 15 to 17 days and 22 to 37 days respectively (Table 1). In both the species, the lowest time required for the completion of spawn run was recorded in PS (15 days in *P. florida* and 22 days in *P. ostreatus*). Longest time required for the completion of spawn run (17 days) was noticed in *P. florida* cultivated on BL and in *P. ostreatus* (37 days) cultivated on SB. Between the two species of *Pleurotus*, *P. florida* showed the fastest colonization of mycelia than *P. ostreatus*. Among the substrates used for the present study, colonization was fastest on PS in both the species than other substrates.

Differences in spawn run duration among species were evident in the studies conducted by Ashraf et al. (2013) comparing three *Pleurotus* species (*Lentinus sajor-caju* (Fr.) Fr., *Pleurotus ostreatus* and *Pleurotus djamor* Rumph. Ex Fr.) Boedijn) on three different wastes (cotton waste, wheat straw and paddy straw). Our findings in the present experiment are almost similar to the findings of Lalithadevi and Many (2014) who reported that spawn running day was between 16–25 days on paddy straw. The findings of the spawn run on sugarcane bagasse did not agree with the report of Hossain (2017) who stated that *P. ostreatus* completed the spawn run in 17 days on sugarcane bagasse. Increase in number of days for spawn running on lingo-cellulosic waste materials might be due to slow hyphal growth of mushroom on substrates (Mandeel et al., 2005).

Mycelium development and colonization is the initial step, which provides suitable internal environment for the development of basidiocarp. Thus, exponential growth of mycelium is a key feature in mushroom cultivation (Sharma et al., 2013). The variation in the days

might be due to the difference in the chemical constituents and C : N ratio of the substrates (Bhatti et al., 1987). These results were similar to the findings of Vanathi et al. (2016) they have cultivated *P. florida* and reported 16–19 days for spawn running, it was highest in sugarcane trashes. Iqbal et al. (2016) reported that oyster mushroom cultivated upon sugarcane bagasse took 28.5 days for spawn running. The present study is corroborated with these findings. The occurrence of influential proportion of lignin, hemicellulose and alpha-cellulose in the growing medium was the assumed factor for higher rate of spawn running in banana leaves and rice straw substrate (Mondal et al., 2010).

### 3.2 NUMBER OF PINHEADS AND PERCENTAGE OF FRUITBODIES DEVELOPED FROM PINHEADS

*Pleurotus* species produced significantly different numbers of pinheads on different substrates (Table 1). In both the species, maximum numbers of pinheads (212 in *P. florida* and 51 in *Pleurotus ostreatus*) were recorded on PS followed by pinheads developed on the MIX in *P. florida* (137) and banana leaves (33) in *P. ostreatus* while minimum numbers of pinheads were observed on SB (32 in *P. florida* and 12 in *P. ostreatus*). Between the two species of *Pleurotus*, *P. florida* showed the highest number of pin heads (212) than *P. ostreatus* (51). From the present study, it was concluded that maximum numbers of heads were noticed on PS in both the species than other substrates. The percentage of fruit bodies developed from heads was very low (39 %) on PS though maximum numbers of pinheads produced on the same. In both the species, the highest percentage of fruit bodies developed from pinheads was maximum on BL (74 % in *P. florida* and 84 % in *P. ostreatus*) followed by the MIX (68 % in *P. florida* and 75 % in *P. ostreatus*).

Our findings are further supported by Hague (2004) and Al Amin (2004), who reported that the highest number of pinheads of Oyster mushroom was found on paddy straw. Minimum numbers of pinheads were observed on sugarcane bagasse (12). Almost similar results reported Hasan et al. (2015) who observed minimum number of pinheads of oyster mushroom on sugarcane bagasse. The results were in accordance with the findings of Al Amin (2004) who reported maximum number of primordia and fruiting bodies of oyster mushroom on paddy straw. Formation of higher number of fruiting bodies may be due to the occurrence of glucose, fructose and trehalose in the substrate (Kitamoto et al., 1995). Poppe (1973) reported that presence of indole acetic acid (IAA) induces the formation of maximum fruiting body of mushroom.

### 3.3 TOTAL YIELD (G)

The present study confirmed that the use of different substrates brought about a significant effect on yield of *P. florida* and *P. ostreatus* (Table 1). In *P. florida*, the harvest yield ranged from 158 g to 622 g while in *P. ostreatus*, the harvest yield ranged from 102 g to 588 g. From the present study, we concluded that there was a difference in the yield between the selected *Pleurotus* species however, the difference is not significant. In *P. florida*, the average yield of mushroom fruitbodies was the highest on BL (622 g) followed by mushroom fruitbodies cultivated on PS (583 g) while in *P. ostreatus*, the average yield of mushroom fruitbodies was maximum on PS (588 g) followed by mushroom fruitbodies cultivated on BL (571 g). In both the species, minimum yield was obtained in mushroom fruitbodies cultivated on SB (102 g in *P.*

**Table 1**: Effect of different substrates on the growth performance of *Pleurotus florida* and *Pleurotus ostreatus*

| Species | Substrates | Spawn running days | Yield (gram) | No. of pin heads | Fruiting body Developed From Pinheads (%) | Biological Efficiency (%) | Organic Mass Loss (%) |
|---|---|---|---|---|---|---|---|
| P. florida | PS | 15 ± 0.71 | 583 ± 18 | 212 ± 17 | 39.2 | 77. 7 | 26.9 |
| | SB | 16 ± 2 | 158 ± 21 | 32 ± 5 | 54.2 | 21.1 | 15.7 |
| | BL | 17 ± 1.5 | 622 ± 30 | 110 ± 11 | 73.6 | 82.9 | 39.6 |
| | MIX | 16 ± 1 | 460 ± 16 | 137 ± 16 | 67.8 | 61.3 | 24.8 |
| P. ostreatus | PS | 22 ± 0.6 | 588 ± 21 | 51 ± 9 | 66.2 | 78.4 | 26.3 |
| | SB | 37 ± 1.5 | 102 ± 17 | 12 ± 4 | 63. 9 | 13.6 | 12.5 |
| | BL | 29 ± 1 | 571 ± 37 | 33 ± 8 | 83.8 | 76.1 | 24.1 |
| | MIX | 28. 7 ± 1.5 | 526 ± 12 | 29 ± 7 | 75 | 70.1 | 21.6 |

*ostreatus* and 158 g *P. florida*). The increase in the yield of *P. florida* and *P. ostreatus* on PS is due to easier way of getting sugars from cellulosic substances (Ponmurugan et al., 2007). Superiority of paddy straw over other substrates in cultivation of *Pleurotus* species with respect to yield had been reported earlier by Pala et al. (2012). Our results also agree with the result of Ragunathan et al. (1996) who reported that maximum yield was obtained by cultivation *Lentinus sajor-caju* on paddy straw.

### 3.4 BIOLOGICAL EFFICIENCY (%)

The highest percentage biological efficiency of *P. florida* was found on BL (82.9 %) followed by PS (77.7 %) while in *P. ostreatus* the highest percentage biological efficiency was noticed on PS (78.4 %) followed by BL (76.1 %) as given in Table 1. Higher biological efficiency of different substrates represents their higher suitability for the cultivation of mushroom. The lowest biological efficiency (21.1 % in *P. florida* and 13.6 % in *P. ostreatus*) was obtained on SB. Our results agree with the result of Sardar et al. (2016) who reported that lowest biological efficiency was obtained on sugarcane bagasse.

### 3.5 PERCENTAGE OF BIOMASS LOSS

The mushroom has the ability to degrade lignocellulosic materials during the idiophase stage following severe nitrogen and carbon depletion (Manson et al., 1989). In *P. ostreatus*, biomass loss was maximum (26.3 %) in PS while in *P. florida*, biomass loss was maximum in BL (39.6 %) which shows that degradation and solubilization was more intensive in the PS and BL.

### 3.6 EFFECT OF SUBSTRATE ON LENGTH AND DIAMETER OF PILEUS AND STIP

Among mushroom quality characteristics, pileus diameter, stipe length, stipe diameter are very important attributes (Mondal et al., 2010). Maximum length of pileus and stipe (7.3 ± 0.8 cm and 3.1 ± 0.6 cm) was obtained on paddy straw while maximum width of pileus and stipe (8.4 ± 1.8 cm and 1.8 ± 0.1 cm) was noticed on SB and MIX respectively.

In the present study, maximum length and width of pileus was obtained (11.3 ± 2.2 cm and 21.5 ± 6.7 cm) on BL followed by the MIX (11.3 ± 3.4 cm and 20.3 ± 6.1 cm) respectively. The minimum length and width of pileus was noted (5.8 ± 0.8 cm and 8.2 ± 2.9 cm) on SB. Our results are in consistence with the findings of Sardar et al. (2016) who observed minimum diameter of pileus (4.10 ± 0.07 cm) on sugarcane bagasse.

Stipe length and width of *P. ostreatus* was observed on different substrates in the present study and significant difference on different substrates used was found. Maximum length of stipe (3.1 ± 1.5 cm) was obtained on the MIX and PS alone (3.0 ± 1.5 cm). Similarly, maximum width of stipe (2.6 ± 1.1 cm) was obtained PS alone and on the MIX (2.3 ± 0.7 cm). Minimum length of stipe (1.3 ± 0.3 cm) was observed on BL while the minimum width was noticed (1.2 ± 0.3 cm) on SB.

Between the two species, *P. ostreatus* showed the maximum length and width of pileus than *P. florida*. From the present study, it was concluded that maximum length and width of pileus were noticed on BL and MIX than other substrates. Oyster mushroom quality depends on the length of stipe. Mondal et al. (2010) found that the higher the stipe length, the poorer the quality of the mushroom. Hence growers should use substrates that do not promote excessive growth of stipe length at the expense of marketable yield.

The size of the fruiting bodies is depended on the water holding capacity of the substrate (Chukwurah et al., 2013) and environmental conditions (Sanchez, 2004). It was also identified temperature, relative humidity, fresh air and compact material as the major external factors that affect stalk length, stalk width and mushroom cap shape AMGA (2004). The quality of oyster mushrooms relies upon its stalk length, higher the stalk length lesser will be the mushroom quality (Zadrazil, 1978).

## 4 CONCLUSION

In all over the world edible mushrooms are eaten and appreciated for their flavor, economic and ecological values and medicinal properties. Two species of *Pleurotus* namely *Pleurotus florida* and *Pleurotus ostreatus* were cultivated using three different substrates such as paddy straw, sugarcane bagasse, banana leaves and their mixture in 1:1:1 ratio. These three different substrates were investigated to determine the growth and yield of *Pleurotus* species. *P. florida* showed the fastest colonization cultivated on paddy straw and maximum numbers of pin heads were observed in the same species on the same substrate. In both the species, the percentage of fruitbodies developed from pin heads was maximum on banana waste. *P. ostreatus* showed the maximum length and diameter of pileus on banana waste. In both the species, yield of mushroom fruitbodies, biological efficiency and biomass loss were high on banana waste and paddy straw. Growth parameters and yield were found to be low in both the species cultivated on sugarcane bagasse. It is

**Table 2**: Effect of different substrate on the morphological parameters of *Pleurotus florida* and *Pleurotus ostreatus* (Results with standard deviation)

| | *Pleurotus florida* | | | | *Pleurotus ostreatus* | | | |
|---|---|---|---|---|---|---|---|---|
| | Pileus | | Stipe | | Pileus | | Stipe | |
| Treatment | Length (cm) | Width (cm) | Length (cm) | Width (cm) | Length (cm) | Width (cm) | Length (cm) | Width (cm) |
| PS | 7.3 ± 0.8 | 8.2 ± 0.7 | 3.1 ± 0.6 | 1 ± 0.3 | 9.8 ± 2.2 | 18.5 ± 7.8 | 3 ± 1.5 | 2.6 ± 1.1 |
| SB | 6.7 ± 1.2 | 8.4 ± 1.8 | 1.4 ± 0.7 | 1.1 ± 0.4 | 5.8 ± 0.8 | 8.2 ± 2.9 | 2.6 ± 1 | 1.2 ± 0.3 |
| BL | 7.0 ± 0.9 | 10.7 ± 2.6 | 1.7 ± 0.2 | 1.7 ± 0.5 | 11.3 ± 2.2 | 21.5 ± 6.7 | 1.3 ± 0.3 | 2 ± 0.4 |
| MIX | 6.1 ± 1.3 | 8.1 ± 1.3 | 2.7 ± 0.4 | 1.8 ± 0.1 | 11.3 ± 3.4 | 20.3 ± 6.1 | 3.1 ± 1.5 | 2.3 ± 0.7 |

concluded that mushrooms are a clear example of how low value waste, which is produced primarily through activities of the agricultural, forest and food processing industries can be converted to higher value material useful to mankind.

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# Performance and genetic diversity of some sesame (*Sesamum indicum* L.) accessions based on morpho-agronomic traits and seed proximate composition in Kwara State of Nigeria

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**Performance and genetic diversity of some sesame (*Sesamum indicum* L.) accessions based on morpho-agronomic traits and seed proximate composition in Kwara State of Nigeria**

**Abstract:** This study evaluates eleven sesame accessions in Nigeria for performance and genetic diversity using morpho-agronomic traits, chlorophyll contents and nutrient composition in a complete randomized experimental design with five replicates. The results showed 'Igboho Black', '02M' and 'Kenan 4' had the best growth attributes. Although 'NGB0090' and 'Exsudan' matured early, 'E8', 'Bogoro Local' and 'Kenan 4' had the best yield attributes. Seeds produced were predominantly milky-white, an accession had black seeds, while three produced white seeds. Plant height positively correlated with number of leaves and leaf area as well as peduncle length. Days to 50 % flowering positively correlates with days to maturity. So also the number of capsule per plant, capsule dimension and seeds per capsule. The moisture content in seeds of the accessions was < 3.5 %, ash (4.5-5.9 %), crude protein (5.3-7.4 %), fat and oil (53.6-60.5 %), and carbohydrate < 30 %. Out of the eight components that accounted for the observed variations, the PC-1 and PC-2 contributed 65.42 %. The dendrogram revealed that 'NGB00960' and 'NGB00963' which had ''Kenan 4' as a distant member are the closest relatives, while 'NGB00390' and '01M' are the most diverse. The study concludes that the accessions are genetically and phenotypically varied and the existing diversity can be harnessed for selecting high yielding and adaptable variety for the development of improved cultivars.

**Key words: c**hlorophyll content; genetic diversity; morphometric attributes; oilseed

**Preučevanje uspevanja in genetske raznolikosti nekaterih akcesij sezama (*Sesamum indicum* L.) na osnovi morfoloških in agronomskih lastnosti ter zgradbe semen v državi Kwara, Nigerija**

**Izvleček:** V raziskavi je bilo ovrednoteno sedem akcesij sezama iz Nigerije glede na uspevanje in genetsko raznolikost z uporabo morfološko-agronomskih lastnosti, vsebnosti klorofila in hranil v popolnem naključnem bločnem poskusu s petimi ponovitvami. Rezultati so pokazali da so imele akcesije 'Igboho Black', '02M' in 'Kenan 4' najboljše lastnosti. Čeprav sta 'NGB0090' in 'Exsudan' dozorevali zgodaj, so imele 'E8', 'Bogoro Local' in 'Kenan 4' najboljše lastnosti pridelka. Semena so bila pretežno mlečno bela, ena akcesija je imela črna semena, tri pa popolnoma bela. Višina rastlin je bila v pozitivni korelaciji s številom listov kot tudi z dolžino cvetnih pecljev. Dnevi potrebni do 50 % cvetenja so bili v pozitivni povezavi z dnevi do zrelosti. V podobni povezavi je bilo tudi število plodov na rastlino, dimenzija glavic in število semen na glavico. Vsebnost vlage v semenih je bila večja od 3,5 %, vsebnost pepela 4,5-5,9 %, celokupnih beljakovin 5,3-7,4 %, maščob in olj 53,6-60,5 % in ogljikovih hidratgov več kot 30 %. Od osmih komponent, ki so pripspevale k ugotovljeni raznolikosti sta komponenti PC-1 in PC-2 pripevali 65,42 %. Dendrogram je pokazal, da so 'NGB00960' in 'NGB00963', ki vključujeta oddaljeno akcesijo 'Kenan 4' najožji sorodniki, med tem, ko sta 'NGB00390' in '01M' najbolj raznoliki. Na osnovi raziskave je bilo ugotovljeno, da so akcesije genetsko in fenotipsko različne in da bi se njihova raznolikost lahko izkoristila za izbor visoko donosnih in prilagodljivih različic pri razvoju izboljšanih sort.

**Ključne besede:** vsebnost klorofila; genetska raznolikost; morfometrične lastnosti; vsebnost olja v semenih

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## 1 INTRODUCTION

Sesame (*Sesamum indicum* L.) also known as 'benniseed' is an important member of the Pedaliaceae family. It is an ancient tropical crop of high economic importance, widely known for its seed edible oil. The origin of the cultivated sesame is controversial. There are claims that it originated in the Savannah of tropical Africa and was introduced into India and China by early humans (Purseglove, 1977; Naturland, 2002; Bedigian, 2004; Behera et al., 2017). There is also a concept of Indo-Asian centre of origin and diversity of sesame. While the presence of wild types and abundance supports Africa as the centre of origin (Ram et al., 1990), interspecific crosses and lignan analyses refuted the same (Bedigian et al., 1985; Bedigian & Harlan, 1986) and pointed at Indo-Asia origin of progenitor sesame.

The world sesame production is approximately 5.5 million metric tonnes, of which 70 % are produced in Asia while Africa accounted for about 26 %. African major producers include Ethiopia, Sudan, Uganda and Nigeria (FAOSTAT, 2011; Pathak et al., 2014). India ranks top in global sesame production followed by China (Anthony et al., 2015). However, the average yield per hectare is highest in China, followed by India, and Tanzania. Meanwhile, China is the world's largest consumer of sesame (FAOSTAT, 2020). Despite the peachy production of sesame in Nigeria, productivity is comparatively low. There is a need to accelerate its production through the use of improved genotypes and farming practices.

Sesame is a herbaceous annual plant that can grow up to 1.5 m in height. The fruit has many seeds enclosed in a capsule. The seeds are small, oval, and almost oblate in shape. They vary from cream-white to charcoal-black but often white or black and may also be, yellow, red or brown depending on the variety (Naturland, 2002; Bennet, 2011). Although sesame is adapted to various ecological conditions, it thrives better on well-drained, fertile soils. The temperature requirement is between 20-35 °C (Misganaw et al., 2015). Most varieties are drought and insect resistant with negligible economic loss from pests (Langham et al., 2008). The seed oil is rich in sesamol which is an important anti-oxidant, and polyunsaturated fatty acid that is safe for human and animal consumption (Ashri, 1998). Also, the oil is used majorly for cooking, for making margarine, soap, paints, lubricants and lamp fuel. The seeds are equally nutritious, containing protein (18–25 %), carbohydrate (about 13 %), calcium, phosphorus, iron, essential minerals and vitamins (Bedigian et al., 1985; Bedigian, 2004). Sesame seed contains more oil than major oilseed crops such as peanut and soybean (Ashri, 1998). However, seed oil quantity and quality may vary, depending on the genotype and growth conditions (Myint et al., 2020). The medicinal usage of sesame oil as an anti-oxidant, anti-tumour, anti-cholesterol and anti-microbial agent have been reported (Sankar et al., 2005; Costa et al., 2007).

Sesame improvement to meet the growing demand for seed oil requires adequate knowledge of the genetic diversity and relationship among the available germplasm. The genetic diversity among sesame accessions and genotypes based on morphological parameters has been established from different studies conducted over the years (Bisht et al., 1998; Baydar, 2005; Arriel et al., 2007; Furat & Uzun, 2010; Parameshwarappa et al., 2010; Frary et al., 2015; Kiranmayi et al., 2016; Azeez et al., 2017; Iqbal et al., 2018). The authors reported significant variations among the genotypes studied and elucidated the genetic relationship among the accessions. Furthermore, more recently, Adu-Gyamfi et al. (2019) employed morphological traits in the assessment of the diversity of selected sesame genotypes cultivated in Northern Ghana. However, Nigerian accessions of sesame have not been well characterised, more so, the number of available accessions cannot be accurately ascertained. So there is a need to characterise as many accessions that are available to generate basal information that could be used for the crop cultivation and improvement

Although Nigeria is one of the leading producers of sesame in Africa, there is insufficient information on the diversity of sesame accessions in cultivation, hence only a little progress is made on sesame breeding and the development of elite cultivars in the country. To improve sesame production for seed and oil yield in Nigeria, there is a need for characterization, performance evaluation and assessment of genetic diversity of the available genotypes. To this end, the present study assessed the performance, morphometric variation, and proximate composition of eleven accessions of cultivated sesame (*S. indicum*) in the Kwara State of Nigeria with the view of identifying and selecting the best and promising accessions in terms of yield and nutritional quality that could be improved for higher productivity.

## 2 MATERIALS AND METHODS

### 2.1 COLLECTION OF THE SESAME ACCESSION

Eleven accessions of *Sesamum indicum* L. collected from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State, Nigeria were used for the study. NACGAB is the National Agency responsible for the collection, evaluation and conservation of genetic resources in Nigeria. The accessions (Igboho black, 02M, 03M, E8, 01M, Exsudan, Kenan 4,

NGB00960, NGB00963, Bogoro local and NGB00390) are part of the mini-core collection of cultivated sesame from the growing regions of Nigeria. The collections are maintained at NACGRAB genebank facility (All information and details of the accessions are available at NACGRAB, Nigeria).

## 2.2 THE EXPERIMENTAL SITE

The pot experiment was conducted at the University of Ilorin Botanical Garden, Ilorin, Kwara State, Nigeria. The garden is at the Latitude 8° 24′N - 8° 36′N and Longitude 4°10′E - 4° 36′E in the Guinea savanna zone of Nigeria. The annual rainfall is 990-1200 mm and the temperature is between 33 – 37 °C (Olaniran, 1988; Ajadi et al., 2011). The relative humidity is about 75 % during the rainy season, and 65 % during the dry season. During the dry season (November – May), the sun shines for 6.5 - 7.5 hours per day (NIMET, 2018)

Ten clean and healthy seeds of each accession were sown separately into a pot (30 × 25 cm), filled with loose soil taken from 0-15 cm soil depth, from a location with distinct textural characteristics of sandy-loamy in the garden. The experiment was laid out in a complete randomized design (CRD) with 0.5 m between the pots in five replicates for each accession in a screen house facility. On germination, the seedlings were thinned to 2 plants per pot. Adequate watering and weeding practices were carried out as required. Meanwhile, no fertilizer or nutrient supplement was added to the plant through the weeks of evaluation. The performance of the accessions was evaluated at two-week intervals until 14 weeks after sowing (WAS) using the International Plant Genetic Resources (IPGRI, 2004) descriptors for sesame. The growth variables evaluated include; plant height, number of leaves per plant, leaf dimension, petiole length, number of primary branches and stem girth. Furthermore, at maturity data were collected on the flowers and seed related characters such as days to 50 % flowering, the number of flowers per plant, number of capsules per plant, capsule length and width, 1000-seed mass and mass of seeds per plant. Qualitative characters such as stem base colour, leaf and flower colour, seed colour and shape were also recorded.

## 2.3 CHLOROPHYLL AND CAROTENOID DETERMINATION

The photosynthetic pigment extraction and quantification were carried out as described by Porra et al. (1989). Fresh leaf samples collected from the accessions early in the morning were used for the pigment analysis. In brief, chlorophyll extraction was performed by dipping 12.5 mg of each leaf sample into a sample bottle containing 3.5 ml acetone in five replicates. The set-up was left for 72 hours in a dark cupboard at room temperature, after 72 hours, the bottle was vigorously shaken, and then the bleached leaf was removed, leaving behind the leaf homogenates in the bottle. The chlorophyll and carotenoid contents were quantified using a spectrophotometer (Jenway, Model 6305, Bibby Scientific, USA). The amount of chlorophylls a, b, carotenoid, and total chlorophyll was calculated using the Porra et al. (1998) equations:

- Chlorophyll a (mg $ml^{-1}$) = [(12.21 x $A_{663}$) – (2.81 x $A_{646}$)]
- Chlorophyll b (mg $ml^{-1}$) = [(20.13 x $A_{646}$) – (5.03 x $A_{663}$)]
- Total chlorophyll = Chlorophyll a + Chlorophyll b
- Carotenoids = [(1000 x A470) – (3.27 x Chl.a) – (104 x Chl.b)] / 198

Where *A* is the absorbance wavelength read from the Spectrophotometer.

## 2.4 DETERMINATION OF THE PROXIMATE COMPOSITION OF SESAME SEED

The proximate analysis followed the standard protocol of AOAC (2000). The moisture content was determined by oven-drying the seeds at 105 °C for 24 hours. The dried seeds were grounded and the moisture content was estimated. Crude lipid was extracted from the samples with petroleum ether as solvent using the Soxhlet apparatus technique, then the percentage crude lipid was determined as described in the protocol (AOAC, 2000). The nitrogen composition was determined by Micro-Kjeldahl's method using Electrothermal (Model MQ3868B/E, Fisher Scientific, Austria). The total nitrogen was estimated using the relationship N x 5.95, and the resultant values are taken as the percentage crude protein of the seeds. The percentage of fibre and ash contents (% minerals) was also obtained using a standard procedure (AOAC, 2000). The total carbohydrate present in the seeds was derived by the differential method as:

% Total carbohydrate = 100 – (% moisture + % crude protein + % crude lipid + % ash + % crude fibre)

## 2.5 STATISTICAL ANALYSIS

Data collected were subjected to Analysis of Variance (ANOVA) using SPSS statistical package version 21. The means were separated by New Duncan's Multiple Range Test (N-DMRT), and the probability value of 0.05

was used as a benchmark for separating a significant difference in the means. Growth and yield parameters were correlated using Genstat 19th Edition (Payne et al., 2007). Genetic relationship and cluster analysis were conducted, based on the agglomerative technique of the unweighted pair group of arithmetic average (UPGMA) method. The genetic relationships of the accessions were graphically presented as a dendrogram.

## 3 RESULTS

### 3.1 QUANTITATIVE GROWTH PARAMETERS OF THE ACCESSIONS

The plant height varied across the weeks of evaluation (Fig. 1). The average plant height for the accessions are 2.45, 7.85, 21.11, 43.79, 57.61 and 61.03 cm for 2, 4, 6, 8, 10 and 12 WAS respectively. Accessions 02M, Kenan 4 and 01M had the best growth performance across the weeks. Four accessions (Igboho Black, 03M, E8 and Exsudan) recorded plant height below the average over the period (Figure 1a). At 2 WAS, 'Bogoro Local' had a 6.0 mean number of leaves per plant which is far higher than the average of 3.60 leaves per accession (Fig 1b). At the same time, 'Exsudan' had 1.60 leaves per plant. Similarly, at 4-12 WAS, 'Bogoro Local' maintained the best performance in terms of leaf formation while 'Exsudan' showed the least. Other accessions that exhibit good leaf formation are; Igoho Black, NGB00390 and 01M. Meanwhile, 03M, E8 and NGB00963 had the number of leaves less than the average. However, a fall was recorded for most

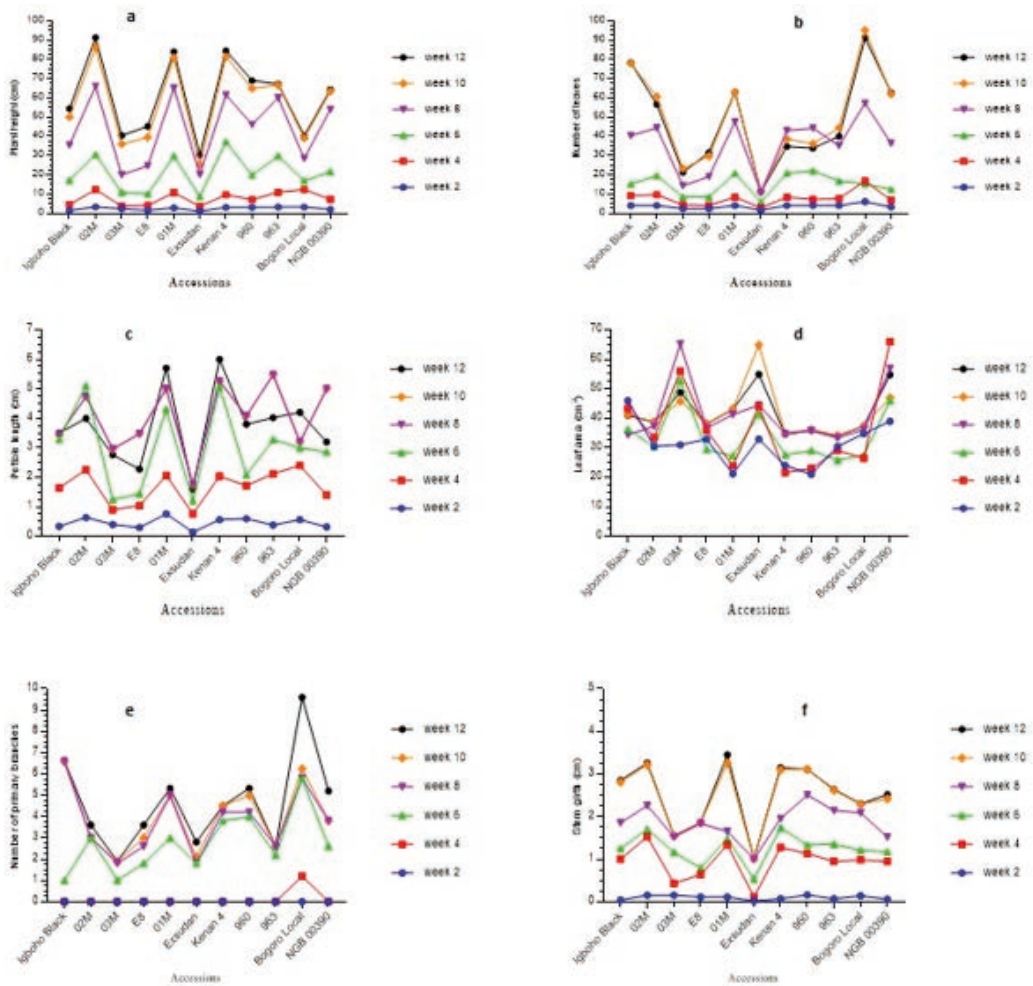


**Figure 1**: Growth parameters (2 – 12 weeks after sowing) of the eleven accessions of *Sesamum indicum* evaluated for variability and genetic diversity of accessions. Kenan 4 and 00960 were the first to begin senescence, observed from the 10 WAS, followed by six accessions (Bogoro Local, 00963, Kenan 4, 02M, NGB00960 and 03M) at 12 WAS with a corresponding decrease in stem girth. Bogoro Local had the highest number of primary branches, an average of 9.60, closely followed by Igboho black (6.65), 01M and NGB00960 both having 5.33. Altogether, Eksudan and 03M had the poorest performance in quantitative characters observed

accessions at 10-12 WAS, indicating the inception of senescence.

The petiole length was progressive till 10 WAS (Fig 1c). Five accessions (01M, 02M, Kena 4, NGB00960 and NGB00963) consistently had petiole length higher than the average. Worthy of mention is that 'Exsudan' which had the least number of leaves per plant also had the shortest petiole. The accessions were diverse in leaf area which did not follow a definite trend, though most accessions' leaf area was above the average (Fig 1d). However, at 8-12 WAS, '01M' had the largest assimilative surface followed by '02M' and 'Bogoro Local'. The least leaf area occurred in 'Exsudan' over the period. No branching occurred in the accessions at 2-4 WAS except 'Bogoro Local' that had 1.20 branches per plant. Only two accessions (NGB00960 and Bogoro Local') had the number of branches ≥ 4.0 per plant at 6 WAS, even at 8 WAS, only 1.80 branches were found on 'Exsudan 'and 03M. More branches were observed in 'Igboho' black at 6-8 WAS reaching its peak in week 8 (6.60). Generally, the average number of branches for the accession was low across the weeks of evaluation (Fig 1e). Most accessions fell below the average, only four (Igboho Black, 01M, NGB00960 and Bogoro Local) had a higher number of branches. The stem diameter of the accessions progressed with the week of evaluation, with most accessions recording stem diameter above average, the least occurred in 'Exsudan' (Fig 1f).

At maturity, growth parameters varied significantly ($p < 0.05$) for the accessions (Table 1). The average plant height of the accessions was 62.19 cm, 'Igboho Black', '03M', 'E8', 'Exsudan', and 'Bogoro Local' plants are significantly short. The tallest accession (94.84 cm) was 02 M, while the Exsudan was the shortest (27.76 cm). Whereas 'Bogoro Local' was the best in terms of leaf formation with over 78 leaves per plant which is significantly higher than the accessions' average (31.54). In contrast, 'Exsudan' showed poor performance with just 8.66 leaves per plant. The accessions petiole length ranged from 1.60 to 6.01 cm, five accessions had petiole lengths greater than the average (3.85 cm), and the longest occurred in 'NBG00390'. Accession 01M produced leaves with the highest surface area (43.06 $cm^2$). The 'Exsudan' which had the least number of leaves and shortest petiole also produced the smallest assimilatory surface (14.91 $cm^2$). The most branched of the accession is Goboro Local with over 9 branches per plant. There are other five accessions (Igboho Black, 01M, Kenan 4, 00960 and NGB00390) with more than 4 branches per plant. Branch formation remained poor in '03M', attaining only 1.90 primary branches per plant at maturity which is well below the average of all accessions (4.65). The stem girth of the accessions was between 1.76 and 3.41 cm, while nine accessions had stem girth > 2 cm, two accessions (03M and Exsudan) had less (Table 1).

## 3.2 YIELD ATTRIBUTES PERFORMANCE

There are significant differences ($p < 0.05$) in the performance of the yield attributes of the sesame accessions. Among the accessions, E8 showed superior yield features (Table 2). Flowering was delayed in 'NGB00390' and 'Exsudan', both took longer days to attain 50 % flowering (54.25 and 50.11 days respectively). 'E8', 'Bogoro Local' and 'Kenan 4' attained 50 % flowering in 36.33, 37.35 and 39.56 days respectively, about a week earlier than the average (43.14 days) for the accessions. The average number of days to attain physiological maturity for the accessions was 100.96 days which was not significantly different. Whereas, 'NGB00390' matured much earlier at 89.33 days. The last accession to reach maturity was 02M (108.66 days), followed by 03M (105.54 days) and NGB00960 (104.28 days). The highest number of capsules per plant (56.66) occurred in 'E8', followed by 'Bogoro Local' (54.02 capsules). Although the average number of capsules per plant for the accessions was 40.84, however, seven accessions (Igboho Black, 03M, 01M, NGB00960, NGB00963, Exsudan and NGB00390) produced less, and the least occurred in 'NGB00390' which produced 24.08 capsules per plant.

Furthermore, the number of seeds per plant varied significantly ($p \leq 0.05$) among the accessions. All accessions had more than 30 seeds in each capsule, with an average of 42.08 seeds per capsule. The highest seeds per capsule were recorded in 'Bogoro Local' (58.2 seeds), 10 seeds more than 02M (47.01 seeds) which is the second in the ranking. Fewer seeds per capsule were found in 'NGB00390' (34.10) which is not statistically different from 'Exsudan' (36.02). The average mass of a thousand seeds (MTS) of the accessions was 2.48 g. Three accessions (03M, Exsudan and NGB 00390) had MTS less than 2.00 g and the least obtained in '03M' (1.22 g). Nevertheless, 'E8' produced the heaviest seeds (4.31 g), about 2.0 g more than the average for the accession. Besides, 'E8' had the highest (8.52 g) total mass of seeds per plant (TMSP), which means the accessions is superior in term of seed mass. Next to 'E8' was 'Bogoro Local' with 6.28 g TMSP. Both accessions also recorded the best capsule features, having capsules lengths of 2.23 and 2.58 cm, capsules width 0.64 and 0.92 cm respectively. In contrast, three accessions (03M, Exsudan and NGB00390 performed poorly for MTS and TMSP. Whereas the three accessions had capsule length and width below average, 'NGB00390' had the least capsule length of all the acces-

**Table 1**: Vegetative growth variables of eleven accessions of *Sesamum indicum* at physiological maturity

| Accessions | Plant height (cm) | Number of leaves | Petiole length (cm) | Leaf Area ($cm^2$) | Number of primary branches | Stem girth (cm) |
|---|---|---|---|---|---|---|
| Igboho Black | 56.80e | 68.81b | 3.12de | 41.02a | 6.65b | 2.87cd |
| 02M | 94.84a | 37.47d | 5.01ab | 38.74ab | 3.60e | 3.25ab |
| 03M | 41.28fg | 12.80gh | 3.33de | 15.84f | 1.90g | 1.94f |
| E8 | 44.84f | 23.20ef | 2.28ef | 38.12ab | 3.60e | 2.09ef |
| 01M | 88.04ab | 41.83c | 5.51ab | 43.06a | 5.33bc | 3.41a |
| Exsudan | 27.76h | 8.66i | 1.60g | 14.91f | 2.80ef | 1.76f |
| Kenan 4 | 84.24b | 14.42g | 3.50d | 34.63cd | 4.50de | 3.02ab |
| NGB960 | 69.84c | 22.25ef | 3.01e | 36.02bc | 5.33bc | 3.01ab |
| NGB963 | 68.20c | 13.80g | 4.50bc | 34.10cd | 2.60ef | 2.29de |
| Bogoro Local | 41.72fg | 78.45a | 4.51bc | 37.40bc | 9.60a | 2.49de |
| NGB 00390 | 66.48cd | 25.25ef | 6.01a | 24.87e | 5.20bc | 2.53cd |
| Mean | 62.19 | 31.54 | 3.85 | 32.61 | 4.65 | 2.61 |
| Max | 94.84 | 78.45 | 6.01 | 43.06 | 9.60 | 3.41 |
| Min | 27.76 | 8.66 | 1.60 | 14.91 | 1.90 | 1.76 |

Values with the same letter(s) along a column are not significantly different at $p < 0.05$. The mean is the average for all the accessions, the Max is the maximum value and the Min is the minimum value for each parameter

sions (1.68 cm), and 'Exsudan' with the least apsule width (0.58 cm) as presented in Table 2.

The boxplot analysis (Fig. 2) revealed that the plant height of the accessions lies closer to the average (62.19 cm), the little deviation was accounted for greatly by 'Exsudan'. The plots also showed that most accessions performed above the average in leaf formation, petiole length, the number of days to 50 % flowering, number of capsules per plant and number of seeds per capsule. The accessions' performance aligned on the median values for stem girth, days to maturity and the total mass of seeds per plant. For other characters such as the number of primary branches, leaf areas, capsule length and breadth, most of the accessions performed below the average. This indicates the presence of significant variation in both growth and yield characters of the accessions.

### 3.3 QUALITATIVE CHARACTERS

The summary of the qualitative characters of the sesame accessions observed at maturity is presented in Table 3. The accessions leaf colour was either dark or light green. Only three accessions (Igboho Black, 02M and E8) exhibited dark-green leaves, others produced pale or light green leaves. The basal stem colour varied from greenish-brown, dark-green, pale green and green with specks of yellow. '03M', '01M', 'Exsudan' and 'Kenan 4', produced white flowers, 'Bogoro Local' and 'Igboho Black' had purple, '02M', 'E8', 'NGB00960' and 'NGB00963' had milky-white while 'NGB00390' flowers are brownish white. The accessions' capsules were elongated in shape and predominantly brown, enclosing numerous oval seeds which are either white, milky, brown or black (Table 3).

### 3.4 THE PRINCIPAL BIPLOT AND CORRELATION ANALYSES

The principal biplot analysis of the agro-morphological and seed related characters revealed that eight components (PC) contributed significantly to the observed variations among the sesame accessions. Out of the components, PC-1 and PC-2 which accounted for 45.17 and 20.25 % respectively, and cumulated to 65.42 % of the variables are the most important (Fig. 3). In the PC-1, plant height, the number of leaves per plant, number of primary branches, leaf areas, number of capsules per plant, number of seeds per capsule and a thousand seed mass are the main sources of variation of the accessions under PC-1. The variables that significantly contributed to the observed variation in the PC-2 are; plant height, petiole length, leaf area and the number of seeds per capsule. On the contrary, stem girth, number of days to 50 % flowering, days to maturity, capsule length and

**Table 2**: Yield attributes of seed related characters of eleven accessions of *Sesamum indicum* evaluated for variability and genetic diversity study at maturity

| Accession | DT 50 % F | DTM | NCP | NSC | CL (cm) | CD (cm) | MTS (g) | TMSP (g) |
|---|---|---|---|---|---|---|---|---|
| Igboho Black | 42.02b | 98.45ab | 37.10c | 39.02b | 2.01bc | 0.62de | 2.24bc | 3.48d |
| 02M | 46.25ab | 108.66a | 44.21b | 47.01ab | 2.22ab | 0.79b | 2.76b | 5.52ab |
| 03M | 42.05b | 105.54a | 39.33c | 37.99b | 1.89cd | 0.68cd | 1.22d | 2.45de |
| E8 | 36.33c | 100.45a | 56.66a | 42.01ab | 2.23ab | 0.64d | 4.31a | 8.52a |
| 01M | 42.06b | 101.25a | 37.25c | 38.02b | 2.12bc | 0.61de | 2.20bc | 4.42bc |
| Exsudan | 50.11a | 98.28ab | 28.44d | 36.02c | 2.02bc | 0.58e | 1.24d | 2.01e |
| Kenan 4 | 39.56bc | 101.58a | 51.33a | 43.01ab | 2.21ab | 0.72bc | 2.92ab | 5.82a |
| NGB00960 | 42.25b | 104.28a | 38.55c | 38.02b | 2.11bc | 0.67cd | 2.70b | 3.95c |
| NGB00963 | 42.33b | 100.56a | 38.25c | 42.02ab | 2.21ab | 0.66cd | 2.72b | 4.02c |
| Bogoro Local | 37.35c | 102.20a | 54.02a | 58.20a | 2.58a | 0.92a | 2.96ab | 6.28a |
| NGB 00390 | 54.25a | 89.33b | 24.08d | 34.10c | 1.68d | 0.72bc | 1.96c | 2.08e |
| Average | 43.45 | 100.68 | 40.77 | 42.08 | 2.12 | 0.70 | 2.52 | 4.54 |
| Max | 54.25 | 108.66 | 56.66 | 58.20 | 2.58 | 0.92 | 4.31 | 8.52 |
| Min | 36.33 | 8933 | 24.08 | 34.10 | 1.68 | 0.58 | 1.22 | 2.01 |

Values with the same letter(s) along a column are not significantly different at $p < 0.05$. The mean is the average for all the accessions, the Max is the maximum value and the Min is the minimum value for each parameter. DT 50 % F: Days to 50 % flowering; DTM: Days to physiological maturity; NCP: Number of capsules per plant; NSC: Number of seeds per capsule; CL: capsule length; CW: capsule width; MTS: Mass of a thousand seeds per plant; TMSP: Total mass of seeds per plant

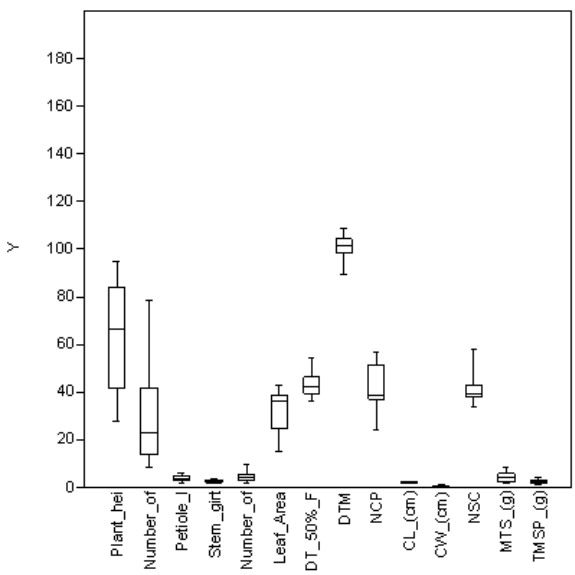


**Fig 2**: The boxplot of the growth and reproductive characters of eleven accessions of *Sesamum indicum* evaluated for performance and genetic diversity. The boxes revealed the skewness of the data from the mean proportion of the studied accessions with values below or above the mean. The y-axis shows the magnitude of the measured characters while the x-axis contains the characters

breadth are without significant effect. Thus, plant height, number of leaves, leaf area, days to flowering, days to maturity, number of capsules per plant, seeds per capsule and seed mass are the major factors of variation in the sesame accessions.

The matrix of correlations between the vegetative and reproductive characters of the sesame accessions is presented in Figure 4. At a significant level of $p < 0.01$, the number of leaves is associated with the number of primary branches (r = 0.8701). Also, a strong positive correlation occurred between capsule length and the number of seeds per capsule (r = 0.8869); the number of capsules per plant, capsule width, and the number of seeds per capsules (r = 0.8393). Besides, the mass of a thousand seeds strongly related to the total mass of seeds per plant, and the total mass of seeds per plant was strongly linked with the number of capsules per plant (r = 0.9192). So also, is the number of primary branches and number of leaves (r = 0.8700); stem girth and plant height (r = 0.8803). Furthermore, at $p < 0.05$, plant height and leaf area (r = 0.5876), days to maturity and number of capsules per plant, the number of seeds per capsule and days to maturity, capsule length and days to maturity are linked (with r values above 0.4 and less than 0.8). Likewise, leaf area correlated with plant height, number of primary branches, number of leaves, number of capsules per plant and seed mass parameters. Other correlated characters are; the number of capsules per plant, number of seeds per

**Table 3:** Summary of the qualitative characters of eleven accessions of sesame for similarities and differences in their morphological features at maturity

| Accession | Leaf colour | Stem base colour | Flower colour | Capsule colour | Capsule shape | Seed shape | Seed colour |
|---|---|---|---|---|---|---|---|
| Igboho Black | Dark green | Greenish brown | Whitish purple | Brown | Elongated | Oval | Black |
| 02M | Dark green | Yellowish green | Milky white | Brown | Elongated | Oval | Milky white |
| 03M | Light green | Dark green | White | Brown | Elongated | Oval | Milky white |
| E8 | Dark green | Dark green | Milky white | Brown | Elongated | Oval | Milky white |
| 01M | Light green | Light green | White | Brown | Elongated | Oval | White |
| Exsudan | Light green | Light green | White | Greenish brown | Elongated | Oval | White |
| Kenan 4 | Light green | Greenish white | White | Greenish brown | Elongated | Oval | White |
| NGB00960 | Light green | Greenish white | Milky white | Greenish brown | Elongated | Oval | Milky white |
| NGB00963 | Light green | Light green | Milky white | Brown | Elongated | Oval | Milky white |
| Bogoro Local | Light green | Greenish-white | Purple | Yellowish-brown | Elongated | Oval | Brownish-yellow |
| NGB00390 | Yellowish green | Yellowish green | Brownish white | Brown | Elongated | Oval | Brownish-yellow |

Values with the same letter(s) along a column are not significantly different at $p < 0.05$. The mean is the average for all the accessions, the Max is the maximum value and the Min is the minimum value for each parameter

plant and capsule dimension. More associations of significant interaction between the morphometric and seed related characters are shown in Figure 5. However, a negative correlation ($r < 0$) existed between the number of days to 50 % flowering and the number of seeds, capsules per plant, capsule length, and mass of seeds. There was approximately no correlation between plant height and the number of seeds per plant, number of leaves and the number of primary branches.

### 3.5 PHOTOSYNTHETIC PIGMENTS CONTENT

Chlorophyll analysis was conducted on each accession every four (4) weeks till maturity to determine the quantity and variations in chlorophyll a, b, and carotenoids contents. Over the period of vegetative growth (2-12 WAS), chlorophyll-a was relatively more abundant in the leaf compared to other photosynthetic pigments (Fig. 5). At 4 WAS, 'Exsudan' had the highest chlorophyll-a (4.78 mg $l^{-1}$) and chlorophyll-b (1.54 mg $l^{-1}$), same for the total chlorophyll (6.32 mg $l^{-1}$) (Fig. 5), followed by 'Bogoro Local', 'NGB00390', 'NGB00960', and '02M' which all had chlorophyll-a > 3.00 mg $l^{-1.}$ and chlorophyll-b > 4.00 mg $l^{-1}$. At the same period, '01M' had the least total chlorophyll (1.65 mg $l^{-1}$). Likewise, the highest carotenoid content (1.21 mg $l^{-1}$) was found in 'Exsudan'. Four accessions (03M, Igboho Black, E8 and 01M) had carotenoid content < 0.70 mg $l^{-1}$. Chlorophyll-b was generally low at the seedling stage, although notably high in 'Exsudan' (1.54 mg $l^{-1}$), '01M' recorded the least amount of the pigments.

At 8 WAS, the total chlorophyll content of the accessions increased for some accessions, 'Exsudan' yet had the highest chlorophyll (11.56 mg $l^{-1}$) (Fig. 5). The carotenoid contents of all the accessions were similar except 'NGB00960' and 'NGB00963' which showed a decline. Carotenoid increased as much as 43 % in '01M', and 'Exsudan' by 25 %, amounting to 1.58 mg $l^{-1}$ which was the highest recorded while the accession NGB00963 recorded the least carotenoid (0.38 mg $l^{-1}$). Although, the total chlorophyll contents of the accessions are similar, not-

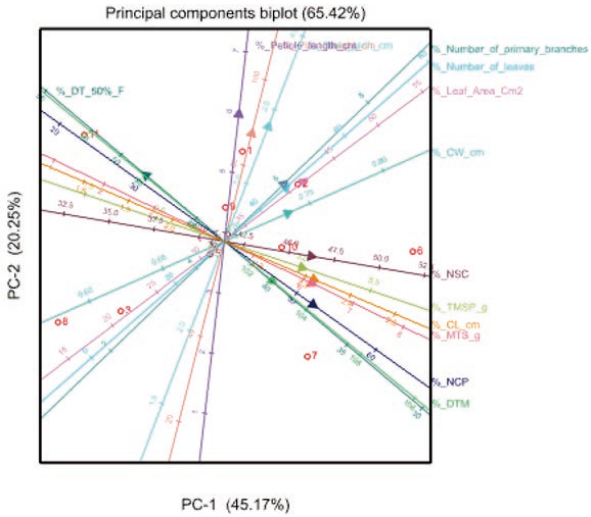


**Fig. 3**: The principal biplot of PC-1 vs PC-2 of contributions of the growth and seed related parameters to the observed variations in eleven accessions of cultivated sesame. Eight attributes contributed significantly to the observed variation of which PC-1 and PC-2 accounted for 65.42 % cumulative effects. The direction of the arrows shows the loading effects of the parameters in marking the variation among the accessions

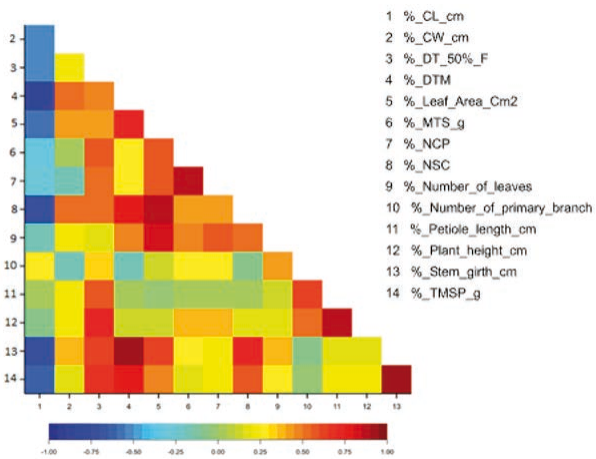


**Fig. 4:** The matrix of correlations between the vegetative and reproductive characters of the eleven sesame accessions evaluated for performance and genetic diversity. The deep brown coloured signifies significant trait associations at $p < 0.01$ with $r$ values ≥ 0.75, light brown shows significant trait association at $p < 0.05$ with $r$ values ≥ 0.45 ≤ 0.74. The yellow colour had $r$ values ≥ 0.02 ≤ 0.30. DT 50 % F: Days to 50 % flowering; DTM: Days to physiological maturity; NCP: Number of capsules per plant; NSC: Number of seeds per capsule; CL: Capsule length; CW: Capsule width; MTS: Mass of a thousand seeds per plant; TMSP: Total mass of seeds per plant

withstanding, 'Exsudan' had the highest total chlorophyll (11.57 mg $l^{-1}$). Chlorophyll contents of 'Exsudan', '03M' and 'Bogoro Local' are at per at 12 WAS, and the trio recorded the highest. Likewise, 'Kenan 4' and '01M' had a statistically similar amount of carotenoid. Whereas the lowest (0.73 mg $l^{-1}$) occurred in 'NGB00960', 'Exsudan' has the highest chlorophyll-a (8.37 mg $l^{-1}$) and b (4.35 mg $l^{-1}$). At maturity, '03M', 'Exsudan' and 'Bogoro Local' are not significantly different in carotenoid contents. 'Exsudan' had the highest total chlorophyll (12.87 mg $l^{-1}$) with chlorophyll-a, and b contents of 8.31 mg $l^{-1}$ and 4.56 mg $l^{-1}$ respectively. All through the study, 'Exsudan' had the highest photosynthetic pigments content (chlorophyll-a, b and carotenoids) among the accessions. However, there was a general decline in the synthesis of the plant pigments.

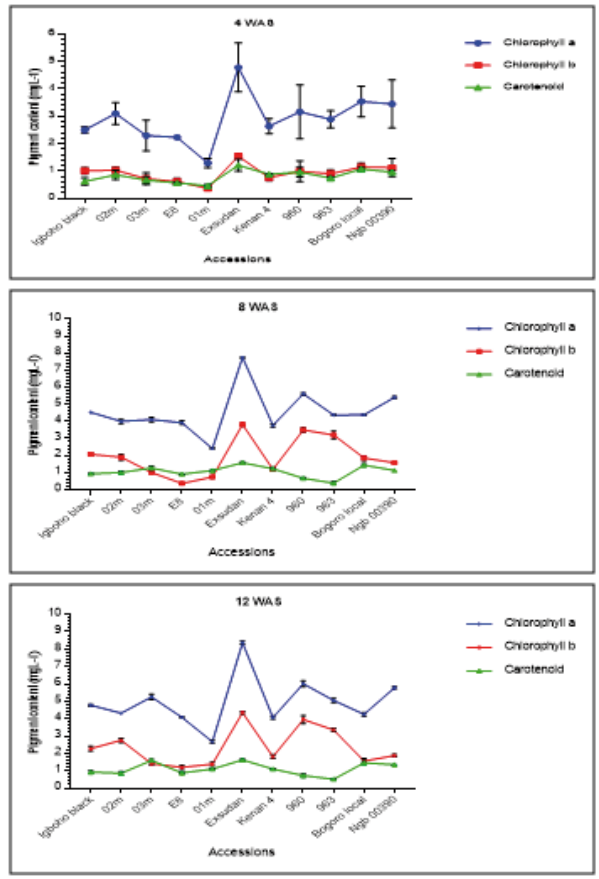


**Fig. 5**: Chlorophyll and carotenoid contents of eleven accessions of *Sesamum indicum* evaluated for performance and genetic diversity at 4, 8 and 12 weeks after sowing

## 3.6 PROXIMATE COMPOSITION OF THE ACCESSIONS

The proximate composition of the sesame accessions varied significantly ($p \leq 0.05$) as shown in Table 4. 'Igboho Black' and 'E8' had the highest moisture

**Table 4**: Proximate composition of the eleven accessions of *Sesamum indicum* evaluated for variability and genetic diversity

| | Percentage composition (%) | | | | | |
|---|---|---|---|---|---|---|
| Accession | Moisture | Ash | Crude protein | Crude fibre | Fat and oil | Carbohydrate |
| Igboho Black | 3.40a | 5.98a | 5.60g | 5.77a | 53.61j | 27.63b |
| 02M | 3.10e | 4.84cd | 5.33i | 4.69e | 57.25e | 24.79c |
| 03M | 3.25b | 4.97bc | 5.80h | 4.75d | 59.83b | 21.49e |
| E8 | 3.38a | 5.10b | 7.21c | 4.99b | 60.50a | 18.81h |
| 01M | 3.20bc | 4.92bc | 7.35ab | 4.81bc | 58.76d | 21.47e |
| Exsudan | 3.18cd | 4.85cd | 6.80e | 4.78cd | 58.95c | 21.33f |
| Kenan 4 | 2.68g | 4.60ef | 6.90d | 4.49g | 60.46a | 20.79g |
| NGB00960 | 2.74f | 4.80d | 7.40a | 4.62f | 56.80f | 23.76d |
| NGB00963 | 2.03i | 4.55ef | 5.85g | 4.38h | 54.47h | 28.62a |
| Bogoro Local | 2.04h | 4.65e | 6.58f | 4.47g | 54.25i | 27.62b |
| NGB00390 | 3.15d | 4.88cd | 6.81e | 4.80c | 55.58g | 24.79c |

Values with the same letter(s) along a column are not significantly different at $p < 0.05$

contents of 3.40 % and 3.38 % respectively, followed by '03M' (3.25 %), '01M' (3.20 %), 'Eksudan (3.18 %), and 'NGB00390' (3.15 %). Whereas moisture contents of '01M', 'Eksudan' and 'NGB00390' are not statistically similar, the least amount of moisture (2.03 %) was found in 'NGB00963'. The ash content which indicates the mineral element composition of the accessions ranged was 4.55- 5.98 %, only two accessions, Igboho Black and 03M had ash composition above 5 %. Like the moisture content, the least percentage of the mineral was found in 'NGB00963'. Generally, all the accessions had a percentage crude protein higher than 5 %, the highest (7.4 %) occurred in '00960' and the lowest (5.33 %) in '02M'. The highest crude fibre content was found in 'Igboho Black' (5.77 %), followed by 'E8' (4.99 %), none of the accessions had crude fibre composition of less than 4 %. Furthermore, there is variation in the fat and oil composition of the accessions. Sesame seeds are mainly used for oil production; therefore, the percentage of oil composition is important. The accession E8 had the highest oil yield (60.5 %) which was not significantly different from the percentage composition of fat and oil in 'Kenan 4' (60.45 %). Meanwhile, none of the accession recorded crude fat and oil percentage less than 50 %, the least (53.61 %) was found in 'Igboho Black'. As an oil-bearing crop, the carbohydrate composition was expectedly low, ranging from 18.81-28.62 %. The accessions E8 and Kenan 4 with high fat and oil had 18.81 and 20.79 % carbohydrate respectively. The percentage of fat and oil composition of the accessions was inversely proportional to the carbohydrate content (Table 4).

### 3.7 DIVERSITY OF THE ACCESSIONS

The dendrogram partitioned the accessions into two major groups (Fig. 6). Group 1 consisted of two accessions; Igboho black and Bogoro Local which are separated at a genetic distance below 24. Group 2 consisting of 9 accessions was further divided into two sub-groups; 2A and 2B. The sub-group 2A had three accessions separated into two clusters; 2A(i) and 2A(ii) at a genetic distance below 34, a single accession (E8) made the cluster 2A(i) while two accessions (03M and Exsudan) with distance scale of 15 constituted the cluster 2A(ii). On the other hand, the sub-group 2B members were segregated into two distinct clusters; 2B(i) and 2B(ii). The former comprised of four accessions (NGB00390, Kenan 4, NGB00960 and NGB00963), whereas 'NGB00390' was the most distant member, followed by 'Kenan 4' with less genetic distance while the duo of 'NGB00960' and 'NGB00963' are the most related members of the cluster. Besides, two other related accessions, 02M and 01M formed the clustered 2B(ii).

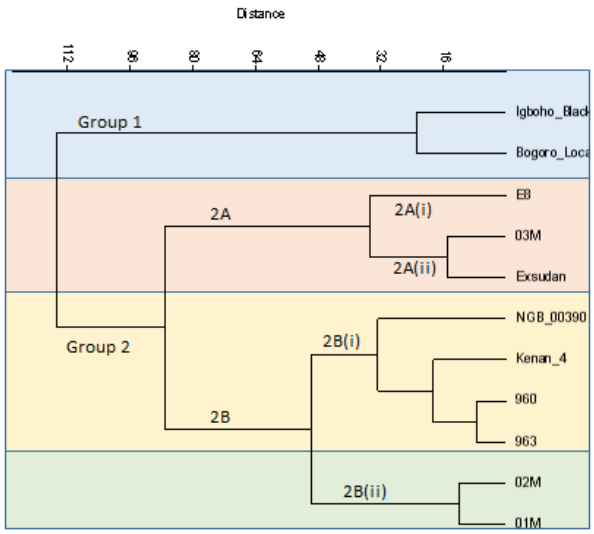


**Figure 6**: Dendrogram of the genetic relationship of the eleven sesame accessions based on agro-morphometric and yield-related attributes. The dendrogram was constructed using the agglomerative technique of the unweighted pair group of arithmetic average (UPGMA) method

## 4 DISCUSSION

The qualitative and quantitative traits of the accessions revealed variations in the studied variables, this showed there is existing genetic diversity amidst the accessions. Factors such as genetic drift, natural and artificial selection, exchange of breeding materials could contribute significantly to diversity among the accessions. (Sabesan et al., 2009; Banumathy et al., 2010). The observed variability in the morphological attributes obtained in the present study could provide useful information for the identification and selection of accessions with superior traits that could be used for sesame production, and as parents for breeding programs (Arriel et al., 2007). Significant dissimilarities in parameters such as plant height, leaf attributes, and stem girth as well as yield-related characters are essential for the assessment of variability and genetic divergence in plant genetic resources (Bisht et al., 1998; Baydar, 2005; Sharmila et al., 2007; Furat & Uzun, 2010; Kiranmayi et al., 2016; Animasaun et al., 2017; Azeez et al., 2017; Iqbal et al., 2018; Adu-Gyamfi et al., 2019).

The significant differences in the growth and yield characters of the evaluated *Sesamum indicum* accessions concurred with earlier reports (Adebisi et al., 2005; Parameshwarappa et al., 2010; Pham et al., 2010). The observed differences may be due to the genetic system of the accessions. The plant height range (27.76-94.84 cm) of the accessions in the current study was lower than 104-161 cm reported for some genotypes in South-East Asia by Pham et al. (2010). In terms of growth attributes, '02M' and 'Kenan 4' are the most promising accessions. Although 'Bogoro Local', 'E8', '03M' and 'Igboho' black had heights below the accessions average, notwithstanding, they had good yield attributes suggesting that vegetative growth and grain yield are under different genetic control systems. The short accessions may not be a complete disadvantage as Baydar (2005), opined that non-branching sesame varieties of medium heights and uniform maturity may be desirable for mechanized cultivation, and development of modern sesame breeding programmes. In addition, shorter canopy might lead to redistribution of assimilate which promotes higher yield.

The ability of the accessions to achieve 50 % flowering between 37 to 54 days showed most of the accessions are elite with regards to maturity. In a similar study, Arriel et al. (2007) reported the commencement of flowering varied from 30 to 48 days in some accessions. Also, the 89-108 days to maturity reported in the current study agreed with the authors' report. Meanwhile, Pham et al. (2010) documented much earlier time (24-31 days) for some accessions. The accession E8 that attained early maturity in this study could be potential mother material for the development of elite sesame cultivars. Since sesame is cultivated mainly for its seed and oil, therefore, seed yield attributes are of great importance in the selection of accession for commercial production. Besides early maturity, accession E8 showed impressive yield-related traits. According to Parameshwarappa et al. (2010), sesame genotypes with delayed maturity may record low seed yields. This is true for this study; 'NGB00390' which attained maturity late also had poor seed yield. Compared to other oilseed crops, sesame yield is low owing to its early senescence and vulnerability to some abiotic stress and photosensitivity (Pathak et al., 2014). Also, legumes generally have low yield, since the construction cost of oil is high compared to that of the carbohydrates in cereals. The high seed mass of 'E8' further reinforced the good quality of its seeds, making it a good parent material for sesame breeding programmes. The variations in the number of capsules per plant and seeds per capsule obtained in this study corroborate the earlier works (Ogbonna & Ukaan, 2012), who demonstrated considerable variation in some sesame seed characters, and this suggest yield component are controlled by multiple genes (Basu et al., 2009).

In green plants, the assimilatory surface is one of the major factors that determine growth and yield (Beheshti & Fard, 2010). Thus, a larger and increased assimilatory surface may translate to higher photosynthate, and ulti-

mately a better growth and yield. Sesame is highly sensitive to day length which coupled with temperature have a significant effect on its flowering rate (Suddihiyam et al. 1992). There is a close correlation between photosynthetic active radiation (PAR) absorption and yield (Yadav et al. 1988). But the reverse is the case for the duo of 'Exsudan' and 'NGB00960', which despite their high chlorophyll contents, they performed poorly in growth and yield. This suggests the two accessions had inherent characters for their non-impressive performance and therefore may not be suitable as raw material for the crop improvement. The gradual decline in chlorophyll contents of the accessions after 10 WAS could be due to increasing senescence, as the plant shift from the vegetative growth phase to the reproductive and maturity stage, this, of course, explains the slight increase recorded in the carotenoid contents. Carotenoids are essential pigments that compliment chlorophyll, it also acts as photoprotectors, antioxidants, colour attractants, and precursors of plant hormones in non-photosynthetic organs of plants. As the plant attains maturity and gene switches from vegetative to reproductive phase, more carotenoids are produced to manage the decrease in the Chlorophyll a and b functionality. In addition, carotenes contribute to photosynthesis by transmitting the light energy they absorb to chlorophyll and protect plant tissues by helping to absorb the energy from singlet oxygen, an exciting form of the oxygen molecule $O_2$ which is formed during photosynthesis.

Traits that are significantly correlated are highly linked and are possibly controlled by a genetic system. The strong associations between plant height, leaf area and seed yield showed there is a connection between growth and yield components. Significant correlations have been established in crop plants (Kiranmayi et al., 2016; Animasaun, et al., 2021; Azeez et al., 2017; Olorunmaiye et al., 2019), in all cases, it was argued that traits that are correlated are linked, and can be improved simultaneously. Meanwhile, Parameshwarappa et al. (2010), reported a negative relationship between the number of days to 50 % flowering and seed yield, indicating the higher the number of days to flower, the less the seed yield. Characters that showed significant negative association are not genetically linked and may be improved or selected individually. The concept of correlated traits is of great importance to a breeder as it gives concise information on character linkage on which selection could be made.

The nutrient composition of the seeds also varied, oil yield was generally high above 50 % in all accessions, a bit higher than values reported by Kiranmayi et al. (2016). The high oil yield obtained from 'E8' and 'Kenan 4', makes them the potential candidates for improved oil yield breeding programmes. The low carbohydrate contents of the accessions qualify the seed oil as low calories and safe for human and animal health.

The Principal Component Biplot Analysis enables the plant breeders to sort genotypes and select promising varieties using multivariate methods to estimate the contribution of each trait that constitute an ideal plant (Yan & Rajcan, 2002, Iqbal et al., 2018). Thus, it is an important tool to reveal the degree of similarity or variability among evaluated accessions. The effectiveness of a trait as a morphological marker is measured by its discriminating power among the accessions and its stability of expression (Arriel et al., 2007). The presence of '02M' and '01M' on a plain revealed they had similar morphological traits. They are both tall, with a relatively similar number of leaves per plant. The co-occurrence of three accessions; E8, 03M, and Exsudan in a cluster indicates a shared resemblance in yield characteristics. Relatedness and genetic diversity among the accessions based on growth and yield characters were further established by the dendrogram, which partitioned the accessions into four distinct groups. The higher the genetic distance, the less the relatedness, consequently, accessions having less distance scale are more related. The dendrogram obtained in the present study revealed that 'NGB00960' and 'NGB00963' are the most related accessions, and they are distant relative to 'Kenan 4'. A similar study (Tyagi et al., 2014) also demonstrated heterogeneous genotypes in a cluster, because, genetic relationship is based on related genetic markers and not necessarily the origin of the accessions (Animasaun et al., 2015). The use of cluster analysis to unravel genetic similarity in crop genetic resources have been demonstrated (Adewale et al., 2015; Pandy et al., 2015; Animasaun et al., 2017). The genetic divergence information present by the dendrogram is of great importance because it reveals accessions that are likely from a common progenitor. Besides, such information is a prerequisite for the selection and breeding of improved cultivars with novel alleles mix as well as developing an effective conservation strategy for the genetics (Upadhyay et al., 2011). Since members of a cluster share similar genetic composition regardless of origin, therefore, duplication and misidentification of accessions can be prevented.

## 5 CONCLUSION

The current study evaluated the performance and assessed the genetic diversity of eleven accessions of cultivated sesame using morpho-agronomic traits, photosynthetic pigment and nutrient composition. The result showed significant variations among the accessions for

growth, seed yield and nutritional composition. In terms of growth attributes, '02M' and 'Kenan 4' are the most promising while 'E8', 'Bogoro Local', and 'Igboho Black' showed good yield attributes. However, 'Kenan 4' showed the optimum performance. The study concludes that the accessions are genetically and phenotypically diverse and the existing diversity could be harnessed for selecting high yielding and adaptable variety as possible parents for the development of improved cultivars for commercial cultivation.

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## 7 CONFLICT OF INTEREST

The authors declare there is no potential conflict of interest.

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# Seed longevity characteristics of tomato (*Solanum lycopersicum* L.) genotypes stored with different packaging materials under ambient tropical humid conditions

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**Seed longevity characteristics of tomato (*Solanum lycopersicum* L.) genotypes stored with different packaging materials under ambient tropical humid conditions**

**Abstract:** Tomato seeds have a high commercial value, and the loss of seed physiological quality over time is demonstrated by their low storability unless hermetic conditions are used. This study aimed to store and preserve seed quality under ambient conditions using different packaging materials such as plastic bottles, glass bottles, paper envelope, earthen pot, polyethylene bag, galvanized iron tin. Freshly harvested seeds of four tomato genotypes were packed inside different containers and then sealed and stored for eight months under ambient humid tropical conditions. Data collected were subjected to Analysis of Variance (ANOVA) and means were separated using Tukey's HSD test at 5 % probability level. The result revealed that envelope and earthen pot were not ideal for tomato seed storage for long time, because seed stored in air tight containers maintained desired seed quality than non- airtight packaging materials. Glass bottle was identified as the best packaging material in maintaining seed quality of tomato throughout the storage period. Tomato seeds could be stored up to between 120 and 180 days under ambient conditions, depending on genotype and storage medium of the seed lot.

**Key words:** seed deterioration; seed quality; storage life; storage container

**Vzdrževanje vitalnosti semen različnih genotipov paradižnika (*Solanum lycopersicum* L. ) shranjenih različno v vlažnih tropskih ambientalnih razmerah**

**Izvleček:** Semena paradižnika imajo veliko tržno vrednost in izguba njihove fiziološke kakovosti nastopi hitro, če niso shranjena v zrakotesnih razmerah. Namen raziskave je bil shranjevati in ohranjati kakovost semen v ambientalnih razmerah z uporabo različnih materialov kot so plastične steklenice, steklenke, papirnate vrečke, glineni lonci, polietilenske vrečke in pocinane železne posode. Sveža semena štirih tipov paradižnika za bila shranjena v različne shranjevalnike, ki so jih zapečatili in hranili osem mesecev v vlažnih tropskih bivalnih razmerah. Pridobljeni podatki so bili obdelani z analizo variance (ANOVA), poprečja so bila ločenam s Tuckeyevim tesom (Tukey's HSD test) pri 5 % verjetnosti. Rezultati so pokazali, da papirnate vrečke in glinene posode niso primerne za daljše shranjevanje semen, kajti, semena ki so bila shranjena v zrakotesnih shranjevalnikih so ohranila zaželjeno kakovost v primerjavi s tistimi, ki so bila shranjena v zračnih ovojih ali posodah. Za daljše shranjevanje semen paradižnika so se izkazale najboljše steklenice. Semena paradižnika so v ambientalnih razmerah lahko shranjena od 120 do 180 dni, odvisno od genotipa in materiala, v katerem so shranjena.

**Ključne besede:** propadanje semen; kakovost semen; dolžina shranjevanja; shranjevalniki

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## 1 INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a member of the Solanaceae family which is famous for a number of medicinal and nutritional properties. Botanically, this fruit is known as berry (Salunkhe et al., 2005). Though it is a perennial crop but some of its cultivars are grown as an annual crop in various parts of the world (Nunes et al., 1996; Knapp, 2002). Tomato is one of the most important vegetables grown for edible fruits consumption in virtually every home in Nigeria. There are thousands of varieties of tomatoes in array of shapes, colours and size. The most common shapes are round (beefsteak and globe), pear shaped (roma) and the tiny cherry sized (cherry and grape) (Demir and Ellis, 1992).

Tomato seeds have a high commercial value, and the loss of seed physiological quality over time is demonstrated by their low storability unless hermetic conditions are used (Tigist et al., 2012). As a result, the development of satisfactory seed vigour test must be used and intensified. Slow, asynchronous and unreliable germination and emergence, within germinable, low vigour seeds, arise due to seed ageing (Mathew, 1980) and lead to problems for successful vegetable production.

The fact that seeds of most species can be dried and stored from year to year has been exploited since the beginning of agriculture. Indeed, the ability of many orthodox seeds (Roberts, 1973) to remain viable for tens or hundreds of years in dry storage (Walter et al., 2005) means that they also provide a convenient vehicle for the long term ex-situ conversation of plant germplasm (Probert et al., 2009).

The principal purpose of seed storage is to preserve economic crops from one season to another. Seed longevity refers to how long a seed can be stored under given set of conditions, how long a seed can remain dormant and still remain viable (Kehinde, 2018). Storage temperature and moisture content are the most important factors affecting seed longevity, with seed moisture content usually being more influential than temperature. The effect of temperature, the availability of oxygen and the greater improvement in deteriorated low vigour seeds were cited as evidence in support of metabolic repair during aerated hydration treatment (Thornton and Powell, 1992).

During storage, seed quality can remain at the initial level or decline to a level that may make the seed unacceptable for planting purpose. Several environmental factors have been reported to affect seed viability during storage (Rindels, 1995). Some of the factors that affect the longevity of seeds in storage could be genotype of seed, initial seed quality, storage conditions, and moisture content among others. Within the same plant species, different varieties may exhibit different storing abilities either from genetic variation or other external factors (Simic et al., 2007). However, irrespective of the initial seed quality, unfavorable storage conditions, particularly air temperature and relative humidity contribute to accelerating seed deterioration (Heatherly and Elmore, 2004). High relatively humidity and temperature cause high moisture content in seeds and result in low germination at the end of storage (McCormack, 2004).

In Nigeria and Ghana, most small holder farmers store their seeds in various containers including: pieces of cloth, black polyethylene bag, galvanized tin, clay pots, and plastic containers, mostly under ambient conditions (Adetumbi et al., 2009; Bortey et al., 2011; Akintobi et al., 2006). Additionally, several studies have indicated that storage containers affected the quality of seeds in terms of germination and viability over a period of time (Bortey et al., 2016). However, it has been reported that the intensity decreasing quality of stored seed under different storage techniques differ among plant species and within plant species (Al-Yahya, 2001) and as well as among varieties. Thus the type and choice of container used in storing seed is crucial in ensuring that the seed longevity characteristics of seed are maintained during storage.

Tomato is a common vegetable used frequently in the households. Thus, it is essential to have statistical estimates of seed longevity in order to decide efficient readily available storage conditions for seed storage and be able to predict how long seeds will store under ambient conditions.

Moreover, since the effect of these storage materials on the quality of seeds of different crops may vary, it is important to investigate and establish the most suitable storage material and condition for various food crop seeds. This would provide seed producers, breeders and farmers information on how to maintain the integrity of the seed during storage. The objectives of this study were therefore to: to evaluate the potential of packaging materials (storage containers) for preservation of tomato seed for seed physiological quality and to estimate potential seed longevity of some tomato genotypes stored under ambient conditions using probit modelling.

## 2 MATERIALS AND METHODS

### 2.1 SEED MATERIALS AND SOURCE

Four genotypes of tomato seeds ('Tropimech', 'Alausa', 'Cobra F1' hybrid and 'Roma VF') used in the study were sourced from National Institute of Horticulture (NIHORT), Ibadan, Nigeria.

## 2.2 STORAGE MATERIALS

Six storage materials were selected for the study, namely: plastic bottles, glass bottles, earthen pot, polyethylene bag, galvanized iron tin and paper envelope (control). The storage containers were selected on the following bases: polyethylene is being recommended as most cost-effective material. It is durable and reusable. In rural area, our fathers put their seeds in the earthen pot. Plastic and glass bottle are always available at ceremonies and common household and can be used as storage materials for some of the vegetables at no cost. Galvanized iron tin has a sealing which are always effective, readily available and easy to get. Most seeds are packaged in small envelope paper bags.

## 2.3 EXPERIMENTAL DESIGN

The trial was factorially arranged and laid out in completely randomized design with three replicates. There were three factors thus: cultivar at four levels; packaging materials at six levels; storage periods at five levels i.e. 0, 60,120,180 and 240 days. The treatments combination was 5 x 6 x 5 = 150 treatments and was replicated thrice.

## 2.4 STORAGE ENVIRONMENT

Storage and every other test were done in the processing and storage unit and in the laboratory of Plant Breeding and Seed Technology Department respectively, College Plant Science and Crop Production (COLPLANT), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria under ambient conditions. The temperature and relative humidity of the storage environment were monitored daily throughout the storage period with a thermo hygrometer.

## 2.5 SEED DATA COLLECTION AND PARAMETERS ANALYSED

Data were collected on the following seed quality characters at 60 days interval for 240 days.

### 2.5.1 Seed viability test

This test was carried out in the laboratory. Hundred seeds in three replications were placed inside Petri dish in an incubator maintained at 7 ml of distilled water and germination count was taken at 7 days and germinated seeds defined as those with a radicle of at least 2 mm long (ISTA, 1995).

Seed viability was then determined as:

(Viability count at 7 days / Number of seeds sown) x 100

### 2.5.2 Rate of germination

This was determined from viability test at 3 days after germination as:

(Normal germination at 3 days / Number of seeds sown) x 100

### 2.5.3 Seedling Length

Shoot length of 10 randomly selected seedlings were measured using a ruler in centimetre (cm).

### 2.5.4 Seedling vigour index

Seedling vigour index was computed as follows:

(Normal germination at 3 days / Number of seeds sown) x 100

## 2.6 DATA ANALYSIS

Data collected were subjected to analysis of variance (ANOVA). Significant means were compared using Tukey HSD test at 5 % probability level. Probit analysis of mean percentage seed viability data was also done using the PROC statements of SAS. Seed longevity parameters were values of *Ki* (an estimate of the probit value of initial seed viability at the time of storage), slope ($1/\sigma$), an estimate of rate of seed physiological deterioration, sigma ($\sigma$), the standard deviation of seed survival curve and an estimate of time taken to lose 1 probit seed viability, and $P_{50}$, a measure of time taken for a seed lot to lose 50 % viability and estimate of absolute seed longevity (Ellis and Roberts, 1980; Daniel, 1997; Adebisi et al., 2008; Kehinde, 2018).

# 3 RESULTS

Table 1 presents the influence of genotype on the four quality parameters of tomato seeds. For the varietal effect on rate of germination, 'Tropimech' hybrid had higher value of 72 %, 'Roma VF' and 'Alausa' were statis-

tically similar but had higher seed viability than 'Cobra F1' hybrid with 51.2 %. 'Roma VF', 'Cobra F1' hybrid and 'Alausa' had statistically similar and higher viability percentage than 'Tropimech' (72.0) which was not significantly different from 'Alausa'. For seedling length, 'Roma VF', 'Tropimech' and 'Alausa' had statistically similar values but higher value than 'Cobra F1' hybrid. For seedling vigour, 'Roma VF' and 'Alausa' were statistically similar but higher than 'Cobra F1' hybrid (7.3) which was also not significantly different from 'Tropimech' vigour 7.6 %.

The effect of packaging material (Table 1) shows that with rate of germination, galvanized iron tin had the highest percentage of 67.4 but was not significantly different from 65.3 and 65.3 % values recorded by polyethylene bag and glass bottle, respectively, and these latter was also not significantly different from that of earthen pot. But 59.8 and 60.6 % of plastic bottle and paper envelope had the lowest rate of germination respectively. For seed viability, glass bottle had the highest value of 81.8 % which was not significantly different from paper envelope (80.0 %) and earthen pot had the lowest value of 70.2 %. The result was similar to that of seedling length except that glass bottle (10.2 cm) was not significantly different from paper envelope (8.1 cm). With seedling vigour, glass bottle had the highest value of 8.4 % which was not significantly different from paper envelope (8.1 %) and galvanized iron tin (7.9 %) followed by polyethylene bag(7.6 %) which was not also significantly different from galvanized iron tin (7.9 %) and earthen pot which had the lowest value of (7.1 %) which was not significantly different from plastic bottle (7.3 %).

Influence of storage time on quality parameters of tomato seeds (Table 1) shows that the storage time of 180 days had the highest percentage the rate of germination was not statistically different from 120 days, followed by the 60 days, then 0 days and 240 days which was the lowest. With seed viability, 120 days was the highest but not statistically different from 0 and 60 days while the lowest viability was recorded at 240 days of storage. For seedling length, 120 and 180 days had similar and the highest value followed by 0 days, 240 days, the 60 days. For seedling vigour, 120 days had the highest value of 10.8 % which was not significantly different from 180 days followed by 0 days, while other storage time had statistically lower but similar values.

**Table 1**: Influence of treatment (genotype, storage time, package material) on quality parameters on tomato seeds under ambient conditions

| Treatment | Rate of germination at 3 days (%) | Seed viability (%) | Seedling length (cm) | Seedling vigour (%) |
|---|---|---|---|---|
| Genotypes | | | | |
| Roma VF | 65.8b | 78.8a | 9.9a | 7.9ab |
| Cobra F1 hybrid | 51.2c | 78.1a | 9.1b | 7.3c |
| Tropimech | 72.0a | 75.7b | 9.9a | 7.6bc |
| Alausa | 65.6b | 77.3ab | 10.2a | 8.2a |
| Standard error | 1.3 | 1.3 | 0.2 | 0.2 |
| Packaging Material | | | | |
| Polyethylene Bag | 65.3ab | 77.9cd | 9.4a | 7.6abc |
| Plastic bottle | 59.8c | 75.4d | 9.5b | 7.3c |
| Glass bottle | 65.3ab | 81.8a | 10.2a | 8.4a |
| Paper envelope | 60.6c | 80.0ab | 9.9ab | 8.1a |
| Earthen pot | 63.5b | 70.2e | 9.8b | 7.1c |
| Galvanized Iron Tin | 67.4b | 79.6c | 9.7b | 7.9ab |
| Standard Error | 1.6 | 1.6 | 0.2 | 0.2 |
| Storage Time | | | | |
| 0 days | 53.7c | 79.8b | 9.5b | 7.6b |
| 60 days | 66.4b | 77.8b | 6.7d | 5.3c |
| 120 days | 82.2a | 88.8a | 12.3a | 10.9a |
| 180 days | 83.1a | 83.4ab | 12.3a | 10.4a |
| 240 days | 32.9d | 57.6c | 7.9c | 4.6c |
| Standard Error | 1.5 | 1.5 | 0.2 | 0.2 |

Means followed by the same alphabet along the column are not different from each other at 5 % probability level according to Tukey's HSD test

Rate of germination as affected by genotype, packaging material and storage time are presented in Table 2. The result reviewed that 'Alausa' stored in galvanized iron tin had the highest viability of 85 % at zero day storage, but was not significantly different from 'Tropimech' stored in polyethylene bag, plastic bottle, glass bottle, and in galvanized iron tin and also 'Roma VF' stored in glass bottle. Whereas 'Cobra F1' stored in glass bottle and paper envelope had similar but the lowest viability rate of 13.3 at zero day of storage. At 60 days of storage, 'Alausa' stored in paper envelope had the highest viability rate of 90 % which was significantly different from the one stored in galvanized iron tin, 'Tropimech' stored in polyethylene bag, glass bottle, earthen pot. Whereas 'Cobra F1' hybrid stored in earthen pot and galvanized iron tin had the lowest viability rate of 33 %. At 120 days of storage, 'Alausa' seeds stored in glass bottle had the highest viability of 95 % which was not significantly different from the one stored in earthen pot and galvanized iron tin, also from 'Roma VF' stored in earthen pot and galvanized iron tin, 'Cobra F1' hybrid seeds stored in earthen pot and galvanized iron tin, 'Tropimech' seeds stored in polyethylene bag. At 180 days of storage, 'Alausa' seeds stored in glass bottle had the highest viability rate of 93 % while 'Tropimech' seeds stored in glass bottle had the lowest rate of 70 % which was not significantly different from the one stored in polyethylene bag, plastic bottle and 'Roma VF' seeds stored in plastic bottle. After 240 days, the viability rate decreased across all genotypes and package materials which 'Roma VF' had the highest rate of 58 %.

**Table 2**: Influence of storage time and packaging material on rate of germination of four tomato genotypes

| Genotypes | Packaging material | Storage time (days) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 60 | 120 | 180 | 240 |
| Roma VF | Polyethylene Bag | 58.3efgh | 53.3ef | 80.0bcd | 91.6ab | 28.3e |
| | Plastic Bottle | 48.3hi | 50.0ef | 73.3d | 80.0b-g | 26.6e |
| | Glass Bottle | 78.3abc | 58.3e | 80.0bc | 90.0ab | 50.0ab |
| | Paper Envelope | 40.0ijk | 78.3bcd | 73.0d | 88.3abc | 30.0de |
| | Earthen Pot | 63.0def | 76.0bcd | 86.0abc | 76.0d | 36.0c |
| | Galvanized Iron Tin | 68.3cde | 73.0cd | 86.0abc | 90.0ab | 58.0a |
| Cobra $F_1$ Hybrid | Polyethylene Bag | 35.0jk | 46.6fg | 86.6ab | 86.6abcd | 21.6efg |
| | Plastic Bottle | 30.0kl | 43.3fgh | 76.0cd | 86.6abcd | 18.3fg |
| | Glass Bottle | 13.3m | 58.3e | 78.3bcd | 78.3c | 11.6g |
| | Paper Envelope | 13.3m | 36.6gh | 83.0bcd | 85.0abcd | 21.0efg |
| | Earthen Pot | 48.0hi | 33.0h | 85.0abcd | 86.0abcd | 25.0e |
| | Galvanized Iron Tin | 23.0l | 33.0h | 86.0abc | 83.0abcde | 20.0efg |
| Tropimech | Polyethylene Bag | 83.3a | 81.6abcd | 86.6abc | 81.6b-g | 58.3a |
| | Plastic Bottle | 81.7a | 70.0d | 80.0bcd | 80.0b-g | 48.3ab |
| | Glass Bottle | 83.3a | 83.3abcd | 78.3bcd | 70.0g | 53.3a |
| | Paper Envelope | 73.3bcd | 75.0bcd | 81.0bcd | 76.6d | 40.0bcd |
| | Earthen Pot | 53.0fg | 85.0ab | 80.0bcd | 71.0f | 40.0bcd |
| | Galvanized Iron Tin | 83.0a | 76.0bcd | 76.0bcd | 73.0e | 53.0a |
| Alausa | Polyethylene Bag | 46.7ij | 76.6bcd | 80.0bcd | 81.6bcde | 40.0cd |
| | Plastic Bottle | 40.0ijk | 76.6bcd | 83.3bcd | 90.0ab | 13.3fg |
| | Glass Bottle | 48.3hi | 75.0bcd | 95.0a | 93.0a | 28.3e |
| | Paper Envelope | 40.0ijk | 90.0a | 78.0bcd | 83.3a-e | 23.0efg |
| | Earthen Pot | 50.0ghi | 78.0bcd | 88.0ab | 83.3a-e | 21.0efg |
| | Galvanized Iron Tin | 85.0a | 83.0abc | 86.0abc | 86.0abcd | 20.0efg |

Means followed by same alphabet along column are not different from each other at 5 % probability level according to Tukey's HSD test at 5 % probability level

Seedling length as affected by genotype, packaging material and storage time is revealed in Table 3. 'Alausa' seeds stored in galvanized iron tin had the highest value of 13.2 cm which was not significantly different from the one stored in paper envelope while 'Tropimech' seeds stored in plastic bottle had the lowest value of 7.4 cm. At 60 days of storage, 'Alausa' seeds stored in polyethylene bag had the highest value of 9.9 cm, which was statistically similar to 'Tropimech' seeds stored in glass bottle while 'Cobra F1' hybrid seeds had the lowest value of 4.3 cm which was not significantly different from the one stored in galvanized iron tin. At 120 days of storage, seeds of 'Alausa' stored in polyethylene bag had the highest value while at 180 days 'Alausa' seeds stored in earthen and galvanized iron tin had similar and the highest seedling length for the storage time. After 240 days, 'Tropimech' seeds stored in plastic bottle had the highest value 11.6 cm which was not significantly different from 'Roma VF' seeds stored in earthen pot.

Table 4 shows data on seed viability as affected by genotype, package material and storage time. Seed of 'Alausa' genotype stored in paper envelope had the highest value of 95 % which was statistically similar to 'Roma VF' stored in polyethylene bag, plastic bottle, galvanized iron tin, 'Cobra F1' hybrid stored in polyethylene bag, plastic bottle, glass bottle, 'Tropimech' stored in polyethylene bag and galvanized iron tin and 'Alausa' seeds stored in galvanized iron tin. For 60 days of storage 'Alausa' stored in polyethylene bag had the highest value of 93 % which was statistically similar to the one stored in paper envelope and plastic bottle. 'Tropimech' stored in galvanized iron tin, glass bottle, paper envelope, 'Co-

**Table** 3: Influence of storage time and packaging material on seedling length (cm) of four tomato genotypes

| Genotypes | Packaging material | Storage time (days) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 60 | 120 | 180 | 240 |
| Roma VF | Polyethylene Bag | 7.5ef | 7.3bcd | 12.9bcde | 11.4gh | 7.2hijk |
| | Plastic Bottle | 8.9cde | 5.4fgh | 12.9bcde | 13.3-e | 6.9ijk |
| | Glass Bottle | 9.6cd | 7.7bc | 12.6bcde | 13.0a-f | 9.6bcd |
| | Paper Envelope | 9.2cd | 6.2defg | 13.4ab | 13.7abc | 9.3cde |
| | Earthen Pot | 9.2cd | 5.8efg | 12.7bcde | 11.3gh | 10.9ab |
| | Galvanized Iron Tin | 8.5def | 7.2cde | 9.6h | 12.7a-g | 9.7bcd |
| Cobra $F_1$ Hybrid | Polyethylene Bag | 8.6def | 7.4bcd | 12.6bcde | 9.5i | 4.9mn |
| | Plastic Bottle | 9.4cd | 5.3fgh | 11.5cdefg | 11.7f | 5.6lmn |
| | Glass Bottle | 8.5def | 7.7bc | 11.7cdefg | 12.2defg | 7.8f-j |
| | Paper Envelope | 9.2cd | 6.2defg | 10.4gh | 11.8fg | 6.6jkl |
| | Earthen Pot | 9.7cd | 4.3h | 13.1abc | 12.1defg | 9.1cdef |
| | Galvanized Iron Tin | 10.1c | 4.8gh | 11.6defg | 11.9efg | 6.1jklm |
| Tropimech | Polyethylene Bag | 9.2cd | 6.7cdef | 13.0bcd | 10.2hi | 8.3d-i |
| | Plastic Bottle | 7.4f | 6.6cdef | 10.9f | 12.5c-g | 11.6a |
| | Glass Bottle | 9.4cd | 8.7ab | 12.5bcde | 12.6b-g | 9.2cdef |
| | Paper Envelope | 8.9cde | 7.7bc | 11.9cdef | 13.1a-f | 8.4defgh |
| | Earthen Pot | 8.6def | 6.6cdef | 11.9cdef | 12.2defg | 10.1bc |
| | Galvanized Iron Tin | 10.1c | 6.4cdef | 11.7c-g | 12.2defg | 8.8cdefg |
| Alausa | Polyethylene Bag | 9.0cd | 9.9a | 14.5a | 13.5abcd | 4.3n |
| | Plastic Bottle | 11.6b | 6.7cdef | 12.7bcde | 11.3gh | 7.4g-k |
| | Glass Bottle | 9.7cd | 6.9cde | 12.8bcde | 11.8fg | 8.8defg |
| | Paper Envelope | 12.9ab | 6.5cdef | 12.3b-f | 14.0ab | 8.1e-i |
| | Earthen Pot | 8.8cdef | 6.0defg | 13.5ab | 14.1a | 5.5lmn |
| | Galvanized Iron Tin | 13.2a | 6.4cdef | 12.8bcde | 14.1a | 6.0klm |

Means followed by same alphabet along column are not different from each other at 5 % probability level according to Tukey's HSD test at 5 % probability level

bra F1" hybrid seeds in glass bottle, 'Roma VF" stored in galvanized iron tin and glass bottle while 'Cobra F1' hybrid stored in earthen pot had the lowest viability of 60 %. At 120 days of storage, seeds of 'Alausa' stored in glass bottle had the highest viability rate from 120 days of storage to 240 days of storage with the value of 100, 98, 75 % respectively.

Table 5 presents seedling vigour as affected by packaging material, genotype and storage time. At zero time of storage, 'Alausa' stored in paper envelope and galvanized iron tin had the highest value of 12.2 and 12.3 respectively, while the lowest value was recorded by the same genotype stored in earthen pot. At 60 days of storage, 'Tropimech' seeds stored in glass bottle had the highest seedling vigour, while at 120 days, 'Alausa' seeds stored in polyethylene bag had the highest value. 'Cobra F1' hybrid stored in earthen pot and 'Tropimech' seeds stored in plastic bottle had the lowest value for 60 and 120 days of storage, respectively. For storage at 180 days, seeds of 'Alausa' stored in galvanized iron tin had the highest value of 12.2 while the one stored in glass bottle for 240 days of storage also had the highest value for that storage time. 'Alausa' stored in earthen pot and galvanized iron tin had similar but the lowest value for 240 days of storage.

Table 6 presents the results of probit analysis of seed viability data in four tomato genotypes and six package materials over 240 days of storage. It is evident by the positive values of the intercept of all the treatments that the seeds maintained its viability, irrespective of the package materials, over 240 days of storage. 'Roma VF' seeds stored in glass bottle had the lowest rate of deterioration (-0.609), followed by 'Tropimech' seeds stored

**Table 4**: Influence of storage time and packaging material on seed viability of four tomato genotypes

| Genotypes | Packaging material | Storage time (days) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 60 | 120 | 180 | 240 |
| Roma VF | Polyethylene Bag | 93.0ab | 71.0fg | 88.0bcde | 91.0abc | 41.0g |
| | Plastic Bottle | 88.0abcd | 63.0h | 73.0h | 81.0b-f | 53.0g |
| | Glass Bottle | 88.0abcd | 86.0abc | 93.0abcde | 92.0abc | 63.0b-f |
| | Paper Envelope | 73.0ef | 78.0b-g | 93.0bcde | 86.0bcde | 60.0cdef |
| | Earthen Pot | 73.oef | 72.0fg | 88.0bcde | 86.0bcde | 55.0ef |
| | Galvanized Iron Tin | 90abc | 88.0ab | 90.0a-f | 92.0abc | 67.0abcd |
| Cobra $F_1$ Hybrid | Polyethylene Bag | 85.0b | 78.0bc | 90.0a-f | 91.0abc | 53.0f |
| | Plastic Bottle | 86.0ab | 61.0h | 86.0c-g | 85.0bcde | 63.0b-f |
| | Glass Bottle | 85.0ab | 86.0abc | 85.0defg | 83.0b-f | 73.0ab |
| | Paper Envelope | 78.0de | 73.0ef | 96.0abc | 81.0b-f | 71.0abc |
| | Earthen Pot | 73.0e | 60.0h | 90.0a-f | 90.0abc | 60.0cdef |
| | Galvanized Iron Tin | 85.0ab | 60.0gh | 95.0abcd | 70.0g | 55ef |
| Tropimech | Polyethylene Bag | 90.0abc | 75.0de | 83.0efgh | 85.0b-f | 66.0a-e |
| | Plastic Bottle | 83.0bcde | 71.0f | 80.0fgh | 75.0efg | 68.0abcd |
| | Glass Bottle | 83.0bcde | 85.0abcd | 85.0defg | 72.0fg | 60.0cdef |
| | Paper Envelope | 80.0cde | 83.0abcde | 91.0a-f | 80.0c-g | 58.0def |
| | Earthen Pot | 65.0fg | 77.0cd | 83.0efg | 70.0g | 27.0h |
| | Galvanized Iron Tin | 90.0abc | 83.0abcde | 78.0gh | 88.0a-e | 53.0f |
| Alausa | Polyethylene Bag | 58.0g | 76.0c-g | 93.0a-e | 78.0def | 66.0a-e |
| | Plastic Bottle | 61.0g | 83.0a-e | 83.0efg | 83.0b-f | 70.0abc |
| | Glass Bottle | 58.0g | 81.0b-f | 100.0a | 98.0a | 75.0a |
| | Paper Envelope | 95.0a | 83.0a-e | 90.0a-f | 78.0def | 62.0b-f |
| | Earthen Pot | 56.0g | 82.0b-f | 90.0a-e | 75.0ef | 30.0gh |
| | Galvanized Iron Tin | 93.0ab | 93.0a | 98.0ab | 87.0bcd | 28.0h |

Means followed by same alphabet along column are not different from each other at 5 % probability level according to Tukey's HSD test at 5 % probability level

in glass bottle (-0.1826). 'Cobra F1' hybrid seeds stored in glass bottle recorded highest value in days to seed half-life (528.23) followed by 'Roma VF' stored in polyethylene bag with 410.7 days. On the other hand, 'Cobra F1' hybrid stored in paper envelope recorded the lowest half-life. Seeds of 'Tropimech' stored in plastic bottle had the highest time taken (793.4 days) to lose one probit viability followed by 'Cobra Fi' hybrid seeds stored in paper envelope (691.3 days). However, 'Alausa" seeds stored in galvanized iron tin had the lowest time taken (51.53) to lose one probit viability.

Moreover, the highest seed storage life of 35.21 months was obtained by 'Cobra F1' hybrid seeds stored in glass bottle followed by 'Tropimech' stored in plastic bottle while 'Cobra F1' hybrid stored in paper envelope had the lowest storage life.

**Table 5**: Influence of storage time and packaging material on seedling vigour of four tomato genotypes

| Genotypes | Packaging material | Storage time (days) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 60 | 120 | 180 | 240 |
| Roma VF | Polyethylene Bag | 7.9bcd | 5.2cdefg | 11.5b-f | 10.5bc | 2.8fg |
| | Plastic Bottle | 8.1bcd | 3.3hi | 9.3hij | 10.9abc | 3.8df |
| | Glass Bottle | 8.5bc | 6.7abc | 11.7bcdef | 11.9ab | 6.1abc |
| | Paper Envelope | 6.7defg | 4.9defg | 12.5abcd | 11.8ab | 5.7abc |
| | Earthen Pot | 6.7defg | 4.2ghi | 11.2c-h | 9.7cdef | 5.1abc |
| | Galvanized Iron Tin | 7.7bcde | 6.3abcd | 8.6j | 11.7ab | 6.4abc |
| Cobra $F_1$ Hybrid | Polyethylene Bag | 7.4cde | 5.8bcdef | 10.5e-i | 8.7ef | 2.8fg |
| | Plastic Bottle | 8.2bc | 3.3hi | 10.0f-j | 9.8cdef | 3.5ef |
| | Glass Bottle | 7.3cde | 6.7abc | 10.0f-j | 10.0cde | 5.7abc |
| | Paper Envelope | 7.2cdef | 4.5fgh | 10.0f-j | 9.6cdef | 4.5cde |
| | Earthen Pot | 7.1cdef | 2.7i | 11.7b-f | 10.8abc | 5.7abc |
| | Galvanized Iron Tin | 8.6bc | 3.2hi | 11.0defg | 8.3f | 3.5ef |
| Tropimech | Polyethylene Bag | 8.3bc | 5.2cdefg | 10.8efgh | 10.0cd | 5.6abc |
| | Plastic Bottle | 6.2efgh | 4.7efgh | 8.8j | 9.8cdef | 6.5ab |
| | Glass Bottle | 8.4bc | 7.4a | 10.4-i | 8.9def | 5.6abc |
| | Paper Envelope | 7.2cdef | 4.5fgh | 10.0f-j | 9.6cdef | 4.5cde |
| | Earthen Pot | 5.5gh | 5.0defg | 9.9g-j | 8.5ef | 2.8fg |
| | Galvanized Iron Tin | 9.1b | 5.4cdefg | 9.2ij | 10.8abc | 4.8cde |
| Alausa | Polyethylene Bag | 5.3gh | 7.2ab | 13.5a | 10.8abc | 2.9fg |
| | Plastic Bottle | 7.1cdef | 5.6c-g | 11.2c-f | 9.4cdef | 5.3a-d |
| | Glass Bottle | 5.7fgh | 5.7b-g | 12.8ab | 11.6ab | 6.7a |
| | Paper Envelope | 12.2a | 5.7b-g | 11.1cdef | 11.9ab | 4.9cde |
| | Earthen Pot | 4.9h | 4.9defg | 12.0abcde | 10.6bc | 1.7g |
| | Galvanized Iron Tin | 12.3a | 5.9a-f | 12.6abc | 12.2a | 1.7g |

Means followed by same alphabet along column are not different from each other at 5 % probability level according to Tukey's HSD test at 5 % probability level

## 4 DISCUSSION

Significant differences observed between the four genotypes of tomato for all the quality parameters suggest that there is an opportunity to select between the four genotypes for better performance. Similarly, significant difference observed among the six packaging materials for rate of germination, seed viability, seedling length and seedling vigour revealed that there is possibility for selecting among the six packaging materials that will give the best performance for the seed quality attributes evaluated. Differences in all the seed quality parameters during the storage period indicated that these seed quality parameters significantly varied with storage time.

**Table 6**: Probit analysis of the seed viability data in four tomato genotypes stored under six package materials over 240 days storage time

| Genotypes | Packaging materials | *Intercept | **Slope | ***Sigma | *%$P_{50}$ | *#Seed storage life |
|---|---|---|---|---|---|---|
| Roma VF | Polyethylene bag | 1.130 | -0.006 | 322.00 | 410.70 | 27.40 |
| | Plastic bottle | 0.944 | -0.004 | 153.80 | 217.20 | 14.50 |
| | Glass bottle | 1.615 | -0.609 | 82.40 | 148.30 | 9.90 |
| | Paper envelope | 0.986 | -0.005 | 294.9 | 234.80 | 15.70 |
| | Earthen pot | 0.881 | -0.002 | 205.07 | 125.80 | 8.40 |
| | Galvanized iron tin | 1.606 | -0.005 | 189.33 | 298.80 | 19.90 |
| Cobra F1 Hybrid | Polyethylene bag | 1.428 | -0.006 | 616.67 | 326.93 | 21.80 |
| | Plastic bottle | 0.960 | -0.002 | 303.19 | 209.63 | 13.90 |
| | Glass bottle | 1.316 | -0.003 | 498.53 | 528.23 | 35.21 |
| | Paper envelope | 0.906 | -0.000 | 691.30 | 467.63 | 31.21 |
| | Earthen pot | 0.633 | 0.000 | 280.80 | 45.97 | 3.10 |
| | Galvanized iron tin | 1.230 | -0.005 | 257.37 | 256.00 | 17.10 |
| Tropimech | Polyethylene bag | 1.255 | -0.004 | 240.07 | 303.93 | 20.30 |
| | Plastic bottle | 1.026 | -0.003 | 793.40 | 455.37 | 30.40 |
| | Glass bottle | 1.467 | -0.007 | 351.57 | 384.63 | 25.60 |
| | Paper envelope | 1.319 | -0.186 | 83.73 | 131.27 | 8.80 |
| | Earthen pot | 1.062 | -0.007 | 151.03 | 143.80 | 9.60 |
| | Galvanized iron tin | 1.602 | -0.007 | 335.53 | 385.85 | 25.70 |
| Alausa | Polyethylene bag | 0.534 | -0.001 | 571.60 | 344.47 | 22.90 |
| | Plastic bottle | 0.594 | -0.002 | 498.67 | 117.36 | 7.80 |
| | Glass bottle | 0.750 | -0.003 | 171.83 | 75.58 | 5.00 |
| | Paper envelope | 2.030 | -0.010 | 97.23 | 189.97 | 12.70 |
| | Earthen pot | 0.928 | -0.000 | 220.20 | 204.77 | 13.70 |
| | Galvanized iron tin | 3.026 | -0.020 | 51.53 | 147.18 | 9.80 |

*Intercept is PROBIT estimate of initial seed viability
**slope is the rate of seed deterioration
***Sigma is time taken for seed lot to lose 1 probit viability
*% $P_{50}$ is seed half- life in days
*# Seed storage life estimated as $P_{50}$ value multiplied by 2 then divided by the 30 days of a month

The significant effect of packaging materials and storage time for all the seed quality parameters except rate of germination implies that the differences recorded for these two parameters were influenced by storage time. The significant genotype and packaging material effect indicated that rate of germination of the genotypes was influenced by packaging material investigated. This support earlier findings by Alegiledoye et al. (2018) and Kehinde et al. (2020) who reported influence of packaging material on seed quality of African yam beans and water melon respectively.

Likewise, significant effect of genotype and storage time on all the seed quality parameters revealed that differences in these characters among the four genotypes were modulated by storage time examined. The significant effect of genotype, storage time and packaging material on rate of germination, seed viability, seedling length and seedling vigour revealed that the variation in these characters between the four genotypes was influenced by both the storage time and packaging material.

Among the packaging materials, glass bottle gave the best performance in terms of all the seed quality param-

eters compared with the other five packaging materials and was also significant across the storage time. Kehinde et al. (2020) also identified glass bottle as the best storage container in maintaining seed quality of water melon out of three storage containers used in the study. The result also showed significant differences among the five storage times for rate of germination, seed viability, seedling length and seedling vigour. The seed quality sharply declined after 120 days of storage. It can be concluded that tomato seeds can be stored under ambient conditions for at least 120 days (4 months) before sowing and still retain good emergence and seedling vigour characters. The result, however, revealed that seeds stored and sown at 240 days had significantly lower rate of germination and seed viability (32.8 and 57.5 %, respectively) and seedling vigour of value (4.6). This indicates the superiority of seeds stored at other earlier storage time.

Aliyu and Akintaro (2007) reported that water imbibition in seeds stored for a longer time is associated with leakage of hydrolytes like sugars and amino acids, which often leads to disintegration of cell membrane and thus reduces the quantity of amino acids and peptides that are translocated to embryo axis and this in turns affect the rate of germination.

In this study, it was observed that glass bottle gave significantly the highest values of viability compared to other packaging materials. It also had significantly higher seedling vigour for the storage periods compared to other packaging materials. 'Tropimech' genotype had significantly higher seed viability across the six packaging materials compared to all other genotypes. Also, a sharp decline was observed in values recorded by the all the genotypes for all the seed quality parameters between 180 and 240 days of storage periods which suggests that storage can further be encouraged up till 180 days of storage with a reasonable and moderate seedling performance compared to seeds stored for 240 days before sowing.

The packaging materials used in this study had significant effect on the quality of the seeds of tomato genotypes used. Glass bottle was the best packaging material used in this study. This finding conforms with expectation as seeds stored in air tight containers maintain seed qualities longer than non-air tight packaging materials like envelopes which absorb moisture from the surrounding atmosphere. This finding agrees with the report of Kumar and Singh (1983) that the seeds of sesame stored in glass bottles maintained satisfactory germination throughout storage period while seeds stored in gunny bags lost viability after six months of storage. Majhi and Bandopadhyay (1993) also reported that freshly harvested groundnut seeds dried to moisture content of nine percent stored in glass bottles for one to nine (9) months had the highest seed viability, root and shoot length and seedling dry mass when compared to seed stored in paper and cloth bag.

Earlier reports by Daniel (1997), Adebisi et al. (2004, 2008), Esuruoso (2010), Adebisi (2012), Oni (2012) and Adebisi et al. (2019) have utilized probit modelling to predict storage life of yam, soybean, kenaf, okra, sesame and pigeon pea, respectively under ambient storage conditions. In this study, the result of probit modelling showed that tomato seeds deteriorated at different rate, irrespective of the package material in which it is been stored for a period of 240 days. 'Roma VF' stored in glass bottle had the lowest rate of deterioration (-0.609) and the highest rate (3.1826) was recorded by 'Tropimech' stored in glass bottle. The highest estimate of tomato seed shelf-life was obtained with 'Cobra F1' hybrid stored in glass bottle with 35.2 months followed by 'Tropimech' stored in plastic bottle with 30.4 months while 'Cobra F1' hybrid stored in paper envelope gave the least storage life. However, the Probit modelling predicted that tomato seeds of 'Cobra F1' hybrid stored in glass bottle can be stored for an average of 35 months and still retain high viability characteristics under good storage conditions.

## 5 CONCLUSIONS

Significant differences were observed in rate of germination, seed viability, seedling length and seedling vigour of the four genotypes of tomato examined in this study. Genotype, storage time and packaging material influenced all the seed quality parameters examined.

Envelope and earthen pot are not ideal for tomato seed storage for long time, because seeds stored in air tight containers maintained desired seed quality than non- airtight packaging materials. Glass bottle was identified as the best packaging material in maintaining seed quality of tomato throughout the storage period due to the fact that it withstood all environmental conditions compared to other packaging materials.

The probit modelling result revealed that 'Cobra F1' hybrid tomato genotype stored in glass bottle had the highest seed storage life of 35.21 months.

## 6 RECOMMENDATIONS

Tomato genotype, packaging material and storage period should be considered in maintenance of viability and seedling vigour in tomato preservation. Tomato seeds should be properly stored in the right packaging material for preservation of the seed vigour and for rapid growth characters. Glass bottles are locally available

at very low cost and peasant farmers can easily handle them, therefore these storage materials should be used for storing tomato seeds and can be adopted for other crop seeds. Tomato seeds could be stored up to between 120(4 months) and 180 days (6 months) under ambient conditions, depending on genotype, and storage medium of the seed lot.

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# Preliminary research on seed yield and nutritional traits of desi chickpea (*Cicer arietinum* L.) grown in Central Italy in spring sowing

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**Preliminary research on seed yield and nutritional traits of desi chickpea (*Cicer arietinum* L.) grown in Central Italy in spring sowing**

**Abstract:** In Italy, chickpea (*Cicer arietinum* L.) cultivation of the *kabuli* type is predominant, with local landraces of the *desi* type, including the Apulian black chickpea, occurring almost exclusively in the south. However, increasing interest in the *desi* type on the part of farmers and consumers is based on the nutritional properties constituting a niche area in the market health sector. Information pertaining to both field evaluation of *desi* chickpea in Central Italy and the chemical composition is scarce. Therefore, the aim of this preliminary research was to evaluate the agronomic performance to the agroclimatic environment of Central Italy of thirteen lines of *desi* chickpea accessions of different origins, as well as the 'Apulian Black Chickpea' (ABC). Accessions were sown on March 21 in 2019, without the use of irrigation. The principle qualitative characteristics, namely protein content and raw fiber were determined. Yield was excellent for 57 % of the accessions, some of which exceeded 3.0 t ha$^{-1}$ with the maximum value of 4.1 t ha$^{-1}$ recorded for 'PI598080' (brown seeds of Indian origin). 'W617611' (black seeds of Turkish origin) was the earliest flowering line at 74 days after sowing, permitting this accession to escape the onset of drought and high temperatures. Additional early flowering lines included 'PI533676' (black seeds of Ukrainian origin) and 'PI567850' (black seeds of Pakistani origin), respectively. Plant structure relating to the average height of the plants (47.0 cm), the height of the first pod (30.8 cm) and the number of branches per plant (2.6) rendered most accessions suitable to combine harvesting, an indispensable prerequisite in the maintenance of an economically sustainable crop. The average protein content was 22.7 % with maximum values exceeding 24 % ('PI572520' - black seeds of Syrian origin -, 'W617614' - black seeds of Turkish origin - and 'PI572850'). The fiber content was very heterogeneous ranging from 4.6 % to 12.0 %.The present study provides the basis towards the future introduction of *desi* chickpea in Central Italy, with the potential for sustainable yield and quality.

**Key words:** *Desi* chickpeas; Central Italy spring sowing; *Cicer arietinum* L.

**Preliminarna raziskava o pridelku semena in hranilnih lastnosti čičerke (*Cicer arietinum* L.) tipa *desi*, rastoče v osrednji Italiji pri spomladanski setvi**

**Izvleček:** V Italiji je pridelovanje čičerke (*Cicer arietinum* L.) izključno omejeno na južna območja, kjer gojijo pretežno *kabuli* tip, lokalno tudi domače lokalne rase tipa *desi* , vključno z apulijsko črno čičerko. Povečanje zanimanja za tip *desi* med kmeti in potrošniki temelji na njeni hranilni vrednosti, kar ustvarja nišo na trgu zdrave hrane. Podatki o vrednotenju pridelave in kemijski sestavi *desi* čičerke so v osrednji Itali zelo redki. Zaradi tega je bila izvedena preliminarna raziskava za ovrednotenje uspevanja v agroklimatkih razmerah srednje Italije na trinajstih linijah *desi* čičerke, z akcesijami različnega izvora, hkrati s črno apulijsko čičerko (ABC). Akcesije so bile posejane enaindvajsetega marca, leta 2019, brez namakanja. Določene so bile glavne kakovostne lastnosti, kot je vsebnost beljakovin in celokupnih vlaknin. Pridelek je bilo odličen pri 57 % akcesij, pri nekaterih je presegal 3,0 t ha$^{-1}$. Maksimalni pridelek, 4,1 t ha$^{-1}$, je bil zabeležen pri 'PI598080' (z rjavimi semeni, indijskega izvora). 'W617611' (s črnimi semeni, turškega izvora) je bila najzgodnejša v cvetenju, 74 dni po setvi, kar omogoča tej akcesiji, da pobegne suši in visokim temperaturam. Zgodaj cvetoče linije so vključevale še 'PI533676' (s črnimi semeni, ukrajinskega izvora) in 'PI567850' (s črnimi semeni, pakistanskega izvora). Zgradba rastlin, ki se nanaša na poprečno višino rastlin (47,0 cm), višino prvega stroka (30,8 cm) in število stranskih poganjkov na rastlino (2,6) je pokazala, da je večina akcesij primerna za žetev s kombajni, kar je nepogrešljiv predpogoj za ekonomsko vzdržno gojenje te poljščine. Poprečna vsebnost beljakovin je bila 22,7 %, z maksimalno vsebnostjo, ki presega 24 % ('PI572520', s črnimi semeni, sirijskega izvora, 'W617614', s črnimi semeni, turškega izvora in 'PI572850'). Vsebnost vlaknin je bila zelo različna in je znašala od 4,6 % do 12,0 %. Raziskava daje osnovo za bodoče uvajanje *desi* čičerke v osrednji Italiji, s potencialom vzdržnega pridelka in kakovosti.

**Ključne besede:** *desi* čičerka; osrednja Italija; spomladanska setev; *Cicer arietinum* L.

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## 1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a widely used legume in the Mediterranean diet. After the Second World War, chickpea cultivation in Italy declined sharply, from 110,000 ha in 1950 to approximately 3,500 ha in 1999. However, based on the adoption of a more balanced diet, closer to that of the traditional Mediterranean diet, the importance of all legumes, including chickpea, were taken into consideration. This has been reflected in the increased cultivation, currently at 27,000 ha, of which production is predominantly directed towards the canned seed industry (Casini, 2018).

This notable increase was attributable, not only to the aforementioned food trends, but also to agronomic benefits such as reducing the use of both fertilizers and pesticides. In turn, this served to improve the structure and fertility of the soil and, above all, provide a more sustainable return to crop rotation with cereal crops (Palumbo, 2017; Watson et al., 2017). Together with the expansion of the areas dedicated to chickpea cultivation, yields have also increased. Yield increase is attributable to genetic improvement programs and innovative agronomic techniques, such as the use of symbiotic bacteria and selected mycorrhiza. Chickpea cultivation in Italy is composed predominantly of the *kabuli* type, characterized by large, clear and wrinkled seeds (Sarno and Stringi, 1980). Apart from some landraces, Italian varieties were derived mainly from genetic improvement studies conducted in the 1980s involving both Italian than foreign accessions. However, the availability of these varieties is very limited.

In southern Italy, chickpeas of the traditional type *desi* are widespread. *Desi* seeds are characterized by small, angular shapes of various colors, including either black, light brown or reddish seeds, with clear seeds being the most rare. Among these varieties, one of the most famous is the 'Cece Nero delle Murge' which, generically, is also referred to as 'Apulian Black Chickpea'. 'Le Murge' is a very large karst plateau, located between the regions of Apulia and northeastern Basilicata. In the north, red chickpeas, named 'Ceci Rossi di Orco Feligno' in Italian, also occur within small areas of the hinterland in the province of Savona in Liguria.

Consumers have become increasingly more attentive to quality, and are constantly looking for novel products that can ensure added value to the diet in the form of nutritional and/or nutraceutical properties. A good source of both protein and fiber is also one of the most sought-after characteristics. The chemical composition of *kabuli* and *desi* chickpeas differ significantly, especially in the raw fiber content, which is generally higher in the dark seeds (Rossi et al., 1984; Kaur and Singh, 2005; Costa et al., 2006; Ghosh et al., 2019). An additional important difference is in the oligosaccharide content, composed of raffinose, stachyose and verbascose, respectively. These oligosaccharides are responsible for impacts on flatulence, that is reported to be lower in *desi* seeds (Singh et al., 1982; Rossi et al., 1984). Bioactive compounds contained in the seeds also include carotenoids, anthocyanins, other phenolic compounds and phytate (Summo et al., 2019).

There are no statistics on the increase in the consumption of *desi* chickpeas in Italy. Generally, consumers in the market are attracted by their unusual attributes, as well as by differences in flavor compared to *kabuli*, despite the difficulties encountered in preparation (cooking), attributable to a high fiber content. In the past, this feature was a distinguishing characteristic of the *desi* chickpeas and for this reason black seeds were mainly destined for livestock feed. However, informal reports from organic product distributors and the appearance of canned *desi* chickpeas on the markets provided useful pointers on the merit of these seeds for human consumption. This coincided with the demand from farmers in both Central and Northern Italy for this type of chickpea. However, the availability of seed was limited to the few varieties that were cultivated exclusively in the area of origin, and preferably in organic farming, in order to maintain market value. In Italy, this type of chickpea, for which only landraces are available, is mostly characterized by modest yields. Moreover, the plant structure does not always facilitate mechanical harvesting and weed control. Reasons for this include the semi-prostrate structure of the plants, the tendency towards branching, and the positioning of pods closer to the ground.

There is very limited information on the field evaluation of *desi* chickpea in Italy (Casini, 1983) and, in particular, on the chemical composition (Rossi et al., 1984; Pavan et al., 2017; Summo et al., 2019).

The aim of this preliminary research was to evaluate the agronomic performance to the agroclimatic environment of Central Italy of thirteen lines of *desi* chickpea accessions of different origins, as well as the Apulian Black Chickpea (ABC).

To this end, sowing in spring was performed, and seeds produced were then analyzed for the principle qualitative characteristics in terms of protein content and raw fiber.

## 2 MATERIALS AND METHODS

The field experiment was carried out in Tuscany, Central Italy at the DemoFarm of 'Tenuta di Cesa-Terre Regionali Toscane (Province of Arezzo)', 43° 18' N; 11° 47' E; 242 m a.s.l. in 2019. The cultivation environment

**Table 1:** Origin and characteristics of the chickpea accessions

| Accessions | Name | Origin | Seed color | Seed shape | Bearing |
|---|---|---|---|---|---|
| W611345 | USSR-05-03-BD | Tajikistan | Black | Angular | Erect |
| PI533676 | Sovhoznii 14 | Ukraine | Black | Angular | Erect |
| PI598080 | Desi chana | India | Brown | Angular | Erect |
| PI559361 | Desi chana | India | Black | Angular | Erect |
| PI559362 | Negro vicos | Spain | Brown | Smooth | Erect |
| W663498 | ICC 4475 | Syria | Black | Angular | Erect |
| PI572520 | ICC 6328 | Syria | Black | Angular | Erect |
| W617614 | 070689-0101 | Turkey | Black | Angular | Erect |
| W617611 | 140689-0601 | Turkey | Black | Angular | Erect |
| ABC | Apulian Black Chickpea | Italy | Black | Angular | Erect |
| PI572491 | Porquero Negro | Mexico | Brown | Angular | Erect |
| PI567850 | AOS 30 | Pakistan | White | Smooth | Erect |
| PI518258 | WKS 237E | Spain | Brown | Angular | Erect |
| PI518248 | Myles | USA | Brown | Angular | Erect |

was characterized by a neutral, loamy-sandy soil. The principle physical and chemical characteristics of the soil were as follows: sand 37 %, loam 39 %, and clay 24 %, respectively. The soil pH was 7.0. Total N was 0.11 % and P (Olsen) 13 ppm. Exchangeable Ca, Mg and K were 4123, 595 and 141 ppm, respectively. Meteorological data were recorded though SIAP automatic equipment, controlled and validated by the Regional Hydrological and Geological Sector. Thirteen accessions, provided by the North Central Regional Plant Introduction Station (Iowa State University, USA) and the Italian landrace 'Apulian Black Chickpea' (ABC) were used.

Based on previous experiments carried out in Central Italy (Casini, 1989), the Autumn-winter sowing period was not taken into consideration due to serious damage caused by anthracnose leaf blight. As a result, the sowing date selected was March 21, 2019. No symbiotic bacteria or mycorrhiza were utilized for seed inoculation; active root nodules were observed in all accessions. Plots were arranged according to a complete random block design, with three replicates. The size of the plots were 2.0 x 6.0 m (four rows wide with 0.5 m row spacing, respectively). The sampling area was comprised of two central rows, each of 4.0 m long, respectively. A seed quantity of 195 g per plot was used. In order to obtain the correct planting density of 35 plants $m^{-2}$, seedlings were thinned soon after complete emergence. Plots were hand-weeded twice (40 and 66 days after emergence [DAE]) during the growth cycle.

The agricultural interventions performed during the experiment are reported in Table 2.

**Table 2:** Agronomic techniques employed during the field trial

| Previous crop | Wheat |
|---|---|
| Plowing | September 12, 2018 |
| Harrowing | October 3, 2018 |
| Harrowing | March 12, 2019 |
| Pre-sowing fertilization | March 12, 2019 |
| | N 52 kg $ha^{-1}$ and $P_2O_5$ 114 kg $ha^{-1}$ |
| Sowing | March 21, 2019 |
| Emergence | April 14, 2019 |
| Harvesting | August 28, 2019 |

Plant height, stem number, height of the first pod (or ground clearance) and number of pods per plant were determined at the maturation stage, using a total of 10 plants per sample plot. Yield calculations and the 100 seed mass were performed using seed samples at a standard humidity of 12 %. Fifty percent flowering and maturity were estimated in the sampling plot areas.

Crude protein content of the seeds were performed in triplicate. The protein content was calculated by multiplying the nitrogen content by 6.25. The nitrogen content of 500 mg flour samples was determined by the FlashEA 1112 Series analyser (Thermo Fisher Scientific Inc., Waltham, MA, USA). Crude fiber content was determined according to the AOAC method (2006).

Data collected in the experiments were processed utilizing a one way randomized block analysis of vari-


**Figure 1:** Seed color and shape of chickpea accessions

ance (ANOVA). Differences between response variables were assessed with the COSTAT 6.45 software. Statistical differences were tested at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$. The Tukey's HSD test was used to evidence significant differences between means and homogenous groups. The Pearson correlation coefficient (r) was calculated between all the quantitative variables.

## 3 RESULTS AND DISCUSSION

Figure 1 shows the climatic trends during the experiment. Average minimum and maximum temperatures were 9.7 and 24.6 °C, respectively. Rainfall was regular from immediately after emergence until fruit set (a total of 230 mm), thereby permitting optimal plant development. The subsequent phase, on the other hand, was characterized by a prolonged drought (50 days) and a maximum average temperature of 32 °C. The period of drought was then interrupted two weeks prior to harvesting, with over 200 mm distributed within 10 days with virtually no effect on seed filling.

The ANOVA reported in Table 3 evidenced significant differences at $p \leq 0.001$ for all the attributes examined.

Figure 3 shows the dates of the principle phenological phases (flowering, fruit set and maturity) for each of the accessions. 'W617611' was the earliest in flowering at 74 DAS whilst, ABC was the latest to flower at 90 DAS. As regards fruit set, 'PI533676' and 'PI598080' were the earliest and latest, respectively. The interval between the latter two phenological phases varied from 5 to 15 DAS for ABC and 'W617611', respectively. In Central Italy, the precocity of these phenological phases for chickpea sown in the spring, is of great importance. The capacity to flower earlier under the best conditions of soil moisture, permits maximum reproductive development prior to the onset of the dry period, which is also characterized by high temperatures. Drought periods represent one of the principle abiotic stresses responsible for the reduction in chickpea yield (Yücel, 2018). The occurrence of a short dry period during flowering, but also during fruit set and seed filling has been shown to reduce yield by 15-60 % (Yücel, 2019). These reductions have been shown to be dependent on both the geographical area and duration of the dry spell (Sabaghpour et al., 2006).

The climatic trend facilitated the development of all the respective reproductive phases of chickpea accessions under the best conditions. The lines with the best potential in escaping the first dry periods of the Central Italian cultivation environment were 'PI533676', 'W617611' and 'PI567850'.

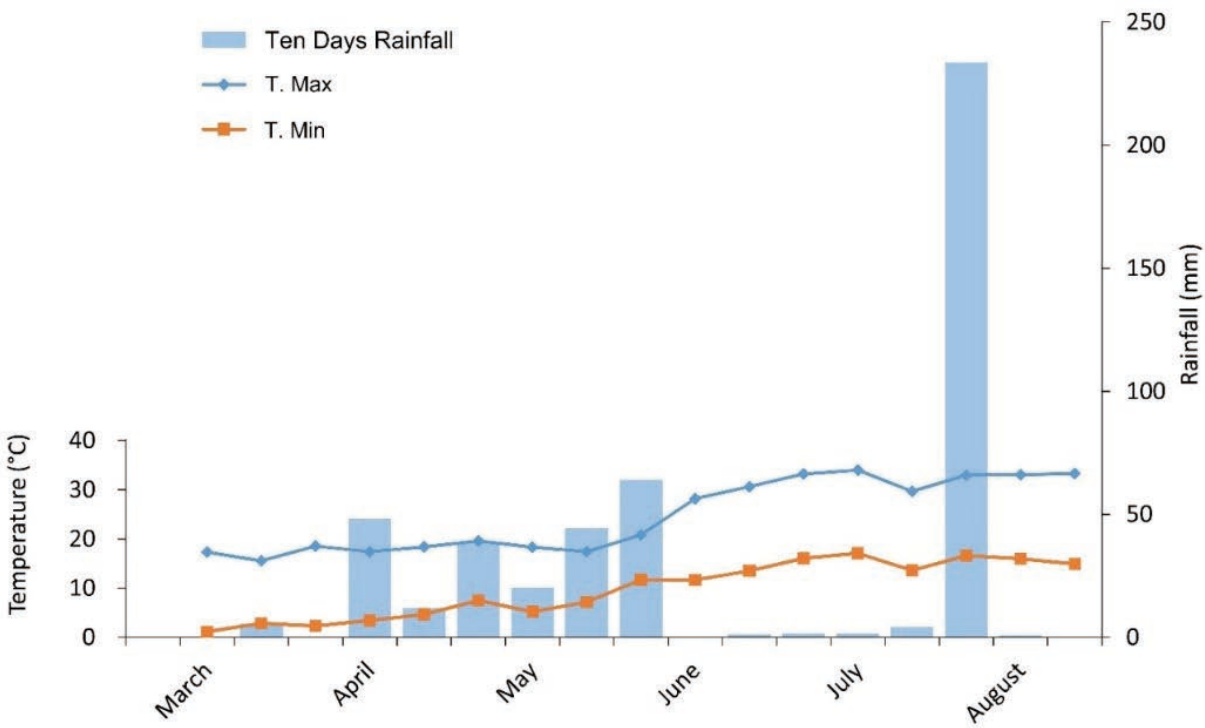


**Figure 2:** Temperature and rainfall recorded during the chickpea field experiment in 2019

**Table 3:** Analysis of variance

| Source of variation | DF | Flowering | Fruit Set | Maturity | Plant Height | Height of First pod | Production strip | Stems per Plant | Filled Pods |
|---|---|---|---|---|---|---|---|---|---|
| Blocks | 2 | 5.33 | 0.14 | 2.90 | 17.83 | 11.00 | 17.33 | 0.17 | 103.37 |
| Accessions | 13 | 1206.11*** | 413.14*** | 423.16*** | 2752.29*** | 893.99*** | 734.31** | 10.79*** | 5613.61*** |
| Error | 26 | 40.66 | 39.85 | 11.76 | 610.34 | 176.23 | 453.78 | 3.78 | 417.75 |
| Total | 41 | 1252.11 | 453.14 | 437.83 | 3380.47 | 1081.22 | 1205.43 | 14.74 | 6134.73 |

| Source of variation | DF | Empty Pods | Seeds per Plant | Seeds per Pod | Seed Mass per plant | 100 Seeds Mass | Yield | Protein | Crude Fiber |
|---|---|---|---|---|---|---|---|---|---|
| Blocks | 2 | 1.14 | 129.13 | 0.10 | 1.04 | 40.67 | 0.84 | 0.02 | 0.03 |
| Accessions | 13 | 66.37*** | 17473.28*** | 4.95*** | 633.38*** | 2197.96*** | 33.97*** | 112.44** | 184.52*** |
| Error | 26 | 7.46 | 501.22 | 3.16 | 4.36 | 158.92 | 5.60 | 3.94 | 0.11 |
| Total | 41 | 74.97 | 18103.64 | 8.22 | 638.78 | 2397.56 | 40.42 | 116.4 | 184.66 |

**, ***: significant at $p \leq 0.01$ and $p \leq 0.001$ respectively

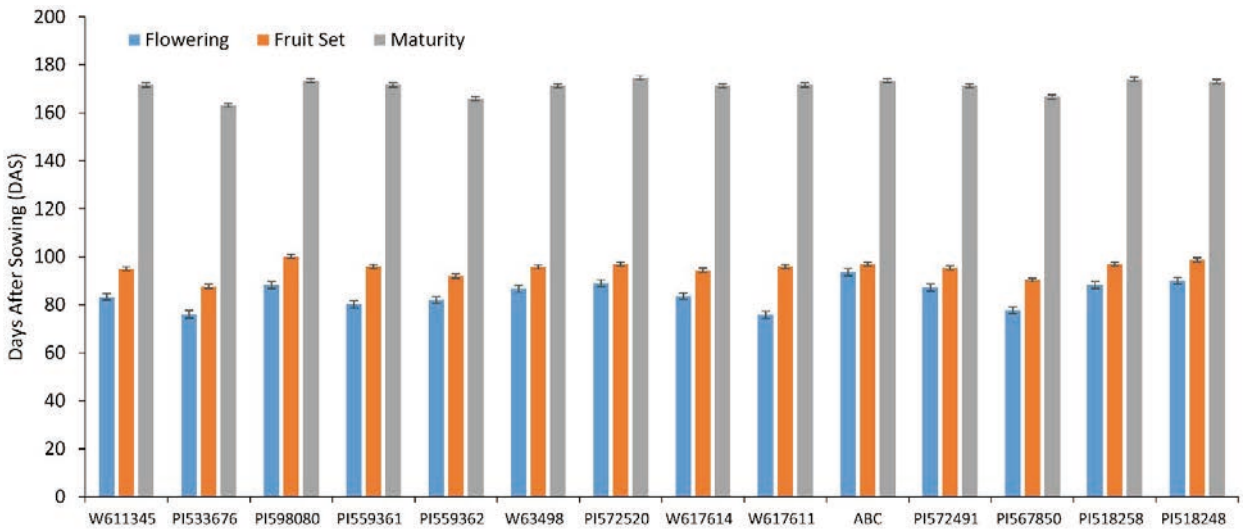


**Figure 3:** Dates of the principle phenological phases of the accessions. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at $p \leq 0.05$

Ripening ended between 160 DAS ('PI533676') and 175 DAS ('PI598080'). Although late ripening, may lead to better seed production, it can lead to problems from an agronomic point of view, especially as regards soil preparation (ploughing) for subsequent cultivations. In Central Italy, it is common to sow an autumn-winter cereal (mainly wheat) after chickpea. With predominantly clay type soils, early soil preparation is essential in order to permit atmospheric agents to effectively disintegrate the clay clumps prior to sowing the cereal crop (late October-November). Even a two-week difference in the ploughing date can make a significant difference. Hence, in this context, early ripening chickpea accessions are preferable. On the other hand, the depth of the chickpea roots and the physic-chemical state of the soil after harvesting may result in the application of less heavy processing techniques than ploughing using, for example, minimum tillage or the two-layer ploughing.

Table 4 shows the quantitative characteristics. Plant structure, plant height, as well as the height of the first pod above ground level, are important traits determining the suitability of the accessions for combine harvesting. In this experiment, the height of the plants varied from 34.1 cm to 59.3 cm reported for 'PI559361' and

'PI567850', respectively. Instead, the height of the first pod from the ground varied from 22.6 cm to 40.5 cm for 'PI559361' and 'PI572520', respectively. Pod height and plant height are positively correlated (r = 0.009**) depending on genotype, corroborating previous research (Singh et al., 2019).

To ensure effective combine harvesters, ground clearance must be more than 30 cm (Chaturvedi et al., 2014; Vishnu et al., 2020). The present results showed that, besides the aforementioned accession, 'PI572520", with the maximum above-ground pod height, that additional eight accessions, with an average height of 34.7 cm (W611345, PI533676, PI598080, PI559362, ABC, PI572491, PI567850 and PI518248) met this require-

**Table 4:** Results of the quantitative characteristics and morphological traits

| Accessions | Plant Height (cm) | Height of first Pod (cm) | Production Strip (cm) | Stems per Plant (n) | Filled Pods per Plant (n) |
|---|---|---|---|---|---|
| W611345 | 45.2 a-e | 31.3 bcd | 13.9 ab | 2.1 bc | 15.5 ef |
| PI533676 | 53.1 abc | 31.3 ab | 21.8 ab | 3.3 a | 43.6 ab |
| PI598080 | 49.1 a-d | 30.7 bcd | 18.3 ab | 2.5 abc | 23.3 cde |
| PI559361 | 34.1 e | 22.6 e | 11.4 ab | 1.5 c | 11.2 f |
| PI559362 | 50.4 abc | 34.3 abc | 16.0 ab | 2.5 abc | 12.3 ef |
| W63498 | 38.8 cde | 25.7 de | 13.0 ab | 3.1 ab | 52.1 a |
| PI572520 | 59.3 a | 40.5 a | 18.8 ab | 3.3 a | 24.1 cde |
| W617614 | 39.6 cde | 27.0 cde | 12.6 ab | 2.2 abc | 17.1 def |
| W617611 | 40.4 b-e | 28.8 cde | 11.6 ab | 2.9 ab | 21.2 def |
| ABC | 54.9 ab | 31.9 bcd | 22.9 a | 2.7 ab | 34.6 bc |
| PI572491 | 53.2 abc | 33.8 abc | 19.3 ab | 2.3 abc | 22.6 c-f |
| PI567850 | 58.6 a | 36.8 ab | 21.7 ab | 2.9 ab | 26.0 cd |
| PI518258 | 35.2 de | 25.1 de | 10.0 b | 2.2 abc | 18.2 def |
| PI518248 | 45.5 a-e | 31.8 bcd | 13.6 ab | 3.2 ab | 13.2 ef |
| Mean | 47.0 | 30.8 | 16.1 | 2.6 | 23.9 |

| Accessions | Empty Pods per Plant (n) | Seeds per Plant (n) | Seeds per Pod (n) | Seed Mass per Plant (g) | 100 Seeds Mass (g) |
|---|---|---|---|---|---|
| W611345 | 2.4 bc | 20.5 de | 1.3 ab | 3.79 ef | 18.7 bc |
| PI533676 | 3.2 ab | 54.7 b | 1.2 ab | 12.4 a | 22.4 b |
| PI598080 | 0.6 d | 23.2 cde | 1.7 ab | 3.8 e-g | 17.1 cd |
| PI559361 | 0.3 d | 14.4 e | 1.2 b | 1.5 h | 11.0 de |
| PI559362 | 1.3 cd | 25.0 cde | 2.0 a | 5.8 bc | 23.6 b |
| W63498 | 2.4 bc | 87.2 a | 1.6 ab | 12.9 a | 13.8 cde |
| PI572520 | 2.5 bc | 34.5 c | 1.4 ab | 8.3 bc | 23.7 b |
| W617614 | 1.8 bcd | 14.0 e | 0.8 b | 2.8 de | 20.7 bc |
| W617611 | 2.5 bc | 25.2 cde | 1.1 ab | 4.4 ef | 16.7 cd |
| ABC | 4.6 a | 62.7 b | 1.8 ab | 8.5 c | 14.1 cde |
| PI572491 | 4.8 a | 33.3 cd | 1.5 ab | 3.7 ef | 10.8 de |
| PI567850 | 2.7 bc | 30.9 cd | 1.2 ab | 9.8 b | 31.0 a |
| PI518258 | 1.57 cd | 30.3 cd | 1.7 ab | 4.4 bc | 17.2 e |
| PI518248 | 1.43 cd | 12.0 e | 0.9 b | 3.8 e | 32.6 a |
| Mean | 2.3 | 33.4 | 1.4 | 6.2 | 19.5 |

Means within rows followed by the same letter(s) are not different at 5% level as per Tukey's test

ment. Mechanical harvesting is advantageous in reducing production costs, even if seed losses must be taken into account in relation to plant structure. According to Haddad et al. (1988), the use genotypes producing tall, erect-structured plants, seed losses ranged from 2.6 to 5.0 % compared to losses of 20 % in semi-erect plants.

In this experiment the concept of "production strip" was introduced. This terminology refers to the productive part of the plant spanning the distance between the height of the first pod to the top of the plant, that can serve as a useful indicator in the regulation of combine harvesters. The production strip was positively and significantly related to plant height (r = 0.018*), height of the first pod (r = 0.001**), the number of empty pods ($r^2$ = 0.017*), the mass of the seeds per plant (r = 0.002**) and the protein content (r = 0.046*).

The number of branches per plant was also related to plant height and the height of the first pod (r = 0.031* and r = 0.022*, respectively). Table 4 shows an average branching number value of 2.3.

The structure of chickpea plants has been shown to be partly modified by sowing density. Singh et al. (2019) reported, with reference to the prerequisite of genotype for mechanical collection, how both the height of the plants and that of the first pod can be increased by increasing plant density. However, the response to a large number of plants per $m^2$ varies according to genotype and the agro-climatic environment.

The number of pods per plant, divided between full and empty, is an indication of the fruit set capacity and production potential. In the present experiment, the average number of pods per plant was 23.9, with a maximum of 52.1 and a minimum of 11.2 recorded for 'W63498' and 'PI559361', respectively. The lowest number of empty pods was shown by the accessions 'PI598080' and 'PI559362' with values of 0.6 and 0.3, respectively, corresponding to 2.6 and 2.7 % of the total pods. The accession with the highest number of empty pods was 'PI572491', accounting for 21.2 % of the total number of pods. However, the data must be evaluated against the number of seeds produced per plant and the number of seeds contained in a pod. As regards the number of seeds per plant, the average value was 38.4. The highest number of seeds per pod was reported for 'W63498' (87.2) and 'ABC' (62.7), respectively, which were significantly higher than the remaining accessions but also significantly different from each other. The number of seeds per plant was positively and significantly correlated with the number of branches per plant (r = 0.009**) and that of both full (r = 0.008**) and empty pods (r = 0.009**).

The accession with the highest number of seeds per pod was 'PI559362' (2.0), and this genotype was followed by the accessions, ABC (1.8), 'PI598080' and 'PI518258' (1.7), respectively. The genotypes that showed a significant minimum number of seeds per pod compared to remaining accessions, were W617614 and PI518248 with values of 0.8 and 0.9, respectively. The mean number of seeds per pod in the present field experiment (1.4) corroborated previous work, reported by Mohibullah et al. (2020).

Seed production per plant, as expressed by mass, is a quantitative character that is closely related to almost all other parameters with the exception of flowering period, height of the first pod and number of seeds per pod (Table 5). The average value was 6.2 g $plant^{-1}$ with the maximum values above 10 g recorded for 'W63498' (12.9) and 'PI598080' (12.4), respectively. Significantly lower seed mass were recorded for group composed of 'PI567850' (9.8), ABC (8.5) and 'PI572520' (8.3), respectively.

The 100 seed mass provides an indication of the size of the seeds which, in the present study, was averaged at 19.5 g. This figure appears to be lower than that observed previously with regard to the *desi* type of chickpea (Ton and Anlarsal, 2017; Mohibullah et al., 2020). The values were similarly lower compared to previous field tests, on other accessions, in the same geographical area (Casini, 1983). This could be attributed to varietal characteristics and to a reduced adaptation to the agroclimatic environment, as well as to the drought period that characterized much of the seed filling phase. However, values above 30 g per 100 seeds were obtained from 'PI518248' (32.6) and 'PI567850' (31.0). Moreover, other accessions, including PI572520 (23.7), PI559362 (23.6), PI533676 (22.4) and W617614 (20.7) recorded values similar to those found in the previously cited literature. According to Ton and Anlarsal (2017), the 100 seed mass has a high degree of heritability and is an important varietal feature to be taken into account in genetic improvement programs for the international scientific community.

The average seed yield was excellent, attaining 3.0 t $ha^{-1}$ despite the aforementioned drought period thanks to the good distribution of rains from April to the first decade of June. The most productive accessions were PI559362 (4.8), PI598080 (4.1) and the group W63498, PI572520 and ABC with an average of 3.7 t $ha^{-1}$, respectively. The accession with the lowest yield was PI518248 with 1.1 t $ha^{-1}$.

The average protein content of 22.7 % was considered excellent, with maximum values exceeding 24% for 'PI572520', 'W617614' and 'PI572850'. These values were shown to be higher than those reported previously (average of 18.5 %) for *desi* type chickpeas cultivated in the same environment (Rossi et al., 1983; Casini, 1983). The protein values were similarly higher than those reported in recent studies conducted outside Italy (Ghribi et al., 2015; Serrano et al., 2017; Rybiński et al., 2019). Contra-

**Table 5:** Pearson coefficient of correlation (r) for traits of investigated accessions

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Flowering | - | | | | | | | | | | | | | | | |
| 2 | Fruit Set | .008** | - | | | | | | | | | | | | | | |
| 3 | Maturity | .009** | .008** | - | | | | | | | | | | | | | |
| 4 | Plant Height | .575 | .052 | .096 | - | | | | | | | | | | | | |
| 5 | Height of First Pod | .518 | .343 | .357 | .009** | - | | | | | | | | | | | |
| 6 | Production Strip | .745 | .018* | .056 | .008** | .001** | - | | | | | | | | | | |
| 7 | Stems per Plant | .857 | .366 | .411 | .031* | .022* | .155 | - | | | | | | | | | |
| 8 | Filled Pods | .948 | .112 | .118 | .324 | .001** | .110 | .003** | - | | | | | | | | |
| 9 | Empty Pods | .303 | .199 | .787 | .003** | .022* | .017* | .073 | .003** | - | | | | | | | |
| 10 | Seeds per Plant | .262 | .245 | .530 | .327 | .941 | .073 | .009** | .008** | .009** | - | | | | | | |
| 11 | Seeds per Pod | .169 | .632 | .862 | .491 | .011* | .330 | .777 | .832 | .180 | .003** | - | | | | | |
| 12 | Seed Weight per Plant | .529 | .002** | .015* | .004** | .894 | .002** | .001** | .008** | .004** | .001** | .117 | - | | | | |
| 13 | 100 Seed Weight | .320 | .093 | .095 | .003** | .849 | .100 | .005** | .587 | .616 | .281 | .358 | .057 | - | | | |
| 14 | Yield | .712 | .887 | .958 | .296 | .448 | .304 | .871 | .166 | .733 | .016* | .009* | .057 | .713 | - | | |
| 15 | Protein | .624 | .114 | .150 | .045* | .074 | .046* | .943 | .252 | .169 | .513 | .889 | .286 | .746 | .043 | - | |
| 16 | Crude Fiber | .911 | .259 | .218 | .006** | .001** | .314 | .852 | .095 | .160 | .341 | .046* | .810 | .153 | .439 | .154 | - |

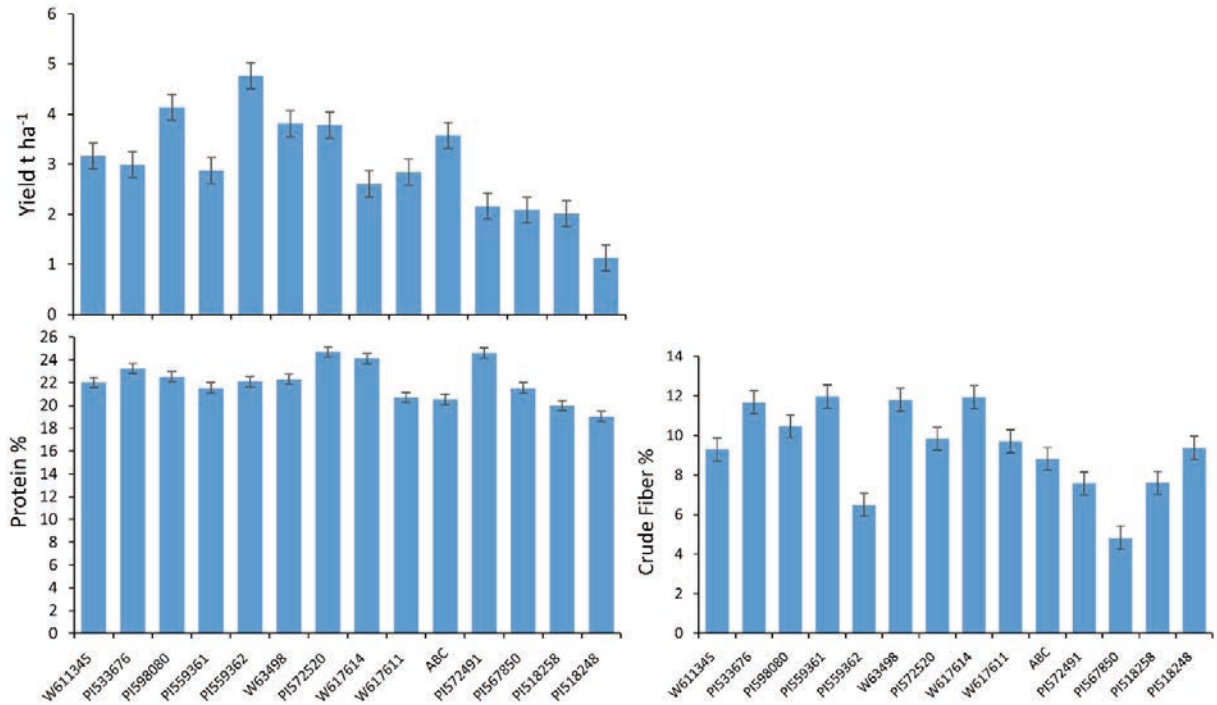


**Figure 4:** Yield, protein and crude fiber content of the accessions tested. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at $p \leq 0.05$

ry to the observations of Kulwal and Mhase (2017), no significant positive correlation between seed mass and protein content was found. Instead there was a significant correlation between protein content and both plant height (r = 0.045*) and production strip (r = 0.046*).

The fiber content was very heterogeneous (Figure 3). A group of four genotypes were shown to have values above 11.0 %, with a maximum of 12.0 % for 'PI559361'. Another group of five lines was characterized by values between 8.0 and 10.0% (W611345, PI572520, W617611, ABC, PI518248). The lowest value of 4.8 % was shown for 'PI567850', characterized by clear tegument. The high fiber content, while characterizing various groups of chickpeas from a qualitative point of view, also denotes a prolonged mean cooking time, generally exceeding 120 min. This is also associated with low hydration capacity (≥ 0.16 g), as attested by Khan et al. (1995).

## 4 CONCLUSIONS

In view of the scarce experimental information on the possibility of cultivating *desi* chickpea genotypes in Central Italy, outside the cultivation area of Southern Italy, the present results permitted us to make some interesting observations.

Grain yield was considered of an excellent level for 57 % of the accessions tested, some of which exceeded 3.0 t ha$^{-1}$, with the maximum value of 4.1 recorded by 'PI598080'. This yield capacity was perfectly comparable to that obtained on the same experimental farm in 2016 and 2017 using *kabuli* chickpea accessions, selected for the production of canned seeds (Casini, 2018). In practice, the present results show that by selecting the most adaptable genotypes for the agroclimatic environment, even the cultivation of *desi* chickpea can be cost-effective for farmers, whilst demonstrating all the well-known agronomic benefits characteristic to legumes. The market share in Italy currently occupied by the *desi* chickpeas is a niche area limited to the health sector. In part, this serves to attenuate the highly unstable chickpea market prices, attributable to the high yield variability and competition from cereal crops, such as rice and wheat, considered as commodities, that also receive a price support policy by governments (Merga and Haji, 2019).

Taking into account the type of Italian market for which the production of *desi* chickpea is intended, the excellent average protein and dietary fiber content are able to meet the needs of the consumer, thereby placing this type of seed in the category of foods considered complementary to the Mediterranean diet. These products, especially if produced in organic farming, have an added market value, resulting in a higher retail price that the consumer is generally willing to support after acquiring

knowledge of the beneficial characteristics. In Italy, from a commercial point of view, the size of chickpea seed is an important factor influencing consumer preference. Taste in size has been based on the prevalent spread of *kabuli* chickpea (mass of 100 seeds > 45-50 g). This preference has also been "transferred" to the *desi* chickpea, and for this reason, genotypes allocated to the Italian market, will need to favor larger seed accessions with a 100 seed mass exceeding at least 30 g. From this point of view, only two tested genotypes satisfy this requisite, namely 'PI518248' (brown seed) and 'PI567850' (black seed).

Most of the accessions, in addition to possessing a good yield, also possess a plant structure, suitable for mechanical harvesting. The height of the first above-ground pod exceeding 30 cm, with fewer branches and an average production strip of 16.1 cm, are all characteristics that correspond to an ideotype of chickpea in which production is concentrated at the top of the plant, thereby facilitating all cultivation operations, from weeding, to hilling, to harvesting. From this point of view, the genotypes that conform perfectly to the ideotype, and with good yields, were PI598080, PI559362, PI572520 and ABC.

With regard to the ABC, the only accession of Italian origin, the grain yield was of an excellent level, even when cultivated in the environment of Central Italy. However, as reported by Summo et al., (2017), this genotype is characterized by a significantly lower protein content than all other accessions. The same authors also pointed out that the chemical, nutritional and functional characteristics of ABC, are generally distinctive from both *kabuli* chickpeas and foreign-sourced *desi* chickpeas.

Finally, the overall indications emerging from the present research provide the basis towards laying the foundations for the future introduction of *desi* chickpea in Central Italy, with the potential for sustainable yield and quality.

## 5 ACKNOWLEDGMENTS

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# Umerjanje merilnikov in določitev vodozadrževalnih lastnosti tal za natančno namakanje na podlagi meritev vsebnosti vode v tleh

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**Calibration of soil moisture sensors, determination of soil water retention properties for precision irrigation based on soil water content measurements**

**Abstract:** Water is becoming a scarce commodity. Therefore, proper water management in precision irrigation is crucial to increase productivity and reduce the cost of crop production. Precision irrigation is based on measuring the soil water content, which is often measured with dielectric sensors that measure the apparent permittivity of the soil. Although the sensors are already equipped with a factory calibration function that converts the measured permittivity into volumetric water content, the function does not work properly for all soil types. It is therefore recommended to check whether a soil-specific calibration is required for accurate soil water content measurements. Precise irrigation also requires adequate determination of soil water retention properties, which can be determined using various procedures and methods. In this paper, we presented the results of applying different approaches to determine soil-specific calibration functions using two types of dielectric sensors (SM150T, Delta-T Devices and MVZ 100, Eltratec) in different soil samples obtained from locations in Slovenia where precision irrigation is performed. In addition, the results of determining the water retention properties of the soil using different methods were also compared.

**Key words:** precision irrigation; soil moisture sensors; water retention properties; calibration methods

**Umerjanje merilnikov in določitev vodozadrževalnih lastnosti tal za natančno namakanje na podlagi meritev vsebnosti vode v tleh**

**Izvleček:** Voda postaja redka dobrina, zato je strokovno pravilno upravljanje vode za natančno namakanje ključnega pomena za povečanje primarne rastlinske produkcije in zmanjšanje stroškov rastlinske pridelave v kmetijstvu. Natančno namakanje temelji na meritvah vsebnosti vode v tleh, ki jo običajno merimo z merilniki, ki merijo dielektričnost tal. Merilniki so opremljeni s tovarniško umeritveno funkcijo, ki zaznano dielektričnost pretvarja v volumsko vsebnost vode, vendar slednja ne deluje ustrezno v vseh talnih tipih. Zato je za točne meritve vsebnosti vode v tleh smiselno preveriti, ali je potrebna uporaba talno specifične umeritve. Poleg tega je za natančno namakanje potrebna tudi ustrezna določitev vodozadrževalnih lastnosti tal, ki jih lahko določimo z uporabo več različnih postopkov in metod. V prispevku smo na izbranih vzorcih tal, pridobljenih iz lokacij po Sloveniji, kjer se izvaja natančno namakanje, prikazali rezultate različnih načinov določanja talno specifičnih umeritvenih funkcij z dvema tipoma merilnikov (SM150T, Delta-T Devices in MVZ 100, Eltratec). Primerjali smo tudi rezultate določanja vodozadrževalnih lastnosti tal z uporabo različnih metod.

**Ključne besede:** natančno namakanje; merilniki vsebnosti vode v tleh; vodozadrževalne lastnosti tal; umeritev

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## 1 UVOD

Voda postaja redka dobrina, ne le v sušnih regijah, temveč tudi v regijah z obilnimi padavinami (Pereira in sod., 2002). Kmetijstvo velja za največjega porabnika sladke vode na svetu, zato so tehnološke inovacije, ki lahko izboljšajo trajnost namakanja v kmetijstvu, pomembne za optimalno izrabo vodnih virov in varovanje okolja (Adeyemi in sod., 2017).

Pri tem imajo pomembno vlogo namakalni sistemi in različni načini kmetijske pridelave. V izogib manjšanju primarne rastlinske produktivnosti zaradi sušnega stresa (raba premalo vode za namakanje), kmetje praviloma namakajo z večjimi obroki in porabijo več vode, kot je potrebno, kar vodi v prekomerno obremenjevanje okolja. Tako se zmanjšuje pridelovalna sposobnost določenih sistemov in se porablja večja količina vode in energije (Kamienski in sod., 2018). Pri natančnem namakanju vodo uporabljamo bolj učinkovito, pri čemer se izognemo tako nezadostnemu kot prekomernemu namakanju. Pametno upravljanje vode za natančno namakanje v kmetijstvu je ključnega pomena za povečanje pridelave na določeni površini in zmanjšanje stroškov, hkrati pa prispeva k okoljski trajnosti (Neupane in Guo., 2019). Konvencionalne kmetijske prakse upravljajo namakalne površine enakomerno, brez vključitve topografskih lastnosti površin, različnih karakteristik tal ter različnih razmer rasti namakanega pridelka in drugih agronomskih dejavnikov. To lahko privede do povečanja onesnaževanja okolja, povečanega izpiranja hranil in zmanjšuje dobičkonosnost in pridelovalno sposobnost namakane površine (Khosla in sod., 2002).

Z usmeritvijo v natančno namakanje se površine razdeli na manjše enote za upravljanje ter optimalno pridelavo. Začetki uvajanja natančnega namakanja izhajajo iz začetka osemdesetih let z razvojem različnih tehnologij za ocenjevanje terenskih variabilnosti. Tako je natančno namakanje in z njim natančno kmetijstvo odvisno od podrobnih prostorskih informacij, uporabe različne informacijske tehnologije ter večje zmogljivosti za obdelavo informacij (McBratney in sod., 2015).

### 1.1 DOLOČANJE VSEBNOSTI VODE V TLEH

Vsebnost vode v tleh lahko določimo z različnimi metodami. Osnovna, neposredna je gravimetrična metoda, ki temelji na odstranitvi vode iz tal, pri čemer neposredno izmerimo delež odstranjene vode. Odstranitev vode lahko poteka na več načinov, z ekstrakcijo in nadomestitvijo vode s topilom (kemična reakcija) ali s segrevanjem. Količina odstranjene vode je nato določena s kemično fizikalno analizo topila ali s spremembo mase po segrevanju. Metoda je splošno uporabljena kot standard za ostale neposredne in posredne metode (Topp in Ferre, 2002). Vsebnost lahko določimo tudi s posrednimi metodami, ki merijo kemične ali fizikalne lastnost tal, ki so odvisne od vsebnosti vode v tleh (Hignett in Evett, 2008).

### 1.2 MERILNIKI VSEBNOSTI VODE V TLEH IN NJIHOVA UMERITEV

Večino trenutno uporabljenih merilnikov vsebnosti vode v tleh uvrščamo v skupino elektromagnetnih metod. Gre za posredno metodo meritev, ki temelji na meritvah relativne dielektričnosti tal (ε). V primerjavi z relativno dielektričnostjo trdne faze tal (ε = 2 - 5) in zraka (ε = 1), je relativna dielektričnost vode bistveno višja (ε = 80), zato je dielektričnost tal primarno posledica prisotnosti vode v obliki kapljevine (Topp in Ferré, 2002; Hignett in Evett, 2008). Talne lastnosti, kot so tekstura, predvsem delež gline, mineralna sestava, delež organske snovi, električna prevodnost, gostota tal in temperatura, imajo v večji ali manjši meri vpliv na meritve z merilniki, ki merijo dielektričnost tal (Vaz in sod., 2013; Fares in sod., 2016; Matula in sod., 2016; Hajdu in sod., 2019; Kassaye in sod., 2019). Zato uporaba zgolj tovarniške umeritvene funkcije ne deluje ustrezno v vseh tipih tal. Mnogo avtorjev ob uporabi merilnikov, ki delujejo z nižjo delovno frekvenco, priporoča izvedbo talno specifične umeritve (Parvin in Degre, 2016; Roberti in sod., 2018; Domínguez-Niño in sod., 2019).

Standardna referenčna metoda umeritve merilnikov, ki merijo dielektričnost tal, je termo gravimetrična, kjer vodo iz vzorca tal odstranimo s sušenjem in vsebnost vode v vzorcu določimo s tehtanjem (Topp in Ferré, 2002). Poznamo različne načine izvedbe talno specifične umeritve tovrstnih merilnikov. Umerjanje lahko izvedemo na terenu ali v laboratoriju.

Laboratorijsko umerjanje lahko izvedemo na porušenem ali neporušenem vzorcu tal. Pri umerjanju porušenega vzorca tla najprej posušimo na zraku, jih presejemo skozi sito, zmešamo z znano količino vode ter jih zgostimo na naravno gostoto v primerno veliki posodi. Z vsako tako meritvijo dobimo le eno umeritveno točko, zato moramo postopek ponoviti pri vsaj petih različnih količinah dodane vode (Starr in Paltineanu, 2002). Umerjanje v neporušenih talnih monolitih je po mnenju določenih avtorjev bolj napredna, zaradi ohranitve naravne strukture tal (Provenzano in sod., 2015; Weitz in sod., 1997). V tem primeru merilnik vstavimo v z vodo nasičen talni monolit in ga pustimo, da se naravno suši. Vzorec tal skupaj z merilnikom v določenih časovnih intervalih tehtamo za kasnejšo gravimetrično določitev vsebnosti vode in sočasno odčitamo izhodne podatke

merilnika. Na ta način dobimo serijo merilnih točk na istem vzorcu tal (Holzman in sod., 2017).

Večina proizvajalcev merilnikov, ki merijo dielektričnost tal, v priročnikih za uporabo opiše priporočen način umerjanja njihovih merilnikov. Delta-T Devices Ltd., Cambrige, VB, za umeritev merilnika SM150T priporoča uporabo dvo-točkovne talno specifične umeritve, ki je podrobneje opisana v priročniku (Delta-T Devices, 2016). METER Group Inc., Pullman, ZDA, za svoje merilnike priporoča metodo umeritve kapacitivnih merilnikov v porušenem vzorcu tal, predlagane s strani Starr in Paltineanu (2002). Stevens Water Monitoring Systems Inc., Oregon, ZDA, navaja, da talno specifična umeritev njihovih merilnikov za večino tal ni potrebna.

## 1.3 POMEN LASTNOSTI TAL PRI DOLOČANJU PARAMETROV NAMAKANJA

Vsebnost vode v tleh pomembno vpliva na številne temeljne biofizikalne procese. Vpliva na mikrobno razgradnjo organske snovi v tleh, kalitev semen in rast ter prehrano rastlin. Določitev vsebnosti vode v tleh je potrebna za različne namene, od vnosa podatkov za zagon izdelave globalnih podnebnih modelov do spremljanja vsebnosti vode na terenu za uporabo v različnih kmetijskih praksah.

Za pravilno izbiro in izvedbo namakanja je potrebno poznavanje lastnosti tal, na katerih se bo namakanje izvajalo. Pridobiti moramo podatke, ki določajo lastnosti namakanih tal, med njimi so: informacije o zastopanosti posameznih talnih tipov na namakanih površinah; podatki o globini talnih horizontov ter sposobnosti zadrževanja vode v posameznem horizontu; teksturi tal ter koeficientu infiltracije (Baviskar in Heimovara, 2017; Pintar, 2006).

Kompleksen, trifazni sistem, ki ga predstavljajo tla, je sestavljen iz mešanice plinastih, tekočih in trdnih snovi. Mineralni delci in organska snov predstavljajo trdno fazo, povezano v porozen prostor. Voda s primesmi hranil in drugih snovi sestavlja talno raztopino, ki je del tekoče faze tal, zrak, ujet v talne pore pa predstavlja plinasto fazo tal (Pintar, 2006). Za določitev lastnosti tal, ki vplivajo na tok vode v tleh uporabljamo razmerja med plinasto, tekočo in trdno fazo tal. Teksturne razrede tal predstavljajo deleži različno velikih delcev tal. Tako lahko govorimo o glinenih, ilovnatih, meljastih, peščenih, peščeno meljastih, peščeno glinastih tleh, itn. Mineralna veziva in organska snov med seboj vežejo talne delce, ki dajejo tlom različno strukturo. Struktura, skupaj s teksturo, določa sposobnost tal za zadrževanje vode in vpliva na koeficient infiltracije, ki je pomemben pri izbiri kapacitete namakalnega sistema, predvsem ko namakamo z razpršilci (Baviskar in Heimovara, 2017,Zhang in sod., 2018).

Rastline morajo, zato da lahko sprejemajo vodo skozi korenine, premagati negativni tlak (podan tudi kot pF vrednost, ki je negativni logaritem vodnega stolpca, izraženega v centimetrih), s katerim je voda vezana na talne delce oz. matrični potencial vode v tleh (Ψ). Ko je Ψ -1500 kPa oziroma pF 4,2 (največja vrednost, ki jo večina rastlin lahko premaga), govorimo o točki venenja (TV). Pri takem stanju vode v tleh rastline trajno uvenijo in si ne opomorejo, četudi jih namočimo. Ko količina vode v tleh narašča, voda postaja vedno bolj dostopna rastlinam, z debeljenjem plasti vodnih molekul, ki so vezane na talne delce. Ko je vode veliko in je Ψ od 6 kPa do -33 kPa (pF med 1,8 ter 2,5), je dosežena poljska kapaciteta (PK) tal. To je stanje, ko je v tleh največja količina vode, ki jo tla lahko zadržijo. V tleh je rastlinam dostopna voda, ki se zadrži med PK in TV. Označujemo jo kot razpoložljivo vodo (RV) (Pintar, 2003). Za natančno namakanje je zelo pomembno natančno določiti vsebnost vode pri PK in TV.

PK in TV sta dve najpomembnejši točki na t. i. krivulji vodozadrževalnih lastnosti tal (VZL), ki predstavlja povezanost vsebnosti vode in matričnega potenciala tal (Bittelli, 2011). Pomembna je tudi krtična točka (KT), ki predstavlja točko določene količine vode v tleh, ko rastlina relativno lahko črpa vodo iz tal, pod to količino pa je rastlina v sušnem stresu. PK in TV sta talno specifični lastnosti, enako kot celotna krivulja VZL, medtem ko je KT rastlinsko specifična lastnost (Pintar, 2003). V praksi to pomeni, da na istih tleh različne vrste rastlin pokažejo znake sušnega stresa pri različni vsebnosti vode v tleh.

## 1.4 DOLOČITEV KRIVULJE VODOZADRŽEVALNIH LASTNOSTI TAL

Za določanje krivulje VZL tal lahko uporabimo več različnih postopkov in metod, ki so bile razvite v zadnjih desetletjih (Bittelli, 2011). Uporabimo lahko metodo Richardove tlačne posode, pri kateri s pomočjo nadtlaka izpodrinemo vodo, katere vezava na talne delce je šibkejša kot vzpostavljeni nadtlak. Večji kot je tlak, več vode lahko iztisne. Za določanje TV talne vzorce na keramični plošči izpostavimo tlaku 1500 kPa za približno teden dni (Bittelli, 2011). Vsebnost vode, ki je ostala v talnem vzorcu, določimo z gravimetrično metodo. Na enak način določimo vsebnost vode za katerokoli vrednost Ψ. Dobljene točke povežemo v najbolje prilegajočo se krivuljo.

Napredek tehnologije je privedel do razvoja avtomatizirane naprave, ki precej skrajša postopek izdelave krivulje VZL tal. Naprava HYPROP® (proizvajalec UMS GmbH, München, Nemčija) deluje na podlagi Schin-

dlerjeve metode evaporacije. Omogoča kontinuirano merjenje Ψ s pomočjo dveh tenziometrov in hkratnega tehtanja vzorca tal (Schindler in sod., 2010). Tenziometra sta nameščena na dveh ravneh v neporušenem, z vodo nasičenem vzorcu tal z znanim volumnom (250 $cm^3$).

Vsebnost vode za krivuljo VZL tal analiziranega vzorca izračunamo na podlagi izgube mase vzorca. Naprava meri v območju med 0 do -100 kPa, pri majhnih vsebnostih vode pa meritve modelira z uporabo različnih, v programskem orodju vgrajenih, modelov. Meritve se izvajajo med naravnim postopkom sušenja tal. Čas merjenja znaša, odvisno od tal, od dveh (vzorci glinastih tal) do največ 10 dni (vzorci šotnih in peščenih tal). Ob koncu meritev s pomočjo termogravimetrične metode ugotovimo maso vode v talnem vzorcu in to je podlaga za preračun mase vode v času izvajanja meritev, ki ga izvedemo s pomočjo programskega orodja HYPROP-FIT (HYPROP Operation Manual, 2015).

Iz pridobljenih podatkov se nato s pomočjo programskega orodja izriše krivulja VZL tal (HYPROP Operation Manual, 2015; Durner in sod., 2015). V programskem orodju lahko za izris krivulj uporabimo več različnih modelov hidravličnih lastnosti tal. Matrični potencial vode v tleh v grafičnem prikazu, t. j. krivulji VZL tal izrazimo s pF vrednostjo, kar omogoča bolj pregleden prikaz VZL tal. Nabor vrednosti vsebnosti vode v tleh je relativno majhen (od 0 do npr. 60 vol. %) v primerjavi z naborom vrednosti Ψ (od 0 do -1500 kPa oz. od 0 do 15 000 cm vodnega stolpca), zato je prikaz s semilogaritemskim grafom (logaritmiramo vrednosti vodnega stolpca izraženega v centimetrih, da pridemo do pF vrednosti) precej bolj primeren. Krivulje VZL tal so specifične za posamezen tip tal in so odvisne od teksture, strukture, gostote tal ter vsebnosti organske snovi (Pintar, 2003).

PK in TV lahko določimo tudi iz podatkov o teksturi tal. S t. i. Saxtonovim modelom lahko izračunamo PK (pF 2,5) in TV (pF 4,2) na osnovi masnega deleža peska in gline s pomočjo nelinearne regresijske zveze (Saxton in sod., 1986). Leta 2006 sta Saxton in Rawls model nadgradila še s štirimi dodatnimi parametri, in sicer: vsebnost organske snovi, gostota tal, vsebnost skeleta in slanost tal (Saxton in Rawls, 2006).

Poleg zgoraj opisanih metod ter različnih modelov za določanje vsebnosti vode v tleh, lahko vrednosti PK določimo tudi na terenu (Veihmeyer and Hendrickson, 1949). Po obilnem deževnem dogodku oz. doseženem stanju nasičenosti tal z vodo (voda na opazovani lokaciji stoji na površini), lahko po 2-3 dneh od tega dogodka, ko pronicanja vode v globlje plasti tal ni več, s pomočjo merilnika izmerimo vsebnost vode v tleh, ki predstavlja stanje PK izbrane lokacije (Zotarelli in sod., 2010).

## 2 MATERIAL IN METODE

### 2.1 UMERJANJE MERILNIKOV

Umerjanje smo izvedeli z dvema različnima vrstama merilnikov, ki merijo dielektričnost tal: merilnik SM150T, Delta-T Devices Ltd., ki deluje po principu kapacitivnosti (Delta-T Devices, 2016) in merilnik MVZ 100, Eltratec, trgovina, proizvodnja in storitve d.o.o., SLO, ki po proizvajalčevih trditvah deluje na podlagi merjenja odboja v časovnem prostoru (angl. Time Domain Reflectometry - TDR). Z merilniki SM150T smo umeritev izvedli na tleh iz šestih različnih lokacij po Sloveniji, ki so vključene v projekt EIP Pro-Pridelava (Pro-Pridelava, EIP, BF, Oddelek za Agronomijo, 2020). Merilnike MVZ 100 smo umerili na 35 vzorcih tal iz Vipavske doline, vključenih v projekt LIFE VivaCCAdapt (LIFE VivaCCAdapt; 2020).

Talno specifično umeritev merilnikov smo izvedli v laboratoriju na neporušenih vzorcih tal (Holzman in sod., 2017). Najprej smo za vsak tip merilnika določili volumen vpliva, na podlagi katerega smo izbrali dimenzije vzorčnega valja. Postopek je podrobneje opisan v Vaz in sod. (2013). Za merilnik SM150T smo izbrali valj s polmerom 5,15 cm in višino 8,00 cm, za merilnik MVZ 100 pa s polmerom 3,50 cm in višino 6,00 cm. Kovinsko masko valjaste oblike, znotraj katere je bil nameščen PVC valj, merilniku ustreznega volumna zaznave, smo zabili v tla in tako dobili neporušen vzorec tal. V laboratoriju smo odvečno maso tal odrezali in poravnali z robom valja, za pridobitev vzorca znanega volumna. Na eno ploskev valja smo namestili filter papir in kovinsko mrežico za preprečitev sipanja vzorca. Tako pripravljene vzorce smo postavili v posodo in vanjo postopoma nalili vodo, skoraj do roba valjev. Vzorce smo pustili v posodi toliko časa, da so se popolnoma nasitili z vodo. Nato smo jih vzeli iz posode in pustili, da odvečna voda odteče in na sredino vsakega vstavili merilnik. V določenih časovnih intervalih smo odčitali surovo vrednost merilnika, mV v primeru SM150T in vsebnosti vode pridobljene s privzeto umeritvijo v primeru MVZ 100. Po odčitku smo nemudoma stehtali celoten vzorec, skupaj z merilnikom, za kasnejšo gravimetrično določitev vsebnosti vode v vzorcu. Časovna serija meritev je potekala do vidne suhosti vzorcev, ki je bila dosežena po približno 14 dneh, na sobni temperaturi 22 °C. Po zadnji meritvi smo vzorce pretresli v papirnate vrečke in sušili v pečici na 105 ˚C za določitev mase suhe snovi. Zaradi večjega volumna, kot je naveden v standardu (ISO 11465, 1993), smo jih 48 ur, do konstantne mase.

Z enačbo 1 smo izračunali volumsko vsebnost vode v tleh.

$$\theta\ (\%) = \left(\frac{m_{sk} - m_{ss}}{m_{ss}}\right) \times \frac{\rho_t}{\rho_v} \times 100 \qquad (1)$$

$\theta$ je volumska vsebnost vode, $m_{sk}$ je skupna masa tal in vode, $m_{ss}$ je masa suhe snovi tal, je gostota tal in je gostota vode.

Z regresijsko analizo smo za vsako vzorčno mesto določili večtočkovno talno specifično, linearno ali polinomsko umeritveno funkcijo, ki povezuje gravimetrično določeno $\theta$ z: a) surovimi vrednostmi merilnika ali z b) $\theta$, pridobljenimi s tovarniško umeritvijo.

Zgoraj opisani način umeritve nam je hkrati omogočil tudi izvedbo dvotočkovne talno specifične umeritve merilnikov SM150T po priporočilu proizvajalca. V mokrem in suhem talnem monolitu smo odčitali surovo vrednost merilnika in jo s priloženo enačbo pretvorili v koren iz dielektričnosti. Razmerje med vsebnostjo vode in korenom iz dielektričnosti je linearno. Postopek je podrobneje opisan v priročniku (Delta-T Devices, 2016).

Na lokacijah projekta EIP Pro-Pridelava, smo primerjali umeritvene krivulje merilnikov SM150T, pridobljene na različne načine: a) privzeta umeritvena funkcija, b) dvotočkovna talno specifična umeritvena funkcija, predlagana s strani Delta-T Devices in c) večtočkovna talno specifična umeritvena funkcija, pridobljena z regresijsko analizo. Na primerih umerjanja z merilniki MVZ 100 na tleh iz Vipavske doline (projekt LIFE VivaCCAdapt) smo izrisali grafe, ki prikazujejo napake, povzročene v primeru a) izbire linearne ali polinomske večtočkovne umeritvene funkcije in b) ob uporabi različnega števila umeritvenih točk.

Za analizo smo uporabili 14 vzorcev iz lokacij projekta LIFE VivaCCAdapt ter 22 vzorcev iz projekta EIP Pro-Pridelava. Za vse vzorce je bila določena tekstura in gostota tal. Vrednosti gostote tal ter tekstura so v Preglednici 1, predstavljene za 6 izbranih vzorcev, ki smo jih grafično prikazali v poglavju rezultati.

## 2.2 DOLOČITEV VODOZADRŽEVALNIH LASTNOSTI TAL

Meritve za izris krivulj VZL tal izbranih lokacij smo izvedli s sistemom HYPROP®, za izris krivulj pa smo uporabili osnovni van Genuchtenov model, ki na podlagi meritev, z upoštevanjem strukture izbranega vzorca tal izdela krivuljo VZL (van Genuchten, 1980; Durner in sod., 2015).

V raziskavi smo za določitev PK in TV izbranih lokacij projekta EIP Pro-Pridelava uporabili različne metode. PK smo določili po treh metodah; (1) na terenu, pri čemer smo določili vrednost po več močnih deževnih dogodkih s pomočjo talno specifično umerjenega merilnika SM150T, (2) s sistemom HYPROP® z evaporativno metodo, pri pF 2,5 in 1,8 ter vzeli povprečje teh dveh vrednosti ter (3) uporabili model Saxton (Saxton in sod., 1986; Saxton in Rawls, 2006).

TV je bila v laboratoriju določena z meritvami v Richardovi tlačni posodi, kar nam je predstavljalo referenčno vrednost, in na sistemu HYPROP®, oboje pri pF 4,2, ter po modelu Saxton (Saxton in sod., 1986; Saxton in Rawls, 2006).

## 2.3 METODE STATISTIČNE ANALIZE

Večtočkovno talno specifično umerjanje smo izvedli z uporabo regresijske analize. Funkcija je bila linearna ali polinomska, in sicer do 5. stopnje. Optimalno stopnjo polinoma smo določili z zaporednimi F-testi za dva gnezdena modela.

Merilne napake ob uporabi različnih umeritvenih krivulj in merilne napake za določitev vsebnosti vode pri poljski kapaciteti smo izračunali z enačbo 2.

$$Napaka\ (vol.\%) = \theta i - \theta ref \qquad (2)$$

**Preglednica 1**: Talne lastnosti izbranih vzorcev iz projekta PRO-PRIDELAVA (oznaka vzorca PP1, PP2) ter LIFE VivaCCAdapt (oznaka vzorca L1-L4)
**Table 1:** Soil properties of selected soil samples from the projects PRO-PRIDELAVA (sample ID PP1, PP2) and LIFE VivaCCAdapt (sample ID L1-L4)

| VZOREC | Pesek (%) | Melj (%) | Glina (%) | Tekstura | GOSTOTA (g/cm³) |
|---|---|---|---|---|---|
| PP1 | 43 | 40 | 17 | I | 1,48 |
| PP2 | 15 | 46 | 38 | MGI | 1,27 |
| L1 | 22 | 50 | 28 | GI | 1,59 |
| L2 | 40 | 51 | 9 | MI | 1,37 |
| L3 | 27 | 51 | 22 | MI | 1,51 |
| L4 | 34 | 54 | 12 | MI | 1,53 |

$\theta_i$ je izmerjena oz. določena vsebnost vode in $\theta_{ref}$ je referenčna vsebnost vode; v primeru umerjanja merilnikov je to gravimetrično določena vsebnost vode in v primeru določitve poljske kapacitete je referenčna vsebnost vode določena na terenu po več močnih deževnih dogodkih s pomočjo talno specifično umerjenega merilnika SM150T.

Analize podatkov in grafični prikazi so bile izvedeni s programom R 3.5.3 (R Core Team 2019).

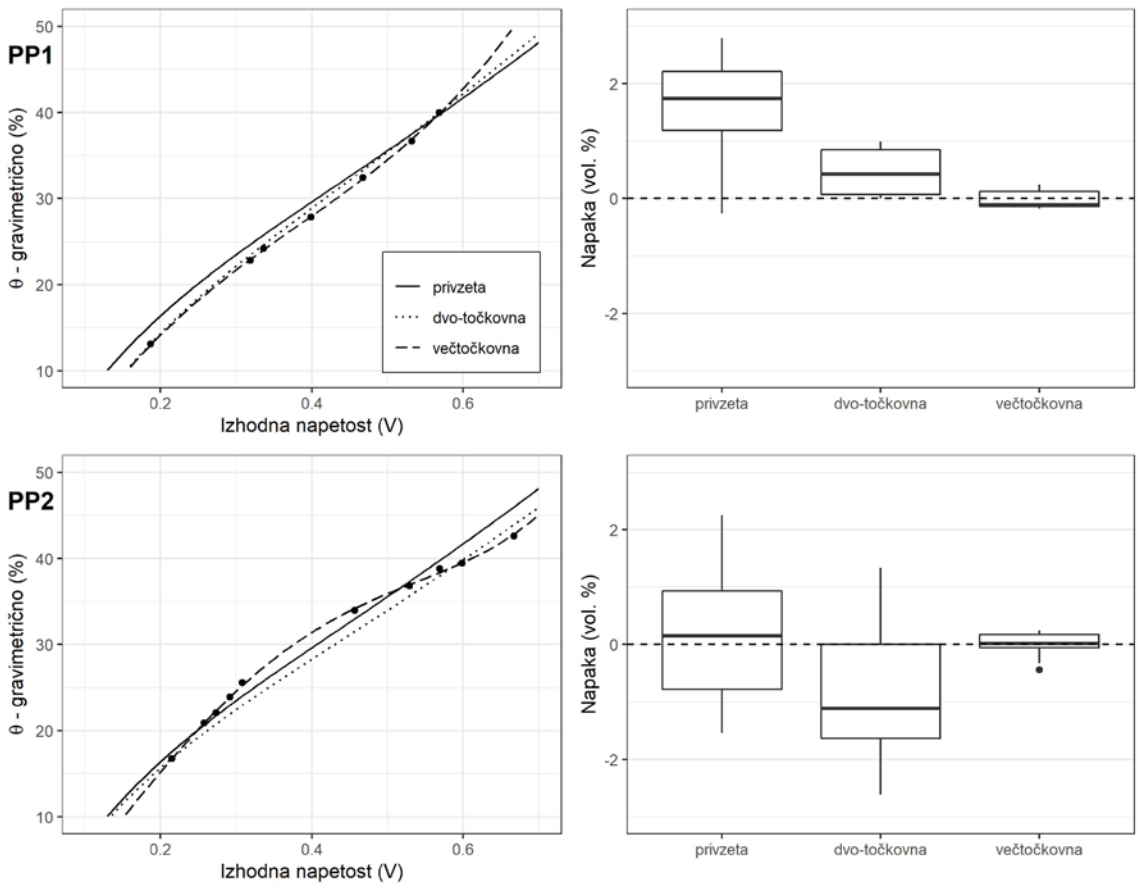
## 3 REZULTATI IN DISKUSIJA

### 3.1 UMERJANJE MERILNIKOV

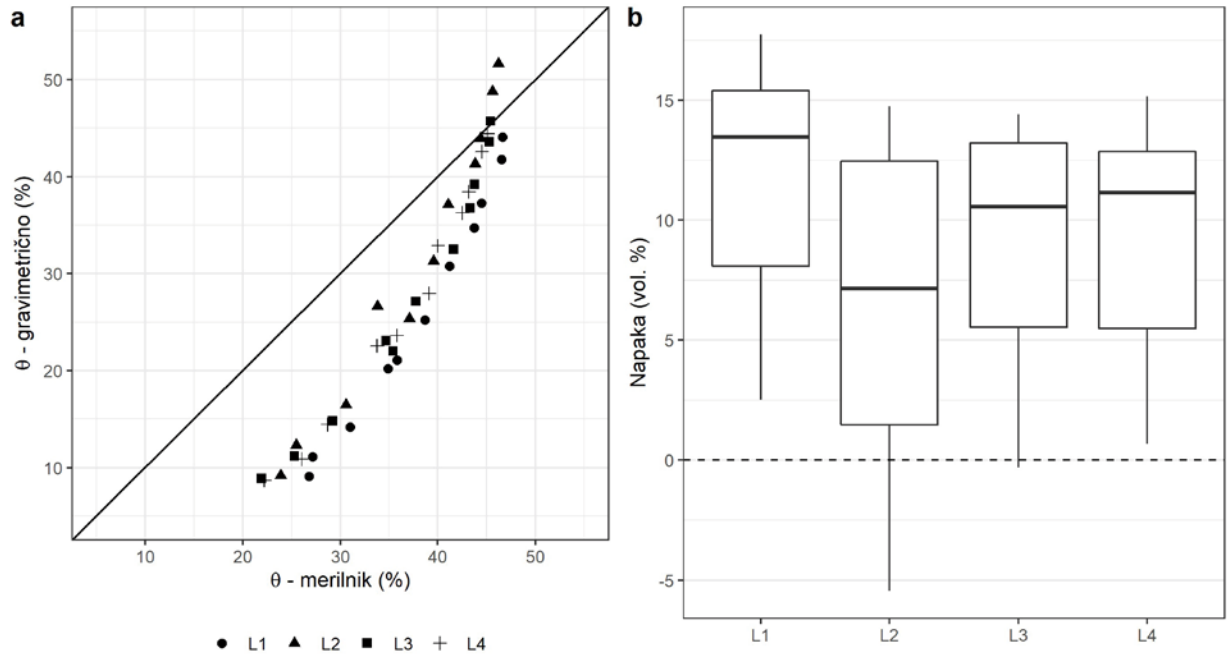
Najmanjšo razliko med izmerjenimi vrednostmi z merilnikom in z gravimetrično metodo določeno vsebnostjo vode v tleh in s tem najbolj natančno umeritev merilnikov SM150T v skoraj vseh primerih dobimo z večtočkovno talno specifično umeritvijo (Slika 1). Mediana napak je v večini primerov manjša kot 0.5 vol. %. Tudi dvotočkovna talno specifična umeritev je v skoraj vseh primerih boljša od tovarniško privzete, a so napake v večini primerov bistveno večje kot pri večtočkovni talno specifični umeritvi. Mediana napak se v večini primerov giblje okoli 1 %. Ugotavljamo tudi, da je bila napaka meritve vsebnosti vode v tleh ob privzeti tovarniški umeritvi v večini primerov v okviru navedbe proizvajalca, t. j. ± 3 vol. % (Delta-T Devices, 2016).

Tovarniško privzeta umeritev merilnika MVZ 100 je zelo netočna (Slika 2). Mediana napak predstavljenih štirih primerov se giblje med 7 in 13 vol. %, največja napaka je celo nad 15 vol. %. Merilnik podaja vsebnosti vode, ki so večje od dejanskih, razen pri zelo velikih vsebnostih vode v tleh. Poleg tega se z zmanjševanjem volumske vsebnosti vode v tleh napaka povečuje.

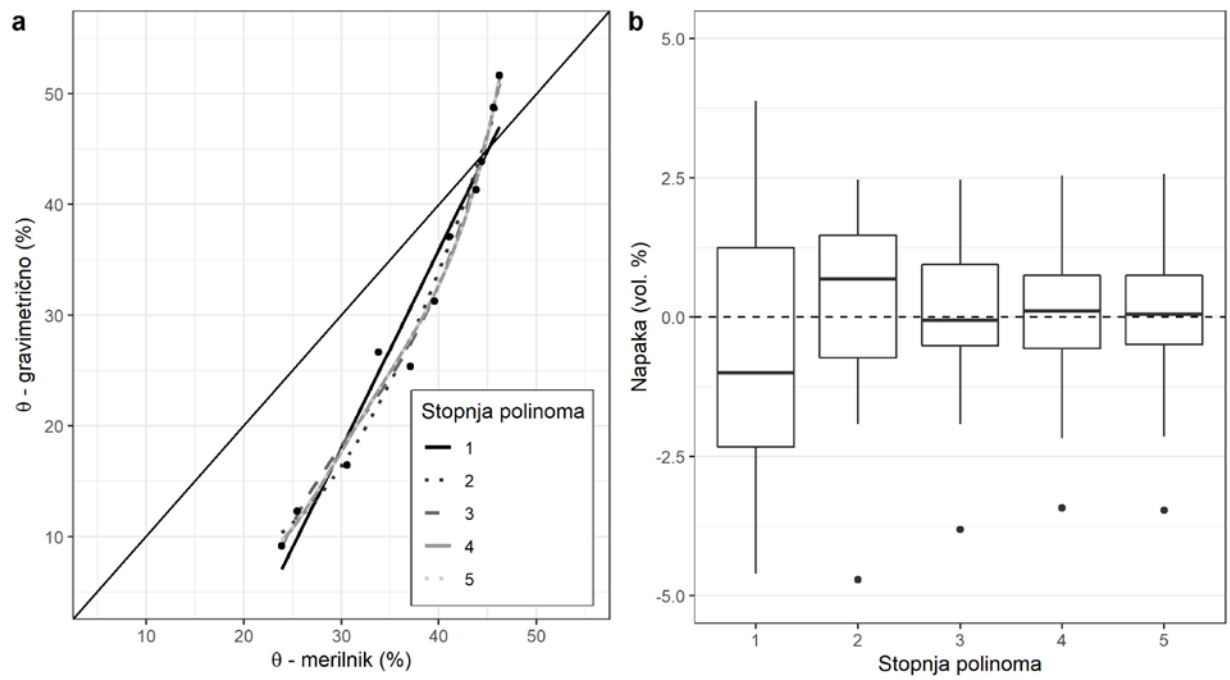
Tudi izbira stopnje polinoma pri določitvi talno specifične umeritvene funkcije je pomembna (Slika 3). V primeru merilnika MVZ 100 linearna funkcija v veliki večini primerov ni primerna, saj so napake v primerjavi




**Slika 1**: Različne umeritvene krivulje (levo) in okvirji z ročaji napak v vsebnosti vode v tleh (desno), za merilnik SM150T za dva izbrana primera tal iz projekta PRO-PRIDELAVA (PP1 in PP2)
**Figure 1:** Different calibration curves (left) and boxplot measurements of soil water content errors (right) for the SM150T sensor for two selected soil samples from the PRO-PRIDELAVA project (PP1 and PP2)




**Slika 2:** Primerjava gravimetrične in z merilnikom izmerjene volumske vsebnosti vode v tleh (a) in okvirji z ročaji napak v točkah (b), za merilnik MVZ 100 za štiri izbrane tipe tal iz projekta LIFE VivaCCAdapt (L1,L2,L3 in L4)
**Figure 2:** Comparison of gravimetric and volumetric soil water content measured with a sensor (a) and boxplot with error points (b), for the MVZ 100 sensor for four selected soil types from the LIFE VivaCCAdapt project (L1, L2, L3 and L4)




**Slika 3:** Primerjava različnih stopenj umeritvenega polinoma (a) in okvirji z ročaji napak v točkah (b), za merilnik MVZ 100 za izbrano lokacijo L2 iz projekta LIFE VivaCCAdapt
**Figure 3:** Comparison of different calibration polynomials (a) and boxplots with error points (b), for the MVZ 100 sensor for the selected site L2 from the project LIFE VivaCCAdapt

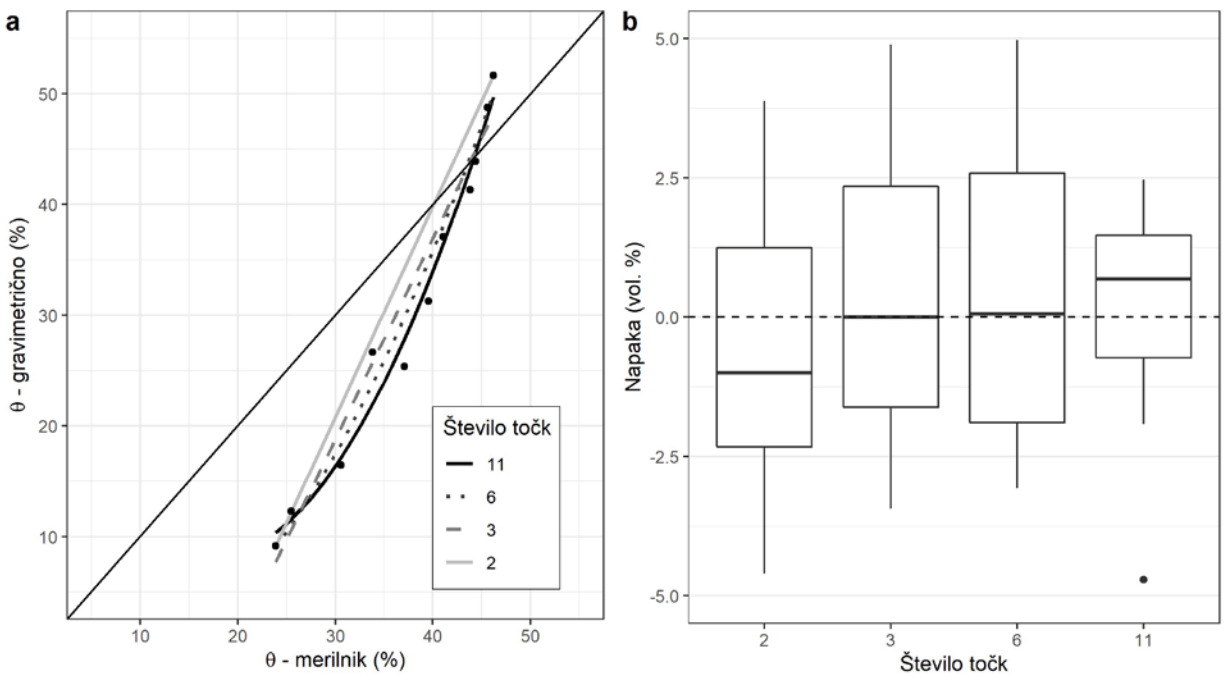
s polinomsko funkcijo višjega reda večje. Ugotavljamo tudi, da je napaka tudi pri izbiri optimalnega polinoma bistveno večja kot pri merilniku SM150T, in sicer znaša do 3 vol. %, t. j. toliko kot pri privzeti umeritvi merilnika SM150T.

Določitev umeritvene krivulje smo izvedli na različnem številu točk, in sicer za vse točke (11), vsako drugo točko (6 točk), prvo, srednjo in zadnjo (3 točke) ter prvo in zadnjo (2 točki). Primer različnih umeritvenih krivulj prikazujemo na Sliki 4. Ugotavljamo, da v redkih primerih zadoščajo 3 točke, v večini zadostuje 6, ponekod, kot v prikazanem primeru, pa potrebujemo za zanesljivo umeritev 11 točk.

Naše ugotovitve kažejo na to, da je pri umerjanju pomembno določiti dovoljšne število točk, vsaj 6 in pravilno izbrati stopnjo polinoma umeritvene funkcije. Uporaba dvotočkovne talno specifične umeritve merilnikov SM150T, v večini primerov zmanjša napako v primerjavi z napako, povzročeno z uporabo privzete umeritve, vendar pa je v določenih primerih, kot npr. PP2 (Slika 1), napaka primerljiva s privzeto umeritvijo oziroma celo večja, zato je potrebno uporabo dvotočkovne umeritve, predlagane s strani proizvajalca Delta-T, še podrobneje raziskati. Matula in sod. (2016) uporabe dvotočkovne talno specifične umeritve merilnikov ThetaProbe istega proizvajalca, ne priporočajo, saj v primerjavi s privzeto, ne doprinese bistveno k zmanjšanju napak. Natančnost meritev je še posebej pomembna v primeru deficitnega namakanja, kjer že napaka nekaj vol. % lahko pomeni veliko razliko v sušnem stresu za rastlino.

Merilniki vsebnosti vode v tleh omogočajo sprotno in neprekinjeno merjenje vode v tleh na različnih globinah z minimalnimi spremembami naravnih razmer v tleh (Paltineanu in sod., 1997). Na točnost delovanja vplivajo talne lastnosti ter delež kamenja in korenin v tleh, v katere so vgrajeni. Po navedbah Vaz in sod. (2013) imajo nekateri merilniki tovarniško določene umeritvene enačbe za mineralna ali organska, njihova natančnost pa se lahko poveča za 2-3 %, če se izvede talno specifična umeritev. Slednje smo z našimi analizami potrdili tudi sami.

V raziskavi, ki so jo izvedli Ferrarezi in sod. (2020), so se z uporabo tovarniške umeritvene funkcije najbolje odrezali merilniki, ki delujejo na podlagi merjenja odboja v časovnem prostoru (angl. Time Domain Reflectometry - TDR). Ravno nasprotno, v naši raziskavi se je slabše odrezal merilnik MVZ 100, proizvajalca Eltratec, ki prav tako deluje na podlagi TDR. Na drugi strani so, cenejša alternativa TDR merilnikom, kapacitivni merilniki, kot je preučevani SM150T, proizvajalca Delta-T Devices Ltd.. Po navedbah Matula in sod. (2016) in Singh in sod. (2018), so se kapacitivni merilniki izkazali za natančne




**Slika 4**: Primerjava umeritvenih krivulj za različno število točk (a) in okvirji z ročaji napak v točkah (b), za merilnik MVZ100 za izbrano lokacijo

**Figure 4:** Comparison of calibration curves for different number of points (a) and boxplot with error points (b), for the MVZ100 sensor for the selected site

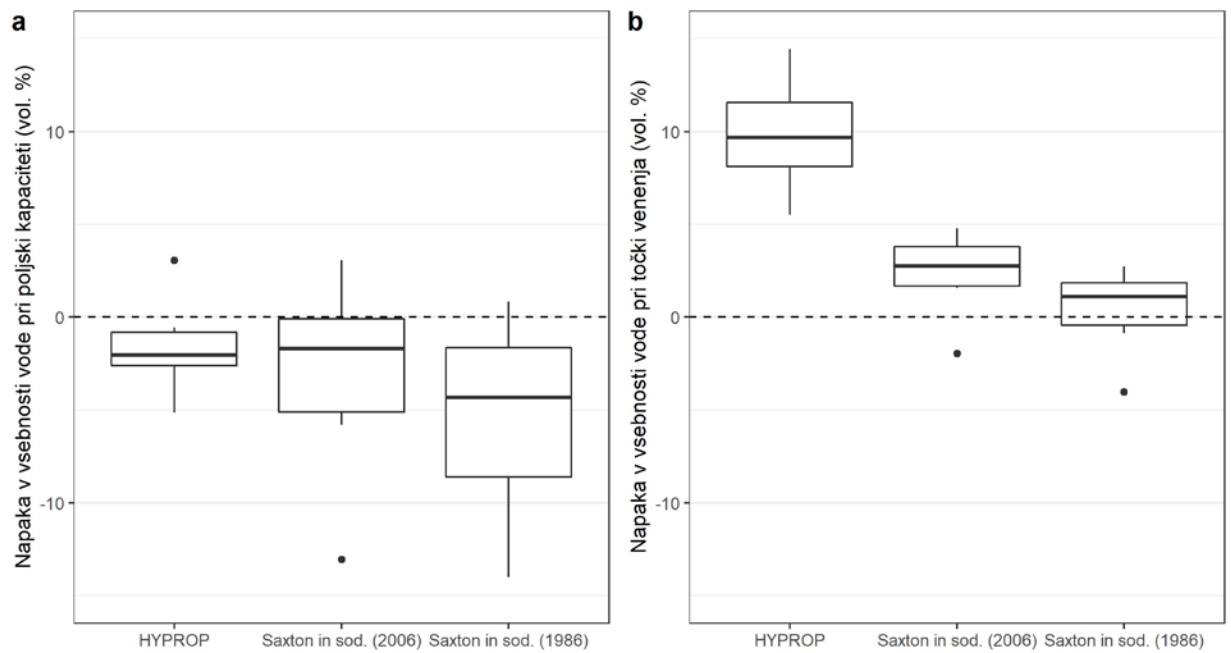
za učinkovito spremljanje vode v tleh v znanstvene ali agronomske namene, če je zagotovljena talno specifična umeritev. Slednje potrjujejo tudi naši rezultati, kjer dobimo z uporabo večtočkovne talno specifično funkcije natančnejšo umeritev merilnika SM150T. Več raziskav je pokazalo, da talno specifične umeritve kapacitivnih merilnikov izboljšajo natančnost meritev vsebnosti vode v tleh (Bircher in sod., 2016; Mittelbach in sod.., 2012; Vaz in sod., 2013).

## 3.2 VODOZADRŽEVALNE LASTNOSTI TAL

Vsebnost vode pri PK in TV smo določili na podlagi različnih metod in jih med seboj primerjali (Slika 5). Pri PK vse tri metode v večini primerov podcenjujejo referenčno vrednost. Najboljše rezultate dobimo z evaporacijsko metodo s sistemom HYPROP® in nadaljnjim modeliranjem s programskim orodjem HYPROP-FIT. Med metodama Saxton pa se bolje obnese novejša (Saxton in Rawls, 2006). Pri TV vse tri metode precenjujejo vsebnost vode v tleh, še posebej evaporacijska metoda izvedena s sistemom HYPROP®, kjer je mediana napake okoli 10 vol. %. Slab rezultat je do neke mere pričakovan, saj HYPROP meri le do-100 kPa, TV, določena pri 1500 kPa je bila modelirana z modelom van Genucthen. Pri tej metodi je ključna izvedba dodatnih meritev pri manjših vrednostih matričnega potenciala, v Richardovi tlačni posodi. Dobljene dodatne vrednosti nato vnesemo v HYPROP-FIT in model priredimo novemu naboru podatkov z večjim razponom. Nasprotno kot pri PK dá pri TV boljše rezultate starejša metoda Saxton (Saxton in sod., 1986).

Po podatkih Evett in sod. (2019) je določanje PK in TV z Richardovo tlačno posodo ter PK z sistemom Hyprop uveljavljena praksa, vendar tako določene vrednosti PK in TV morda niso neposredno uporabne za učinkovito upravljanje namakanja na podlagi podatkov o vsebnosti vode v tleh, izmerjenih na terenu. Te vrednosti so lahko uporabne za oceno hidravličnih lastnosti tal, za natančno določitev vodozadrževalnih lastnosti pa je potrebna kombinacija in uporaba več različnih metod, kar priporočamo tudi v naši raziskavi.

Spremljanje vsebnosti vode v tleh se v času vse večjega povpraševanja po hrani in vse intenzivnejših kmetijskih suš hitro razvija. Natančne meritve vsebnosti vode




**Slika 5**: Okvirji z ročaji za napako v vsebnosti vode pri poljski kapaciteti (a) in točki venenja (b), za različne metode izračuna, pri čemer smo pri poljski kapaciteti kot referenčna vrednost vzeli določeno na terenu po več močnih deževnih dogodkih s pomočjo talno specifično umerjenega merilnika SM150T, pri točki venenja pa v laboratoriju določeno z meritvami v Richardovi tlačni posodi

**Figure 5:** Boxplots for water content errors at field capacity (a) and wilting point (b), for different calculation methods. The field capacity value was determined in the field, after several heavy rains using a soil-specific calibrated SM150T sensor and the wilting point was determined in the laboratory using the pressure plate extractor

v tleh v realnem času omogočajo kmetom, agronomom in hidrologom boljšo obveščenost o količini vode v tleh. Za izboljšanje načrtovanja in upravljanja vodnih virov za kmetijstvo se lahko uporabljajo različne namakalne tehnologije. Ne glede na namakalni sistem je treba vedno natančno določiti količino uporabljene vode, da se zmanjša možnost izgub vode zaradi odtekanja in pronicanja ter poveča pridelek (Ferrarezi in sod., 2020). Če želimo doseči večji obseg uporabe različnih orodij za načrtovanje namakanja, je potreben razvoj novih in dostopnejših tehnologij. Med njimi se po navedbah González-Teruel in sod. (2019) že pojavlja razvoj in umeritev novih kapacitivnih nizkocenovnih merilnikov vsebnosti vode v tleh, ki omogočajo izbiro umeritvene funkcije glede na tip tal. Tudi Cvejić in sod. (2020) pišejo, da je posebno pozornost potrebno nameniti iskanju primernih, nizkocenovnih in nizkoenergijskih merilnikov vsebnosti vode v tleh z umeritvenimi funkcijami, ki pokrivajo širok razpon tipov tal.

Novi dosežki na področju tehnologij natančnega namakanja, obdelave podatkov in upravljanja namakalnih sistemov ponujajo možnosti za optimizacijo odločanja glede režima namakanja. Za zmanjšanje izgub vode čez talni profil je pomembno ne le pravilno določiti potrebe po vodi, temveč tudi prilagoditi pogostost namakanja talnim značilnostim in rasti korenin (Zinkernagel in sod., 2020). To lahko dosežemo z izbiro in uporabo ustreznih merilnikov vsebnosti vode v tleh. Njihov razvoj in dostopnost na trgu se iz leta v leto povečuje. Poleg ustreznih merilnikov, potrebujemo za učinkovito natančno namakanje tudi znanje o modelih za izračunavanje potreb po vodi, ki združujejo podatke iz različnih virov kontinuuma tla-rastline-atmosfera.

## 4 SKLEPI

Ob uporabi merilnikov, ki merijo dielektričnost tal, za meritve vsebnosti vode v tleh, priporočamo izvedbo večtočkovne talno-specifične umeritve, na vsaj 6 točkah. Za merilnike SM150T proizvajalca Delta-T Devices, se je uporaba dvotočkovne talno specifične umeritve, izkazala za ustrezno v določenih tipih tal, v nekaterih pa so napake podobnega velikostnega reda, kot ob uporabi privzete tovarniško določene umeritvene funkcije. Glede na to, da so pri tovarniški umeritvi napake v okviru navedenih napak iz priročnika ± 3 vol. % (Delta-T Devices, 2016), se lahko pri upravljanju namakanja, kjer se vsebnost vode giblje le okoli PK, zadovoljimo tudi samo s privzeto umeritvijo. Podobno velja za primere, kjer želimo spremljati le dinamiko vsebnosti vode v tleh in nas absolutne vrednosti vsebnosti vode v tleh ne zanimajo. Pri merilnikih MVZ 100 proizvajalca Eltratec je privzeta umeritvena funkcija zelo netočna. Merilniki skoraj na celotnem območju meritev močno precenjujejo dejansko vsebnost vode v tleh. Tudi po večtočkovni talno-specifični umeritvi je napaka enakega velikostnega razreda kot pri SM150T ob uporabi zgolj tovarniške umeritve.

Za namen upravljanja namakanja, priporočamo določitev PK na podlagi meritev z merilnikom vsebnosti vode v tleh na terenu po močnejšem deževnem dogodku. V kolikor dežja ne pričakujemo, oziroma je lokacija namakanja v rastlinjaku, mesto, kjer je v tla vstavljen merilnik, namočimo z večjo količino vode. Takšna določitev PK je še bolj smiselna, če ni bila opravljena talno specifična umeritev merilnika. Za TV priporočamo, da se določi z gravimetrično metodo z Richardovo tlačno posodo. Če je namakanje pogosto in ni deficitno ter se vsebnost vode giblje blizu PK, ali pa želimo spremljati gibanje vsebnosti vode v tleh in ne tudi absolutnih vrednosti, pa lahko uporabimo tudi modele, ki TV izračunajo na podlagi teksture tal. Kljub majhnemu vzorcu (6 lokacij), ki ga predstavljamo v prispevku, smo do podobnih ugotovitev prišli tudi na več kot 10 drugih analiziranih lokacijah.

Za natančno določitev in izbiro ustreznih merilnikov, bi bilo potrebno raziskavo razširiti z preučevanjem širšega nabora v merilnikov vsebnosti vode v tleh od več proizvajalcev. Pri izbiri preučevanih merilnikov bi bilo potrebno upoštevati želje in zahteve končnih uporabnikov, največkrat so to kmeti ali drugi raziskovalci, ki se vsak dan na terenu srečujejo z uporabo merilnikov ter težavami z njihovim delovanjem.

Samo z natančno določitvijo parametrov pomembnih za namakanje ter ustrezne izbire in uporabe namakalne opreme, lahko dosežemo želene rezultate, ki nam jih prinaša uporaba natančnega namakanja v kmetijski pridelavi. Najboljše namakalne tehnologije je treba združiti z novimi pristopi za načrtovanje namakanja in sistemi za podporo odločanju o namakanju, ki temeljijo na poznavanju značilnosti tal in potreb po vodi za posamezno namakalno kulturo.

## 5 ZAHVALA

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# Production and bromatological analysis of the oyster mushroom (*Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm.) grown with cocoa, banana, coconut and African palm husk substrates

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**Production and bromatological analysis of the oyster mushroom (*Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm.) grown with cocoa, banana, coconut and African palm husk substrates**

**Abstract:** Oyster mushroom (*Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm. (1871) is a rich food source. It is cultivated on compost and plant waste material. Choosing adequate substrate is essential for oyster production as the substrate can change oyster production in terms of mass and metabolite composition. The different medium substrates for oyster production including T1 (PDA, potato-dextrose-agar), T2 (CCA: PDA + Cocoa Shell), T3 (APR: PDA + African Palm Rachis), T4 (BP: PDA + Banana Peel), T5 (CCO: PDA + Coconut Peel) were used. Based on mycelial diameter, CCO treatment was the best treatment with growth measures of 66.83 mm at 168 hours. CCA treatment with 164.13 g $kg^{-1}$ yield had the highest production that was significantly different from other treatments. For APR treatment, trace production was observed. The bromatological analysis determined that the highest levels of crude total protein were obtained in CCO treatment (30.08 %) while CCA treatment exceeded significantly dry matter (94.05 %), ethereal extract (6.52 %), crude fiber (12.34 %), non-nitrogen matter (56.15 %) and titratable acidity (3.32 %). The substrates with more lignocellulosic compounds like banana and coconut residues are better for producing oyster with a higher percentage of total protein, while substrates that retain moisture like cocoa residues lead to an excellent production. It is recommended to keep fibrous residues moist constantly when they are used in oyster production because of their low absorbent capacity as they quickly lose moisture.

**Key words:** lignocellulosic compounds; PDA; protein; waste material; fruiting body

**Produktivnost in bromatološka analiza ostrigarja (*Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm.) rastočega na ostankih kakavovca, kokosove palme, bananovca in oljne palme**

**Izvleček:** Ostrigar (*Pleurotus ostreatus*) je bogat vir hranil. Goji se na kompostu in ostankih predelave različnih rastlin. Izbira primernega substrata je odločilna za njegovo produktivnost, ker ta vpliva na maso pridelka in njegovo sestavo. Za gojenje so bili izbrani različni substrati in sicer : T1 (PDA, krompirjev dekstrozni agar), T2 (CCA: PDA + ostanki kakavovca), T3 (APR: PDA + osrednja listna rebra oljne palme), T4 (BP: PDA + olupki banan), T5 (CCO: PDA + lupine kokosa). Na osnovi izmerjenega premera micelija je bilo obravnavanje CCO najboljše z izmerjeno hitrostjo rasti 66,83 mm v 168 urah. Obravnavanje CCA je imelo s 164,13 g $kg^{-1}$ največji pridelek, ki se je značilno razlikoval od drugih obravnavanj. Obravnavanje APR je dalo najslabši pridelek. Bromatološka anliza je pokazala največjo vsebnost celokupnih beljakovin pri obravnavanju CCO (30,08 %) med tem, ko je imelo obravnavanje CCA značilno večjo vsebnost suhe snovi (94,05 %), eternega izvlečka (6,52 %), vsebnosti netopnih vlaknin (12,34 %), vsebnost ne dušičnih spojin (56,15 %) in večjo titrabilno kislost (3,32 %). Gojišča z večjim deležem lignoceluloznih spojin kot so ostanki banan in kokosovega oreha so boljši za pridelavo ostrigarjev z večjim odstotkom beljakovin med tem, ko so substrati, ki obdržijo večjo vlažnost kot so ostanki kakavovca odlični za večji pridelek ostrigarja. Priporočamo, da se ostanki, ki vsebujejo vlaknine držijo pri gojenja ostrigarja stalno vlažni, ker zaradi njihove majhne absorpciske sporobnosti hitro izgubijo vodo.

**Ključne besede:** lignocelulozne spojine; PDA; beljakovine; odpadki, trosnjaki

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## 1 INTRODUCTION

In agriculture lots of wastes of plant origin are generated, while several have around 70 % cellulose and lignin. These agro-industrial wastes with a high lignocellulosic content are barely degraded but in the nature, there are a large number of microorganisms that use such compounds as a source of nutrition and some of such microorganisms are used as a food alternative in the world (Del Socorro Fernandez Uribe, 2014). In many cases plant residue wastes are burned or disposed in sanitary landfills where slow degradation biopolymers such as cellulose and lignin remain for years with almost no alterations.

The production of higher fungi, especially that of the oyster mushroom is a very attractive production alternative made from agro-industrial residues of high fiber content because of its unique ability to degrade lignocellulosic residues and its rich protein quality and quantity. Development of efficient technologies for the cultivation of this basidiomycete is increasingly required in order to apply modern methods toward a greater production (Pineda-Insuasti et al., 2013).

This fungus, in addition to presenting nutritional benefits, has a bioremediation capacity. It has a potent lignocellulolytic enzymes such as phenol oxidases (laccase) or heme peroxidases (lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase) as well as cellulose-hydrolysing enzymes, i.e. cellulases basically divided into endo-β-1,4-glucanase, exo-β-1,4-glucanase I and II, and β-glucosidase, all allowing it to detoxify, bioconvert, and bioremediate resistant pollutants (Adebayo & Martínez-Carrera, 2015). The ability of strains of *P. pulmonius* to biotransform herbicide molecules such as atrazine and insecticides like endosulfan has been reported demonstrating its importance in environment protection. Additionally, regarding to the medicinal beneficial effects, *P. ostreatus* presents anticancer activity, immunomodulatory, antiviral, antibiotic, anti-inflammatory attributes and decrease in cholesterol levels (Garzón Gómez & Cuervo Andrade, 2008)tallo de maíz, aserrín y sobras de café de consumo humano. Se evaluó el efecto de los cuatro sustratos de forma individual y en mezclas sobre la producción del hongo y en mezclas sobre la producción del hongo a través de indicadores como la eficiencia biológica, el rendimiento, el número de días en periodo de incubación, el número de días para la aparición de primordios, la frecuencia y el porcentaje de peso de cada cuerpo fructífero y la productividad. El rendimiento de los sustratos que tuvieron café tanto individualmente como en las mezclas varió entre los 265g a 409g y fueron significativamente más altos ($p < 0,05$.

There are many ways to cultivate this fungus species like hanging bags, wooden or stainless-steel slabs. As an advantage, there is a great diversity of organic materials that also can be used as a substrate of the fungus cultivation such as paper, coffee pulp, corn cob and husk, bean shell, leaf litter, grass, bagasse of sugar cane, cotton stalk, leaves and many others those are normally considered as plant wastes (Aguilar-Rivera & de Jesús-Merales, 2010; Rambey et al., 2019; Tesfay et al., 2019; Tsegaye & Tefera, 2017). Moreover, this production activity is 100 % natural and allows the use of by-products derived from the processes of transformation of agricultural products (Cruz et al., 2010).

In a complete production cycle of the fungus, the substrate is used as culture medium, i.e. the residue may be utilized after harvest as a food supplement for cattle, as *P. ostreatus* accelerates the degradation of lignin (Ardon et al., 1998). It also increases digestibility and provides mycelial protein or compost ability to convert it into organic fertilizer for incorporation into the productive cycle of agricultural crops (Del Socorro Fernandez Uribe, 2014).

In this article, a comparative study of production of the oyster mushroom (*P. ostreatus*) on different medium substrates including the residues of cocoa, banana, coconut and African palm rachis is presented. The findings suggest that these substrates, generally available in tropical regions, are efficient to be used in producing oyster mushroom in agroindustry and human consumption.

## 2 MATERIALS AND METHODS

### 2.1 LOCATION

The present research was carried out in the laboratory of Bromatology and Nutritional Metabolism ("RUMEN" standing for its abbreviation in Spanish) of the Experimental Campus "La María", belonging to the Universidad Técnica Estatal de Quevedo (UTEQ), located in the 7½ km of the road from Quevedo to El Empalme.

### 2.2 RESEARCH MATERIALS AND SUBSTRATES

This research work was divided into two phases. The first phase included the radial growth of oyster fungus inoculated in different culture media. The second phase was devoted to analyze the production and chemical composition of the mushrooms cultivated on agricultural waste (cocoa shell and coconut husk) and agro-industrial waste (African palm rachis and banana peel).

## 2.3 PREPARATION OF CULTURE MEDIA

Four culture media were obtained from cocoa shell, African palm rachis, banana peel and coconut peel. Hundred grams of each of the four materials were chopped, washed and placed in four aluminum containers separately, then 1 l of distilled water was added to each container. These containers were put on fire allowing water to boil for 30 minutes. The boiled content of each container as culture medium base was filtered with gauze and cotton to prevent the passage of any impurity. The filtered liquid was placed in the flasks containing 20 g of agar and 20 g of dextrose, and then these solutions of the different stubble were dissolved.

To prepare PDA (potato-dextrose-agar) medium, 200 g of peeled potato were sliced in squares and these pieces were boiled to obtain a solution, which was passed to a flask containing 20 g of agar and 20 g of dextrose. The four prepared solutions were subjected to boil for 30 minutes so that the agar and dextrose were diluted uniformly. Solutions were sterilized in autoclave at 121 °C and ~1 bar for 30 minutes. In total, five culture media were obtained: PDA (Potatoes dextrose agar), CCA (cocoa peel), APR (African palm rachis), BP (banana peel), CCO (coconut peel). In the biosafety cabinet 15 ml of each medium was deposited in the Petri dishes and allowed to solidify.

## 2.4 DETERMINATION OF RADIAL GROWTH CURVE

The PDA invaded by the mycelium of fungus was cut into pieces with 4 mm diameter, which were taken from the Petri dishes previously inoculated by fungus. They were used to obtain inoculums and planted in the center of a 90 mm Petri dish. The Petri dishes contained 15 ml of the culture medium and incubated at 28 °C. A calibrator that measures the diameter of fungi (mm) during its growth time was used to estimate radial growth speed. Measurements of the growth diameter of the fungal mycelium were made every 24-hour.

## 2.5 FUNGUS SEED FOR FERMENTATION IN SOLID MEDIUM

Wheat grains selected for preparing fungus spawn were washed and soaked for 24 hours in potable water, with the aim of reaching to between approximately 50 and 60 % moisture. After this time, they were washed with abundant water. The grains were allowed to drain until being already very dry. Four hundred g of each were weighed and put in the wide-mouth glass jars.

The jars were subjected to sterilization by autoclave at 121 °C and ~1 bar for 30 minutes. Once the bottles were cold, the PDA pieces with mycelium of approximately 3 x 3 cm were cut and 6 to 8 pieces were placed throughout the jar with as much coverage as possible. Mycelium-contained part of PDA pieces was placed in direct contact with the grains aseptically. The bottles were labeled with date, type of fungus, type of grain and were taken to the incubator at 28 °C for an approximate period of 3 weeks.

## 2.6 FERMENTATION ON SOLID MEDIUM

The cocoa, banana, coconut and African palm rachis peel were chopped to an approximate diameter of ± 2 cm to facilitate the invasion of the fungus in FMS (Fermentation in a solid medium). One kg of each substrate including cocoa peel, banana peel, and African palm rachis and coconut shell was washed three times to remove impurities. Substrate mass was recorded for the control. They were put on the canvas for heat treatment. Seventy litter (70 l) of drinking water and 1400 g of lime (2 % of the total water) were deposited in the tank, and the temperature was kept at 100 °C for 1 hour.

After this time, the substrate was allowed to drain and was expected to cool to approximately 25 °C. Then it was weighed and put in bags including 1 kg of media. It was inoculated with the grain spawn of oyster in 10 % (100 g) of the wet mass of substrate, covered by a black cover to have more darkness. All containers were taken to the incubation chamber provided with artificial light and irrigation system for 21-day incubation.

## 2.7 MUSHROOM PRODUCTION IN INCUBATION CHAMBER

The cultivated mushrooms on substrates were incubated for 21 days at 29 °C and relative humidity remained approximately 96 % . After the total colonization of residues developed, the plastic covers were removed and artificial light was supplied to induce mushroom fruiting in order to subsequently weigh production and perform physical and chemical analysis immediately.

## 2.8 EXPERIMENTAL DESIGN AND TREATMENTS

A completely randomized experimental design with

five treatments and six repetitions was used for the first phase of the investigation. In the second phase, a completely randomized experimental design was used with four treatments and six repetitions. For the first phase, five treatments consisting of PDA culture medium plus agricultural by-products were evaluated. Whilst, for the second phase the productive performance and nutritional content were determined, for which the same treatments were evaluated, except for T1 (PDA) (Table. 1).

## 2.9 VARIABLES UNDER STUDY

For the first phase radial growth was estimated. For the second phase, productive yield of mushrooms, moisture, dry matter, fat, fiber, pH, acidity, non-nitrogen elements and protein were measured.

### 2.9.1 Moisture content determination

The total moisture measurement was carried out on the fungal samples, the remaining content of this step was ground in a Thomas Willy mill adapted to a 2 mm sieve and were sterilized at a temperature of 135 °C for 2 hours, following weighed by an analytical balance to record their dry weight.

### 2.9.2 Hygroscopic moisture determination

One g of milled mushroom sample was deposited in a crucible and subjected to 65 °C for 48 hours, next weighed to obtain the percentage of hygroscopic moisture with the following formula (Equation 1) according to the provisions of Association of Official Analytical Chemists (AOAC) (AOAC, 2012).

$$M = \frac{M2-M1}{M} x\ 100 \qquad \text{Equation 1}$$

Where:
M = Moisture
$M_0$ = Sample Mass (g)
$M_1$ = Crucible mass plus sample after drying (g)
$M_2$ = Crucible mass plus sample before drying (g)

**Table 1**: Treatments evaluated in radial growth in the research phases. I and II demonstrate the first and the second phase, respectively

| Treatments | Description | Phase of the investigation |
|---|---|---|
| T1 | PDA | PDA-I |
| T2 | PDA + Cocoa shell (CCA) | CCA-I, CCA-II |
| T3 | PDA + African palm rachis (APR) | APR-I, APR-II |
| T4 | PDA + Banana peel (BP) | BP-I, BP-II |
| T5 | PDA + Coconut shell (CCO) | CCO-I, CCO-II |

### 2.9.3 Dry matter content determination

To calculate the dry matter content, the Equation 2 was used:

$$TDM = 100 - TM \qquad \text{Equation 2}$$

Where:
TM = Total Moisture
TDM = Total Dry Matter

### 2.9.4 Organic matter content determination

To carry out the analysis of organic matter content, with the same sample that remained from the hygroscopic moisture analysis, a muffle was placed at a temperature of 600 ºC for a period of 3 hours, after this time it was weighed in order to obtain the percentage of ash using the following formula (Equation 3):

$$C = 100 - (\frac{M_2-M_1}{M_0} x\ 100) \qquad \text{Equation 3}$$

Where:
C = organic matter content
$M_0$ = Dry sample mass (g)
$M_1$ = Mass of empty crucible (g)
$M_2$ = Crucible mass plus calcined sample (g)

### 2.9.5 Protein content analysis

In order to perform the analysis of protein content a modified Kjeldahl method was used (AOAC, 2012). For this analysis 300 mg of fungal samples were weighed in the dry state and deposited in the digester tubes and a copper catalyst tablet and 5 ml of sulfuric acid 98 % were added to each tube and then the tubes were placed in the programmed digester with the following times: 150 °C for 30 minutes, 280 °C for 30 minutes and 400 °C for 45 minutes, after this process the digested samples were

cooled for 45 minutes. In the distillation process, 10 ml of distilled water was added to each tube and the tubes were placed with the digested sample in the distiller that automatically injected into each tube 40 ml of boric acid solution (80 g of boric acid in 2000 ml of distilled water) and 40 ml of sodium hydroxide solution 6.25M (500 g of sodium hydroxide in 2000 ml of distilled water), where approximately 90 ml of distillate was deposited in a 300 ml flask and took 4 minutes. In the titration process, to the solution product of distillation process, 3 drops of indicator solution (100 ml of 98 % ethanol ,75 mg of bromocresol Green and 100 mg of red methyl) were added, and also a 0.1 N solution of sulfuric acid (2.77 ml of sulfuric acid in 1000 ml of distilled water) added to each tube until a red wine color was obtained.

### 2.10 DATA REGISTERING AND STATISTICAL ANALYSIS

The Excel program was used for the registration and ordering the data. For the statistical analysis, as well as for the comparison between treatments, ANOVA one way and the Tukey multiple range test ($p < 0.05$) were employed using R studio software version 4.0.

## 3 RESULTS AND DISCUSSION

### 3.1 RADIAL GROWTH OF *P. OSTREATUS* GROWN IN DIFFERENT CULTURE MEDIA

The radial growth of the oyster fungus (*P. ostreatus*) inoculated in different culture media is shown in Table 3, where the analysis of variance indicated that there were no significant differences between treatments ($p < 0.05$) at 24 and 48 hours of growth. At 72, 96, 120, 144, and 168 hours there were statistical differences and CCO treatment was the best treatment with 14.00, 24.83, 46.16, 60.16 and 66.83 mm of radial growth in time intervals, respectively. Oyster degraded the available substrates independently and after 72 hours it seems that it reached to a degradation point where substrates were decomposed. This is somehow in accordance with a previous study in which the author reported oyster mycelia grown on a mix of coconut and sawdust were thick, dense and comparatively compact compared to sawdust as control (Vetayasuporn, 2007) taking into account that the substrates were not identical in the compared studies APR treatment showed a difference at 72 and 96 hours with 14.00- and 23.00-mm growth in each interval time. The best radial growth response in CCO treatment was due to the fact that the substrate is rich in lignocellulosic compounds, which causes the fungus to have greater growth (Obodai et al., 2003). del Pilar Rios et al. 2010, in their research evaluated the productive parameters of *P. ostreatus* spawn propagated in different culture media, in which it has been mentioned that substrates with high cellulose and lignin content provide the necessary nutrients for growth of fungus decreasing incubation time (del Pilar Rios et al., 2010). This was also reported by other authors who indicated that rice straw is a good alternate substrate for growing oyster mushroom due to its high cellulose, fiber and lignin (Obodai et al., 2003). The authors used substrates based on cane bagasse and wheat bran extract, obtaining values that were confirmed by the other authors as well, who carried out the evaluation of the growth and production of biomass of three strains of the genus *Pleurotus* in a PDA medium prepared with different solutions of corn residue, achieving greater measures in the radial growth using stubble and corn husk (Rojas Ledesma & Quintana Zamora, 2015). Although degradation was done in different conditions in the above-mentioned studies, the main decomposed substrates were rich in cellulose, fiber and lignin and these coherent results suggest that degradation ability of oyster depends on the available metabolites of used substrate. In our study, this is confirmed by different results for *P. ostreatus* growth for varied substrates.

Rojas Ledesma and Quintana Zamora developed the study of radial growth and biomass production of the species *P. sapidus* (Schultzer) Kalchbrenner inoculated in various culture media using peanut shells (*Arachis hypogaea* L.) and *Cajanus cajan* (L.) Huth., obtaining inferior results, attributing their results due to the little or almost no contribution of linocellulite substances present in these wastes (Rojas Ledesma & Quintana Zamora, 2015).

### 3.2 PRODUCTION OF THE OYSTER MUSHROOM (*P. OSTREATUS*) GROWN ON SUBSTRATES OF COCOA PEEL, BANANA, AFRICAN PALM RACHIS AND COCONUT SHELL

The production of *P. ostreatus* mushrooms harvested in different agricultural by-products is shown in Table 4, where it can be seen that the crop residue on which the highest production was obtained was CCA treatment with 164.13 g ($p < 0.05$), while in APR treatment there was no mushroom growth because in this material there was an accelerated loss of moisture and high temperatures due to its non-absorbent fibrous characteristic, similar to those obtained in the CCO treatment that similarly achieved a lower production associated with this effect.

**Table 3**: Radial growth of *P. ostreatus* inoculated in different culture media

| Variables Hours | Growth diameter (mm) | | | | | p |
|---|---|---|---|---|---|---|
| | T1 PDA | T2 CCA | T3 APR | T4 BP | T5 CCO | |
| 24 | 4.00 ± 0.63 a | 3.66 ± 0.52 a | 3.83 ± 0.41 a | 3.88 ± 0.41 a | 3.66 ± 0.52 a | 0.7639 |
| 48 | 5.16 ± 0.75 a | 6.66 ± 3.14 a | 6.33 ± 2.87 a | 5.00 ± 0 a | 4.66 ± 0.52 a | 0.3268 |
| 72 | 10.66 ± 1.75 ab | 9.00 ± 0.89 ab | 14.00 ± 5.48 a | 7.83 ± 0.75 b | 14.00 ± 5.48 a | 0.0162 |
| 96 | 17.50 ± 2.88 ab | 17.16 ± 1.33 ab | 23.00 ± 10.62 a | 11.83 ± 0.75 b | 24.83 ± 8.93 a | 0.0129 |
| 120 | 30.50 ± 6.83 b | 26.83 ± 2.23 bc | 34.83 ± 5.85 ab | 15.66 ± 0.82 c | 46.16 ± 14.13 a | <.0001 |
| 144 | 50.66 ± 11.48 ab | 37.00 ± 3.1 bc | 49.50 ± 13.1 ab | 20.16 ± 0.98 c | 60.16 ± 14.69 a | <.0001 |
| 168 | 59.66 ± 9.39 ab | 52.33 ± 4.97 bc | 61.50 ± 9.57 ab | 41.33 ± 3.27 c | 66.83 ± 11.58 a | 0.0002 |

T1 PDA = Potato dextrose agar; T2 CCA = cocoa shell; T3 ARP = African palm rachis; T4 BP = banana peel; T5 CCO = coconut shell. Averages with equal letters do not differ statistically, according to Tukey ($p < 0.05$)

This is in accordance with Pineda et al.(2013) who indicated that after 30 °C there is a degradation of growth and therefore low production, demonstrating that *P. ostreatus* has a better growth at temperatures below 20 °C while at temperatures above 30 °C its growth stops or slows. In addition, banana have been used and suggested as a good substrate for oyster production as it has lignocellulosic compounds those accelerate oyster growth (Bonatti et al., 2004). This is confirmed by our findings as banana-based medium is the second one whose oyster production was higher.

These results were similar to those obtained by Quintana Zamora et al.(2018), who obtained 163.75, 132.75 and 114.75 g in production of oyster mushroom species (*P. ostreatus* and *P. sapidus*) in crop media with agricultural residues of soybeans, rice and corn kernels, respectively. However, other authors obtained a greater production of up to 761 g evaluating the growth and production of *P. ostreatus* on different agro-industrial residues using byproducts of cape gooseberry, pea shell and cob of corn (López-Rodríguez et al., 2008). Romero et al. 2010, evaluated the productive capacity of *P. ostreatus* using dehydrate banana leaf (*Musa paradisiaca*(Roatan) and achieved superior results for wheat straw substrate with more than 200 g $kg^{-1}$ compared to other substrates, such as wheat straw (*T. aestivum* L.), barley straw (*H. vulgare* L.), bean straw (*P. vulgaris* L.) and corn stubble (*Z. mays* L.) (Romero et al., 2010).

**Table 4**: Production of *P. ostreatus* mushroom harvested in different agricultural by-products

| Variable | T2 CCA | T3 APR | T4 BP | T5 CCO | p |
|---|---|---|---|---|---|
| Production in grams | 164.13 ± 46.43 a [1/] | ---------- | 142.03 ± 21.99 a | 45.03 ± 21.25 b | 0.0001 |

[1/] Averages with equal letters do not differ statistically, according to Tukey ($p < 0.05$)

**Table 5**: Chemical composition of *P. ostreatus* grown in different agricultural substrates

| Variable | T2 CCA | T3 APR | T4 BP | T5 CCO | p |
|---|---|---|---|---|---|
| DM | 5.94 ± 0.47 b[1/] | ---------- | 13.61 ± 1.49 a | 12.66 ± 0.26 a | <.0001 |
| Mo | 94.05 ± 0.47 a | ---------- | 86.38 ± 1.49 b | 87.34 ± 0.26 b | <.0001 |
| EE | 6.52 ± 0.18 a | ---------- | 6.31 ± 0.24 a | 5.30 ± 0.49 b | <.0001 |
| CP | 19.05 ± 0.68 c | ---------- | 21.23 ± 1.24 b | 30.08 ± 0.71 a | <.0001 |
| CF | 12.34 ± 0.32 a | ---------- | 10.08 ± 0.33 b | 7.86 ± 0.42 c | <.0001 |
| NNE | 56.15 ± 0.97 a | ---------- | 48.24 ± 2.35 b | 43.21 ± 1.06 c | <.0001 |
| pH | 6.65 ± 0.15 a | ---------- | 6.60 ± 0.06 a | 6.55 ± 0.08 a | 0.2426 |
| TA | 3.32 ± 0.12 a | ---------- | 2.78 ± 0.29 b | 2.91 ± 0.22 b | 0.0021 |

Mo = Moisture; DM = Dry matter; EE = Ethereal Extract; CP = Crude protein; CF = Crude fiber; NNE = Non-nitrogen elements; pH = Hydrogen potential; TA = Titratable acidity; P = Probability; [1/] Averages with equal letters do not differ statistically, according to Tukey ($p < 0.05$)

### 3.3 CHEMICAL COMPOSITION OF *P. OSTREATUS* RODUCED IN DIFFERENT AGRICULTURAL RESIDUES

Table 5 shows the results of the chemical composition of mushrooms grown in agricultural residues of coconut, banana and cocoa. There is a statistical difference between the treatments in the analyses of moisture, dry matter, ethereal extract, crude protein, and nitrogen-free elements, while there is no statistical difference between treatments for titratable acidity analysis. *Pleurotus* fungi are considered to have a high percentage of excellent protein value. The fungi cultivated in CCO residues obtained better percentage of protein of 30.08 %.

Paucara Fernández (2014) evaluated the production of the *P. ostreatus* fungus on different types of substrates (barley Tamo, Vicia Tamo, oatmeal straw and paramo straw) enriched by ground cob, barley bran and calcium carbonate. He obtained similar results in protein content as seen in treatments CCA and BP, but inferior to the result of treatment CCO that was the most superior with 30.08 %. On the other hand, Quinrana Zamora et al. 2018 carried out the production of oyster mushrooms (*P. ostreatus* and *P. sapidus*) in crop media with agricultural residues of soybeans, rice and corn kernels, achieving results similar to those obtained in the present study (Quintana Zamora et al., 2018). However, combination of different agricultural wastes and plant residues might be a better solution for oyster production while varied substrates provide different compounds for mushroom growth and yield (Tsegaye & Tefera, 2017).

## 4 CONCLUSIONS

Oyster is a rich protein source. It grows on natural media that are plant-based substrates. Different substrates result in varied oyster yield and metabolite composition. This study was done to find a good substrate as oyster growing medium from plant-waste compostable materials. A medium based on PDA and coconut shell was the best substrate with the most oyster radial growth and protein content. Oyster grown on cocoa husk produced more dry matter and crude fiber compared with the other studied substrates.

In the radial growth phase of *P. ostreatus* on the PDA medium combined with coconut shell solution, greater speed in its growth was seen and therefore the production of fungal biomass. The substrate presented a higher output was the cocoa-based, since this unlike the rest of the residues, conserves a higher moisture. Based on our results, it seems that a mixture of different substrates can be more efficient as oyster growth medium. However, it remains to further study to formulate the substrates to obtain acceptable media for oyster production.

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# Use of watermelon seed meal as a replacer of soybean meal in African catfish diets: effect on growth, body composition, haematology, and profit margin

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**Use of watermelon seed meal as a replacer of soybean meal in African catfish diets: effect on growth, body composition, haematology, and profit margin**

**Abstract:** The effects of replacing soybean meal with watermelon (*Citrullus lanatus*) seed meal (CLM) on growth, body composition, haematology and profit margin in catfish *(Clarias gariepinus)* breeding was evaluated. Juvenile catfish (n = 150) were acclimatised for a week, weighed and allotted into five dietary treatments; D1, D2, D3, D4 and D5 containing 0, 15, 30, 45 and 60 % replacement of soybean meal with watermelon seed meal, respectively. The diets were isonitrogenous and isolipidic. Each treatment was conducted in triplicate with ten fish per replicate. The results from the study indicate that there was no significant difference ($p > 0.05$) in growth, carcass composition, and nutrient utilization. However, a significant variation ($p < 0.05$) existed in the haematological parameters among the fish fed the different dietary treatments. The incidence of cost showed that the production of fish was cheaper when CLM was used as a replacement for soybean meal. The higher carcass yield and profit per kg of fish fed CLM justifies the use of CLM as a substitute for soybean meal in the diet of African catfish.

**Key words:** aquaculture; fish farming; fish; African catfish; *Clarias gariepinus*; animal nutrition; watermelon seed meal; growth; body composition; haematological parameters; economics; profit margin

**Uporaba moke iz lubeničnih semen kot nadomestka sojinih tropin v prehrani afriških somov: vpliv na rast, sestavo telesa, hematologijo in dobiček**

**Izvleček:** V raziskavi smo ocenili učinke zamenjave sojinih tropin z moko iz lubeničnih (*Citrullus lanatus*) semen (MLS) na rast, telesno sestavo, hematološke parametre in dobičkonosnost v prireji afriških somov (*Clarias gariepinus*). Mlade some (n = 150) smo po tednu dni aklimatizacije stehtali in razdelili v pet skupin, ki so prejemale različne krmne mešanice; v poskusnih skupinah smo 0 % (D1), 15 % (D2), 30 % (D3), 45 % (D4) in 60 % (D5) sojinih tropin nadomestili z MLS. Vse krmne mešanice so vsebovale enako količino beljakovin, pa tudi maščob. Vsak tretma smo izvedli v treh ponovitvah z desetimi ribami na ponovitev. Rezultati študije kažejo, da med skupinami ni bilo statistično značilnih razlik ($p > 0,05$) v rasti, sestavi trupa, izkoriščanju krme in hematoloških parametrih. Analiza stroškov je pokazala, da je bila prireja rib cenejša, če smo del sojinih tropin nadomestili z MLS. Boljša klavnost in večji dobiček na kilogram ribjega mesa ob uporabi ribje krme, kjer del sojinih tropin nadomestimo z MLS, upravičuje uporabo MLS kot nadomestka sojinih tropin v prehrani afriških somov.

**Ključne besede:** akvakultura; ribogojstvo; ribe; afriški som; *Clarias gariepinus*; prehrana živali; lubenična semena; moka iz lubeničnih semen; rast; telesna sestava; hematološki parametri; ekonomika; dobiček

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## 1 INTRODUCTION

Soybean meal (SBM) is known for its high protein content, high digestibility, and relatively well-balanced amino acid profile and is widely used as a feed ingredient for many aquaculture species (Storebakken et al., 2000). It is currently the most commonly used plant protein source in fish feed (El-Sayed, 1999; Fadel et al., 2017; Fagbenro et al., 2003; Jimoh et al., 2020a). Lim and Akiyama (1992) and Jimoh (2020) reported that soybean products have been used to replace a significant portion of fish meal in fish feed with nutritional, environmental, and economic benefits. However, the wider utilization and availability of this conventional source for fish feed is limited by the increasing demand for human consumption and by other animal feed industries (Jimoh et al., 2020b; Siddhuraju & Becker, 2001). The rapid expansion of fish culture in recent years requires the development and improvement of low-cost and nutritious fish feeds, mainly because increasing the feed cost may increase the cost of fish production by 50–80 % (Cavalheiro et al., 2007; Jimoh et al., 2019). Feed contributes between 60 and 70 % to the variable cost of fish production (Gabriel et al., 2007), making it one of the factors that determine the profitability of aquaculture production (Jimoh et al., 2019). Hence, the need to focus on using less expensive and readily available vegetable sources of protein to replace soybean meals without reducing the nutritional quality of the feed is imperative (Barros et al., 2002). In the past, research was mostly focused on the underutilised vegetable proteins in the fish diet among which were groundnut cake (Fasakin & Balogun, 1996), lima bean (Adeparusi & Ajayi, 2004), pigeon pea (Adeparasi, 1994), sunflower, sesame (Fagbenro et al., 2010a, 2010b, 2013), and jack bean (Fagbenro et al., 2007; Jimoh et al., 2010).

Watermelon (*Citrullus lanatus*) is a drought-tolerant crop that belongs to the family *Cucurbitaceae*. It is cultivated in a wide range of tropical, semi-tropical, and arid regions of the world (Razavi & Milani, 2006). The seeds of the watermelon have a nutritional quality comparable to that of oilseed proteins including soybean and other conventional legumes (Mustafa & Alamin, 2012). Wani et al. (2011) reported that watermelon seed meal contains an adequate amount of nutritional protein that could be used as an ingredient in feed products. More so, there is a paucity of information on the use of watermelon seeds as a dietary protein source in fish feed. Therefore, this work seeks to study the replacement of soybean meal with watermelon seed meal in the diet of African catfish (*Clarias gariepinus*).

## 2 MATERIALS AND METHODS

### 2.1 SOURCES AND PROCESSING OF INGREDIENTS

The dried watermelon seeds were obtained in Bodija market, Ibadan, Nigeria. The watermelon seeds were rinsed with water and boiled for 15 minutes, after which they were sundried for several days and ground in a hammer mill. The oil therein was removed using the pressure generated from a locally made screw press (cassava-presser type). The cakes and other feedstuffs obtained from commercial sources in Nigeria were separately milled and screened to fine particles size. Triplicate samples were analysed for their proximate composition (AOAC, 2010).

### 2.2 EXPERIMENTAL DIETS

Based on the nutrient composition of different sources of protein (Table 1), the experimental diets were formulated, containing cooked watermelon seed meal (CLM) substituting soybean meal at the shares of 0, 15, 30, 45, and 60 % instead of soybean meal (designated as D1, D2, D3, D4, and D5), respectively (Table 2). The diets were isolipidic and isonitrogenous, containing 40 % crude protein and 12 % crude lipid. The feedstuff was separately ground mixed with hot water, introduced into a Hobart-200T pelleting and mixing machine to obtain a homogenous mass, and then passed through a mincer to produce 2 mm size pellets, which were immediately sundried at 30–32 °C. After drying for three days, the diets were kept in a freezer (–4 °C). The diets were analysed for their proximate composition. It was observed that the value of crude fibre increased as the amount of CLM in the diet increased. However, there was no significant difference ($p > 0.05$) in the proximate composition parameters of the diets.

**Table 1:** Proximate composition of the different sources of protein in the experimental diets

| Parameter | Fish meal | Soybean Meal | CLM** |
|---|---|---|---|
| Moisture | 9.75 | 10.70 | 9.69 |
| Crude Protein | 72.4 | 45.74 | 27.55 |
| Crude Lipid | 10.45 | 9.68 | 11.35 |
| Crude Fibre | - | 5.10 | 4.97 |
| Ash | 8.32 | 4.48 | 5.39 |
| NFE* | - | 30.00 | 41.05 |

* Nitrogen Free Extract
** Watermelon (Citrullus lanatus) meal

**Table 2:** Gross (g/100g) and proximate composition (%) of experimental diets containing watermelon seed meal (CLM)

| Ingredients | D1 | D2 | D3 | D4 | D5 |
|---|---|---|---|---|---|
| Fish meal (72%) | 27.70 | 27.70 | 27.70 | 27.70 | 27.70 |
| SBM (45%) | 44.40 | 37.70 | 31.10 | 24.40 | 17.78 |
| CLM (27.55%) | - | 10.88 | 21.77 | 32.66 | 43.55 |
| Fish oil | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| ϕVitamin premix | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Starch | 17.90 | 13.72 | 9.43 | 5.24 | 0.97 |
| Proximate Analysis | | | | | |
| Moisture | 9.38 ± 0.08 | 9.87 ± 0.31 | 9.95 ± 0.11 | 9.56 ± 0.92 | 9.50 ± 0.72 |
| Crude Protein | 40.17 ± 0.08 | 40.19 ± 0.02 | 40.14 ± 0.01 | 40.19 ± 0.11 | 40.18 ± 0.06 |
| Crude Lipid | 12.00 ± 0.16 | 11.74 ± 0.83 | 12.29 ± 0.45 | 12.00 ± 0.04 | 12.16 ± 0.23 |
| Crude Fibre | 5.51 ± 0.26 | 5.53 ± 0.28 | 5.86 ± 0.59 | 6.20 ± 1.18 | 6.51 ± 0.37 |
| Ash | 6.20 ± 0.06 | 6.30 ± 0.04 | 6.00 ± 0.17 | 5.95 ± 0.33 | 5.44 ± 0.33 |
| *NFE | 26.76 ± 0.32 | 26.42 ± 1.40 | 25.76 ± 0.22 | 26.11 ± 0.44 | 26.19 ± 0.22 |

Means without superscript in the same row are not significantly different ($p > 0.05$) from each other
* Nitrogen free extract
ϕ Specification: each kg contains: Vitamin A = 4,000,000 IU; Vitamin B = 800,000 IU; Vitamin E = 16,000 mg, Vitamin $K_3$ = 800 mg; Vitamin $B_1$ = 600 mg; Vitamin $B_2$ = 2,000 mg; Vitamin $B_6$ = 1,600 mg, Vitamin $B_{12}$ = 8 mg; Niacin = 16,000 mg; Caplan = 4,000 mg; Folic Acid = 400 mg; Biotin = 40 mg; Antioxidant = 40,000 mg; Chlorine chloride = 120,000 mg; Manganese = 32,000 mg; Iron = 16,000 mg; Zinc = 24,000 mg; Copper = 32,000 mg; Iodine = 320 mg; Cobalt = 120 mg; Selenium = 800 mg, manufactured by DSM Nutritional products Europe Limited, Basle, Switzerland.

## 2.3 EXPERIMENTAL FISH AND THE AQUACULTURE SYSTEM

The experiment was conducted at the hatchery unit of the Federal College of Animal Health and Production Technology, Moor Plantation Ibadan, Nigeria. The fingerlings were obtained from a reputable hatchery, Ibadan, Oyo State, Nigeria, and transported to the experimental site inside an aerated bag. The initial average weight of the fish ranged from 10.80 to 10.97 g. A total of 150 fingerlings were acclimated to laboratory conditions for 14 days before the feeding trial while being fed on a commercial pelleted diet. Experimental diets were assigned randomly to the tanks with three replicates per dietary treatment. Each culture tank contained 10 fish that were fed 5 % body weight per day in two equal proportions between 9:00–10:00 a.m. and 5:00–6:00 p.m. for 56 days. Fish – from each tank were batch – weighed every other week and the amount of feed was adjusted accordingly. The mortality was monitored daily and recorded. The growth performance and feed utilization indices were estimated following the method explained in Jimoh and Aroyehun (2011). The water quality parameters were monitored and recorded throughout the experiment (oxygen 6.84 ± 0.55 mg/l, temperature 28.28 ± 0.29 °C and pH 6.88 ± 0.30) using a combined digital YSI dissolved oxygen meter (YSI Model 57, Yellow Spring Ohio) and the pH was monitored weekly using a pH meter (Mettler Toledo – 320, Jenway UK). Eight catfish per treatment were euthanized in clove oil (100 mg/l) at the beginning and end of the feeding trial and analysed for their carcass composition (AOAC, 2010).

## 2.4 BLOOD SAMPLING AND ASSESSMENT

The assessment of the haematological parameters was conducted following the methods explained in Jimoh et al. (2015a). Briefly, fish (n = 6) from each treatment were mildly euthanized with clove oil (100 mg/l) at the end of the feeding trial for blood sampling. The blood (1 ml) was obtained by caudal vein piercing using a 1ml disposable syringe and 25G EDTA treated needle and placed in EDTA treated test tubes for haematological examination. The primary haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb) were measured by the microhematocrit method and the cyanmethaemoglobin method (Coles, 1986; Schalm et al., 1975) and total blood cell counts such as red blood cell count (RBC) and white blood cell count (WBC) were determined by the use of hemocytometer, respectively. The secondary haematological parameters such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Coles, 1986; Schalm et al., 1975).

**Table 3:** Carcass composition of African catfish fed diets containing watermelon seed meal (CLM)

| | Initial | D1 | D2 | D3 | D4 | D5 |
|---|---|---|---|---|---|---|
| Moisture | 77.88 ± 0.18 | 75.57 ± 0.11 | 74.89 ± 0.46 | 74.16 ± 1.09 | 74.20 ± 2.20 | 74.58 ± 3.19 |
| Crude Protein | 15.11 ± 0.14[b] | 17.32 ± 0.16[a] | 17.45 ± 0.12[a] | 17.37 ± 0.12[a] | 17.10 ± 0.44[a] | 17.14 ± 1.21[a] |
| Crude Lipid | 3.13 ± 0.18 | 3.84 ± 0.25 | 3.41 ± 0.21 | 3.59 ± 0.23 | 3.56 ± 0.38 | 3.62 ± 0.33 |
| Ash | 3.89 ± 0.18 | 3.84 ± 0.25 | 4.26 ± 0.13 | 4.88 ± 0.74 | 5.14 ± 1.20 | 4.67 ± 1.66 |

Row means with the different superscripts are significantly different ($p < 0.05$) from each other

### 2.5 ECONOMIC ANALYSIS

The economic analysis of feeding watermelon seeds was assessed following the procedure explained in Jimoh et al. (2015b).

$$\text{Incidence of Cost} = \frac{\text{Cost of Feed}}{\text{Weight of Fish}}$$

$$\text{Profit Index} = \frac{\text{Value of Fish}}{\text{Cost of Feed}}$$

$$\text{Profit / kg} = \text{Value of 1 kg fish} - \text{Incidence of cost}$$

### 2.6 STATISTICAL ANALYSIS

Data obtained from the experiment were expressed in mean ± SD and subjected to one-way analysis of variance (ANOVA) using SPSS version 16.0. Duncan multiple range tests were used to compare differences among individual treatment means to reveal significant differences ($p < 0.05$).

### 2.7 ETHICAL STATEMENT

Standard regulations and guidelines of Federal College of Animal Health and Production Technology, PMB 5029, Ibadan, Nigeria on the care and use of laboratory animals were followed throughout the experiment.

## 3 RESULTS AND DISCUSSION

### 3.1 CARCASS COMPOSITION

Uys and Hecht (1985) reported that the best growth rate and feed conversion efficiency in juvenile and subadult African catfish (*Clarias gariepinus*) are achieved with diets containing 38–42 % crude protein and lipid content of 10–11 %. The carcass composition of African catfish-fed diets containing CLM is presented in Table 3. Significant differences ($p < 0.05$) were observed only in carcass protein content between the fish at the beginning and at the end of the experiment. However, no significant difference was recorded in the carcass protein content of fish fed different experimental diets. A similar observation was reported by Tiamiyu et al. (2015), using CLM as a replacer of soybean meal. The groups fed with the inclusion of CLM up to 30 % (D2 and D3) had higher

**Table 4:** Growth and nutrient utilization of African catfish fed diets containing watermelon seed meal

| | D1 | D2 | D3 | D4 | D5 |
|---|---|---|---|---|---|
| Initial weight (g) | 10.88 ± 0.02 | 10.97 ± 0.04 | 10.80 ± 0.03 | 10.87 ± 0.11 | 10.88 ± 0.03 |
| Final weight (g) | 24.31 ± 2.09 | 32.34 ± 7.81 | 30.95 ± 5.56 | 27.66 ± 6.42 | 21.37 ± 0.45 |
| [1]Weight gain (g) | 13.43 ± 2.11 | 21.37 ± 7.83 | 21.65 ± 7.71 | 16.79 ± 6.53 | 10.49 ± 0.47 |
| [2]% weight gain | 124.50 ± 21.92 | 190.93 ± 76.27 | 200.56 ± 71.89 | 154.78 ± 61.72 | 96.37 ± 4.61 |
| [3]SGR | 1.43 ± 0.16 | 1.90 ± 0.44 | 1.94 ± 0.43 | 1.64 ± 0.44 | 1.20 ± 0.04 |
| [4]FCR | 1.47 ± 0.45 | 1.16 ± 0.08 | 1.30 ± 0.03 | 1.33 ± 0.06 | 1.36 ± 0.04 |
| [5]PER | 1.64 ± 0.76 | 2.16 ± 0.16 | 1.92 ± 0.04 | 1.88 ± 0.08 | 1.84 ± 0.05 |
| [6]% Survival | 77.77 ± 15.71 | 88.89 ± 15.72 | 88.89 ± 15.72 | 88.89 ± 15.72 | 88.88 ± 15 |

Row means without superscript are not significantly different ($p > 0.05$) from each other.
[1] Mean weight gain = final mean weight − initial mean weight; [2] Percentage weight gain = (final weight − initial weight / initial weight) × 100;
[3] Specific growth rate = (In final weight − In initial weight) × 100; [4] Feed conversion ratio = dry weight of feed fed / Weight gain (g);
[5] Protein efficiency ratio = fish body weight (g) / Protein fed; [6] Percentage survival = ((total number of fish − mortality) / total number of fish) × 100

**Table 5:** Haematological profile of African catfish fed the experimental diets

| | D1 | D2 | D3 | D4 | D5 |
|---|---|---|---|---|---|
| PCV (%) | $16 \pm 1.41^{c}$ | $17 \pm 1.41^{bc}$ | $19 \pm 1.41^{ab}$ | $20 \pm 1.41^{a}$ | $20 \pm 1.41^{a}$ |
| Hb (g/dL) | $5.0 \pm 1.41$ | $5.0 \pm 1.41$ | $6.4 \pm 0.57$ | $6.8 \pm 1.41$ | $6.9 \pm 0.14$ |
| RBC ($\times 10^{12}$/L) | $1.42 \pm 0.03^{d}$ | $1.62 \pm 0.03^{b}$ | $1.73 \pm 0.00^{a}$ | $1.50 \pm 0.00^{c}$ | $1.60 \pm 0.00^{b}$ |
| WBC ($\times 10^{7}$/L) | $158.8 \pm 0.28^{d}$ | $205.2 \pm 1.41^{c}$ | $342.6 \pm 3.39^{b}$ | $340.2 \pm 0.28^{b}$ | $400.2 \pm 0.28^{a}$ |
| MCV (fL) | $112.7 \pm 1.41^{c}$ | $123.0 \pm 1.41^{b}$ | $109.8 \pm 2.83^{c}$ | $133.0 \pm 0.00^{a}$ | $125.0 \pm 1.41^{b}$ |
| MCH (ρg) | $35.2 \pm 0.83^{c}$ | $40.4 \pm 0.34^{b}$ | $37.0 \pm 1.41^{c}$ | $40.0 \pm 0.00^{b}$ | $43.1 \pm 1.41^{a}$ |
| MCHC (g/dL) | $31.3 \pm 1.41^{b}$ | $33.3 \pm 0.42^{a}$ | $34.0 \pm 1.41^{a}$ | $34.0 \pm 0.00^{a}$ | $35.0 \pm 1.41^{a}$ |
| WBC Differential | | | | | |
| Lymphocytes (%) | $69.0 \pm 1.41^{c}$ | $78.0 \pm 2.83^{b}$ | $78.0 \pm 2.83^{b}$ | $81.0 \pm 1.41^{b}$ | $88.0 \pm 1.41^{a}$ |
| Neutrophil (%) | $30.0 \pm 2.83^{a}$ | $22.0 \pm 2.83^{b}$ | $21.0 \pm 1.41^{c}$ | $19.0 \pm 0.00^{c}$ | $11.0 \pm 0.71^{d}$ |

Row means with different superscripts are significantly different ($p < 0.05$) from each other.
Hb = Haemoglobin content; PCV = Packed Cell Volume; WBC = White Blood Cell Count; RBC = Red Blood Cell Count; MCHC = Mean Corpuscular Haemoglobin Concentration; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin

carcass crude protein levels than the controls (D1), but the difference was not statistically significant ($p > 0.05$).

## 3.2 GROWTH AND NUTRIENT UTILIZATION

The growth and nutrient utilization of African catfish fed different diets are shown in Table 4. The results of this experiment indicated that the growth and nutrient utilization of *C. gariepinus* were not significantly affected ($p > 0.05$) by up to 60 % replacement level of soybean meal with CLM in the diet. This result agrees with the studies conducted by Davies et al. (2000) using sesame and other oil seeds residue as fish meal replacer in diets fed to Nile tilapia (*Oreochromis niloticus*), Olvera-Novoa et al. (2002), and Sahar et al. (2003) using sunflower seed meal as a protein source in diets fed to red beast tilapia (*Tilapia rendalli*) and common carp (*Cyprinus carpio*), respectively. The D5-fed group had a lower weight gain compared to the control group (D1) but the difference was not significant ($p > 0.05$). Lower growth at higher inclusion is customary of alternative vegetable protein sources used in fish feed, as they may be deficient in some essential amino acids and may possess antimetabolites which may reduce the growth performance of fish (Jobling, 2012). Antimetabolites at higher inclusion can reduce palatability and bioavailability of nutrients in the feed (Jimoh et al., 2014).

## 3.3 HAEMATOLOGICAL PROFILE

Table 5 shows the haematological profile of African catfish fed the experimental diets. Fish fed diets containing CLM had significantly higher ($p < 0.05$) values of PCV, RBC, WBC, and lymphocytes than animals from the control group. The difference in haemoglobin content was not significant ($p > 0.05$) between the groups. The values recorded for haemoglobin contents, PCV, RBC of the fish were all within the range of normal

**Table 6:** Cost of producing 1 kg feed containing watermelon seed meal

| | Price (₦)/kg* | D1 | D2 | D3 | D4 | D5 |
|---|---|---|---|---|---|---|
| Fish meal | 480 | 132.96 | 132.96 | 132.96 | 132.96 | 132.96 |
| SBM | 136 | 60.43 | 51.36 | 42.29 | 33.18 | 24.18 |
| C.L.M | 48 | - | 5.22 | 10.44 | 15.67 | 20.90 |
| Fish oil | 500 | 100 | 100 | 100 | 100 | 100 |
| Vit. Premix | 262 | 52.40 | 52.40 | 52.40 | 52.40 | 52.40 |
| Starch | 200 | 35.80 | 27.44 | 18.86 | 10.48 | 1.94 |
| Cost (₦/kg) | | 381.59 | 369.38 | 356.95 | 344.69 | 332.38 |

* 1 Euro = ₦194.85

healthy fish (Clark et al., 1979; Erondu et al., 1993; Fagbenro et al., 1993; Khan & Abidi, 2010, 2011; Omitoyin, 2006; Rastogi, 2007). According to Lenfant and Johansen (1972), an erythrocyte count greater than $1 \times 10^6/mm^3$ is considered high and is indicative of the high oxygen-carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and with high activity. Watermelon seeds and flour are known to contain bioactive compounds such as tannins, stachyose, phytic acids, raffinose, and verbascose that could have immunomodulatory and immunostimulatory properties thus could enhance the innate defence mechanism of fish (El-Adawy & Taha, 2001; Erhirhie & Ekene, 2014; Tarazona-Díaz et al., 2011). We observed a significant rise ($p < 0.05$) of WBC in fish fed CLM-enriched diets, demonstrating possible ability to boost innate immunity when compared to the control-fed group (Hoseinifar et al., 2020). Fish fed diets D2, D3, D4, and D5 had decreased neutrophil counts compared to the fish fed the control diet. Similar was observed in in African catfish fed dietary combinations of onion-pawpaw where Fawole et al. (2020b) discovered an inverse relationship between lymphocyte and neutrophil counts, while Tiamiyu et al. (2019) discovered the same in African catfish fed *Talinum triangulare*.

### 3.4 COST OF PRODUCING 1 KG OF DIET

Table 6 shows the cost of producing 1 kg of feed containing CLM. There was a reduction in the cost of producing 1 kg of diets with an increasing replacement level of soybean meal by CLM.

### 3.5 ECONOMIC ANALYSIS

Table 7 reveals the incidence of a cost analysis of producing 1 kg of African catfish with diets containing CLM. The cost analysis of producing 1kg of fish, showed that it was cheaper to produce 1 kg of fish with diets containing CLM than with diets containing only soybean meal. A significant difference ($p < 0.05$) was shown in the incidence of cost and profit (₦/kg) of fish fed the different dietary treatments. The cost of the feed D5 was significantly the lowest, while D1 was significantly the highest. A reverse trend was noted for Profit (₦/kg) of fish fed the different dietary treatments. However, there was no significant difference ($p > 0.05$) in the value of fish produced and their profit margin among the fish fed various dietary treatments. The profit index reveals a trend of increasing profitability when feeding CLM based diets to African catfish ($p > 0.05$). A profit index above one (Table 7) shows that it is profitable to feed the fish with the diet. There was a general increase in the profit index observed with an increased dietary level of inclusion of CLM. This agrees with our earlier studies (Jimoh et al., 2012; Jimoh, 2004; Jimoh et al., 2019) that reported a general increase in the profit index with an increase in the replacement level of lesser-known vegetable protein

Gross margin was reported to be a good measure of profitability (Olagunju et al., 2007). The experiment showed that it is profitable to replace soybean meal with watermelon seed meal. This result agrees with the findings of Fagbenro et al. (2001) and Abu et al. (2010), who reported that feeding fish with cheaper and lesser-known feed ingredients left some profit margin. Although the economic implication of using the different dietary treatments might not be well appreciated since the margin is small, it will be much clearer when the magnitude of total cost and expected revenue of its large scale operation is critically and objectively considered (Faturoti, 1989; Jimoh et al., 2019). Adeparusi and Balogun (1999) reported profit margin increasing when the fish meal was replaced by roasted pigeon peal meal in a diet fed to African catfish. Jimoh (2004) also reported an increase in the profit margin in the production of tilapia by replacing up to 30 % of soybean meal with jack bean meal. Jimoh et al. (2019) and Jimoh et al. (2020b) observed similar trends when *Jatropha curcas* was fed African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*), respectively.

**Table 7:** Cost analysis of producing 1kg of African catfish fed diets containing watermelon seed meal

| | D1 | D2 | D3 | D4 | D5 |
|---|---|---|---|---|---|
| Cost of feed fed | 3.55 ± 1.01 | 3.73 ± 3.61 | 3.91 ± 3.44 | 3.51 ± 2.17 | 3.65 ± 0.67 |
| Weight gain of fish | 0.013 ± 0.02 | 0.014 ± 0.02 | 0.015 ± 0.01 | 0.017 ± 0.01 | 0.019 ± 0.01 |
| Value of fish | 5.20 ± 1.05 | 5.60 ± 3.92 | 6.0 ± 3.85 | 6.80 ± 3.27 | 7.60 ± 0.23 |
| Profit index | 1.46 ± 0.01 | 1.50 ± 0.01 | 1.54 ± 0.01 | 1.93 ± 0.57 | 2.08 ± 0.33 |
| Incidence of cost | $273.08 \pm 1.25^{a}$ | $266.42 \pm 2.40^{ab}$ | $260.67 \pm 4.07^{b}$ | $206.47 \pm 8.31^{c}$ | $192.10 \pm 9.86^{d}$ |
| Profit (₦)/kg of fish | $126.92 \pm 1.05^{e}$ | $133.58 \pm 2.43^{d}$ | $139.33 \pm 2.19^{c}$ | $193.53 \pm 4.24^{b}$ | $207.90 \pm 1.21^{a}$ |

Row means without superscript are not significantly different ($p > 0,05$) from each other.
Price/kg of African catfish Clarias gariepinus = ₦400

## 4 CONCLUSIONS

Conclusively, the result of this study indicated that CLM can replace soybean meal up to 60 % without affecting health, and at least up to 45 % without affecting the growth of African catfish. Higher carcass yield and profit per kg of fish fed diets containing watermelon seed meal compared to controls justifies the use of CLM as soybean meal replacer in the diets of African catfish.

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# Pahljačniki (Coleoptera: Scarabaeidae) kot gospodarsko pomembni škodljivci in možnosti njihovega zatiranja z entomopatogenimi glivami

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**Scarab beetles (Coleoptera: Scarabaeidae) as economically important pests and the possibility of using entomopathogenic fungi for their control**

**Abstract:** Scarab beetles (Coleoptera: Scarabaeidae) are a cosmopolitan group of beetles found on all continents except Antarctica. Because of their size, vibrant colors, and above all their role in the ecosystem, they are one of the most recognizable and studied taxons of beetles. Most larvae and adult beetles of species belonging to subfamilies Melolonthinae, Rutelinae, Dynastinae and Cetoniinae feed on plant organs such as roots, leaves, flowers and young fruits and are thus considered to be species of economic importance. In this article we describe some of the most economically important species of scarabs, including their most common host plants. Because the use of chemical insecticides to control scarabs is often limited, the implementation of entomopathogenic fungi as biological control agents is an appropriate alternative based on the rational use of microorganisms to maintain an environmentally balanced level of the pest population. Representatives of the genera *Beauveria* and *Metarhizium* are the most commonly used entomopathogenic fungi to control larvae (white grubs) of scarab beetles. Biological control by entomopathogenic fungi has shown to be effective in some cases, however host range is often species-specific. Therefore, in order to effectively use the entomopathogens against scarab beetles, one needs to identify target species in grub-infested area and consequently select strains that are capable of overcoming the host's defences.

**Key words:** Scarabaeidae; scarab beetles; white grubs; entomopathogenic fungi; biological control

**Pahljačniki (Coleoptera: Scarabaeidae) kot gospodarsko pomembni škodljivci in možnosti njihovega zatiranja z entomopatogenimi glivami**

**Izvleček:** Pahljačniki (Coleoptera: Scarabaeidae) so kozmopolitska družina hroščev. Najdemo jih na vseh celinah, z izjemo Antarktike. Zaradi njihove velikosti in živahnih barv, predvsem pa njihove vloge v ekosistemih, so eden izmed najbolj preučevanih in prepoznavnih taksonov hroščev. Večina ličink in odraslih hroščev iz poddružin Melolonthinae, Rutelinae, Dynastinae in Cetoniinae se prehranjuje z rastlinskimi organi, kot so korenine, listi, cvetovi in mladi plodovi, zato jih uvrščamo med gospodarsko pomembne vrste rastlinskih škodljivcev. V prispevku je opisanih nekaj gospodarsko najpomembnejših vrst pahljačnikov, vključno z njihovimi najpogostejšimi gostiteljskimi rastlinami. Ker je uporaba sintetičnih insekticidov za zatiranje pahljačnikov velikokrat omejena, je uvedba entomopatogenih gliv kot biotičnih agensov ustrezna alternativa, saj temelji na racionalni uporabi mikroorganizmov za ohranjanje populacije škodljivca pod pragom gospodarske škode. Za zatiranje ličink pahljačnikov (ogrcev) so najpogosteje uporabljene glive iz rodov *Beauveria* in *Metarhizium*. Biotično zatiranje ogrcev z entomopatogenimi glivami se je v nekaterih zgledih izkazalo za učinkovito, vendar so sredstva za njihovo zatiranje pogosto vrstno specifična. To pomeni, da moramo za učinkovito uporabo entomopatogenih gliv proti pahljačnikom prepoznati tarčne vrste in posledično izbrati seve, ki so sposobni premagati obrambo gostitelja.

**Ključne besede:** Scarabaeidae; pahljačniki; ogrci; entomopatogene glive; biotično zatiranje

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## 1 UVOD

V družino pahljačnikov (Coleoptera: Scarabaeidae) uvrščamo več kot 31.000 vrst, kar vključuje približno 91 % predstavnikov naddružine Scarabaeoidea (Jameson in Ratcliffe, 2002; Ratcliffe, 2002). V sistematiki je bila pogosta delitev naddružine v tri družine, in sicer Passalidae, Lucanidae in Scarabaeidae, vendar se je zaradi polifiletske narave družine Scarabaeidae vzpostavila trenutna delitev na 14 družin. Družino pahljačnikov sestavlja 19 poddružin in veljajo za raznoliko, kozmopolitsko skupino, katere hrošči so prilagojeni na širok spekter habitatov (Bouchard in sod., 2011).

Med vrstami pahljačnikov obstajajo pomembne ekološke razlike, saj se pri nekaterih vrstah izmenja več rodov na leto, pri nekaterih le en rod (Wagenhoff in sod., 2014; Gyawaly in sod., 2016), nekatere vrste so aktivne ponoči, druge podnevi (Hanski in Cambefort, 1991), način prehranjevanja pa predstavlja glavni ekološki dejavnik, ki vpliva na vedenje pahljačnikov in je hkrati eden najpomembnejših dejavnikov pri razširjanju odraslih hroščev in ličink. Hrošči poddružin Aegialiinae, Aphodiinae in Scarabaeinae se prehranjujejo z mrhovino, iztrebki, glivami in odmrlim rastlinskim materialom (Bai in sod., 2015), medtem ko so hrošči poddružin Melolonthinae, Rutelinae, Dynastinae in Cetoniinae pretežno fitofagni (Eberle in sod., 2014). Hrošči poddružin Melolonthinae in Rutelinae se prehranjujejo predvsem z listi, cvetovi in mladimi plodovi, hrošči iz poddružine Dynastinae s stebli in koreninami rastlin, hrošči poddružine Cetoniinae pa predvsem z nektarjem ter sokom rastlin in sadežev (Ritcher, 1966). Prehranjevanje ličink med posameznimi poddružinami pa ni tako izrazito razdeljeno, saj se mnoge ličinke saprofagnih in koprofagnih hroščev prehranjujejo z rastlinskimi organi in obratno. Tako se večina ličink poddružine Aphodiinae, podobno kot odrasli, prehranjuje z iztrebki, obenem pa so ličinke rodov *Aphodius* in *Ataenius* znotraj te poddružine pomembni škodljivci na naravnem travinju in tratah (Williamson in sod., 2004). Prav tako so ličinke poddružine Cetoniinae, za razliko od odraslih hroščev, detritivori in se prehranjujejo z organsko snovjo v tleh (Ciss in sod., 2019). Gospodarsko najbolj pomembne vrste pripadajo predvsem poddružini Melolonthinae, saj ličinke le-teh v mnogih delih sveta povzročajo poškodbe na koreninah trav, žit, stročnic, različnih sadnih vrst in drugih lesnatih rastlin (Ritcher, 1966).

## 2 PAHLJAČNIKI KOT ŠKODLJIVCI NA OBDELOVALNIH ZEMLJIŠČIH, TRAVINJU IN V GOZDOVIH

Ličinke hroščev pahljačnikov se imenujejo ogrci in se prehranjujejo s koreninami rastlin ali odmrlo organsko snovjo, kar predstavlja hrano z majhno hranilno vrednostjo. Učinkovit prevzem energije jim omogoča modificirano zadnje črevo, ki v anaerobnih razmerah hrani večino črevesne mikrobiote in je glavno območje za prebavo (hemi)celuloze (Huang in sod., 2010). To je ličinkam pahljačnikov omogočilo izkoriščanje različnih ekoloških niš, zaradi česar so postali zelo uspešni v številnih habitatih. V primeru, da niša sovpada s človeškimi dejavnostmi, lahko postanejo pomembni škodljivci tudi v njihovem naravnem okolju. Na kmetijskih zemljiščih, posebno na monokulturah, so posledice množičnih izbruhov toliko večje (Jackson in Klein, 2006).

V centralni Braziliji sta vrsti *Phyllophaga capillata* Blanchard, 1851 in *Aegopsis bolboceridus* (Thomson, 1860) (Coleoptera: Melolonthidae) najpomembnejša škodljivca soje in koruze. Ogrci se pojavijo v začetku pomladi, v času setve soje in koruze, z njihovimi koreninami pa se prehranjujejo do konca poletja. Poškodbe korenin se na rastlinah kažejo v zapozneli rasti, porumenelosti in venenju, lahko pa pride tudi do propada rastlin (Oliveira in Frizzas, 2013). Poškodbe korenin, ki jih povzročijo ogrci prve in druge larvalne stopnje, so sorazmerno majhne. Največjo škodo povzročijo ogrci tretje larvalne stopnje, saj lahko zmanjšajo koreninski sistem pri soji za približno 25 % in 64 % pri koruzi (Oliveira in Frizzas, 2021). Pomemben škodljivec soje in koruze je tudi japonski hrošč (*Popillia japonica* Newman, 1841). Japonski hrošč izvira iz Japonske, kjer pa ne doseže takšne številčnosti in posledično tudi ne povzroča takšne škode kot v državah, kjer ni avtohton. Japonski hrošč je posebno škodljiv zaradi njegovega generalističnega načina prehranjevanja z listi, cvetovi in plodovi več kot 300 različnih rastlinskih vrst (Fleming, 1972). Hrošči se hranijo podnevi, domnevno zaradi večje koncentracije sladkorjev v rastlinah, ki so bolj izpostavljene svetlobi (Bernays in Chapman, 1994). Prehranjujejo se na zgornji strani listov, na katerih žvečijo tkivo med žilami. Vendar ima pri koruzi večji gospodarski pomen obžiranje koruzne svile, kar preprečuje opraševanje in ima za posledico nepravilno oblikovana zrna in zmanjšan pridelek (Shanovich in sod., 2019). Ostale pomembne gostiteljske rastline japonskega hrošča so med drugimi tudi koščičarji iz rodu *Prunus*, beluši, jablana, robida, vinska trta, jagode, vrtnice in

druge okrasne rastline (Bragard in sod., 2018). Ličinke japonskega hrošča se primarno hranijo s koreninami trav, s čimer povzročajo znatno škodo na pašnikih, travnikih in igriščih za golf. Poškodbe korenin zmanjšujejo sposobnost trave, da prevzame zadostno količino vode, kar posledično privede do zaplat odmrle trave. Največ škode nastane pozno poleti in zgodaj jeseni, ko ogrci dosežejo drugo in tretjo larvalno stopnjo (Fleming, 1972). Do sekundarnih poškodb travišč pogosto pride tudi pri gostotah ogrcev, ki same po sebi ne bi nujno povzročile škode, vendar druge živali iščejo ogrce in tako prekopavajo travno rušo (De Goffau, 1996; Laznik in Trdan, 2014). S koreninami trav se prehranjujejo tudi ličinke drugih vrst pahljačnikov, in sicer predstavniki rodov *Phyllophaga, Phyllopertha, Cotinis, Cyclocephala, Rhizotrogus, Anomala* in druge (Hann in sod., 2015; Gyawaly in sod., 2016). Poženel (2007) opisuje znaten porast populacije poljskega majskega hrošča (*Melolontha melolontha* L., 1758) na Idrijskem, kjer je leta 2005 populacija ogrcev narasla na 226 ogrcev na $m^2$. Znatno škodo travne ruše so povzročali ogrci vseh treh larvalnih stopenj, odrasli hrošči pa so objedali listje gozdnega drevja. Znatne poškodbe travišč je poljski majski hrošč med letoma 1989 in 1995 povzročil tudi v Nemčiji (Fröschle, 1996).

V mnogih delih sveta ogrci predstavljajo tudi pomembne škodljivce krompirja. Večina najpomembnejših škodljivcev v Indiji pripada rodovom *Anomala, Brahmina, Melolontha*, *Holotrichia* in *Lepidiota*, v Ameriki rodu *Phyllophaga*, na Kitajskem pa sta najpomembnejša rodova *Holotrichia* in *Amphimallon* (Radcliffe in Lagnaoui, 2007; Xu in sod., 2013; Chandel et al., 2015). Po izleganju se mladi ogrci orientirajo proti koreninam, vendar največjo škodo na krompirju povzročijo ličinke druge in tretje larvalne stopnje. Hranijo se s koreninami in steblom krompirja, zaradi česar odmrejo novo nastale rastline. Prehranjujejo se tudi z gomolji (Slika 1), ki so posledično neprimerni za trženje. V primeru poškodb gomoljev rastlina ne izrazi nikakršnih simptomov, zato se pridelovalci največkrat šele ob spravilu pridelka zavejo škode, ki so jo povzročili ogrci (Chandel in sod., 2013; Xu et al., 2013).

**Slika 1:** Poškodbe na gomoljih krompirja, ki so nastale zaradi ogrcev (foto: Eva Praprotnik)
**Figure 1:** Damage to potato tubers made by white grubs (photo: Eva Praprotnik)

Pahljačniki prav tako veljajo za pomembne škodljivce arašidov v južni Afriki, Aziji in Avstraliji (Wightman in sod., 1994), vinske trte in breskev (Heath in sod., 2002), listov kakavovca (Calcetas in sod., 2021), kokosovih palm v Srednji Ameriki in Aziji (Mariau, 2001; Maniania in sod., 2017), lesk v Turčiji (Sevim in sod., 2010), pšenice v Keniji (LePelley in Goddard, 1952), sladkornega trsa v Afriki in Južni Ameriki (Cock in Allard, 2013), listov jablan in orehov (Pathania in Chandel, 2017), listov evkaliptusa v Avstraliji (Carne in sod., 1974), korenin zelenjadnic (Oliveira in Frizzas, 2013), jagod (Malusá in sod., 2020) in mnogih drugih rastlinskih vrst.

## 3 ENTOMOPATOGENE GLIVE

Pahljačnike so v preteklosti zatirali z obstojnimi sintetičnimi insekticidi (Burkhardt, 1955; Miah in sod., 1986), vendar pa se je zaradi razvoja odpornosti škodljivcev, negativnih vplivov na (agro)ekosisteme in gospodarskih razlogov začelo spodbujati alternativne načine njihovega omejevanja z ustrezno oskrbo tal, uporabo feromonov, izborom kultivarjev, biotičnim varstvom in podobno (Frew in sod., 2016). Pahljačniki del njihovega življenja kot ličinke preživijo v tleh in so tako tesno povezani z rezervoarjem talnih mikrobov, vključno s številnimi vrstami entomopatogenih gliv. To so glive, ki so patogene za žuželke in pršice. Množijo se v mehkih tkivih gostitelja, smrt gostitelja pa navadno nastopi v nekaj dneh po okužbi zaradi izgube vode, pomanjkanja hranil, mehanskih poškodb ali delovanja toksinov (Hajek in St. Leger, 1994; Qu in Wang, 2018). Sledi sporulacija in okužba novih gostiteljev (Lovett in St. Leger, 2017). Za zatiranje pahljačnikov so najpogosteje uporabljeni predstavniki rodov *Beauveria* in *Metarhizium*. Entomopatogene glive se uporabljajo za zatiranje tako odraslih hroščev kot tudi ogrcev, pri katerih pa so prve in druge larvalne stopnje pogosto dovzetnejše za okužbo kot ogrci tretje larvalne stopnje (Erler in Ates, 2015; Laznik in Trdan, 2015; Kim in sod., 2020). S pahljačniki je povezano veliko število patogenov žuželk, zato zastopanost drugih (patogenih) mikrobov (bakterije, virusi), ogorčic in protozojev lahko še dodatno pospeši in poveča občutljivost ličink na okužbo (Marchal, 1976; Ferron, 1978).

### *3.1 BEAUVERIA* SPP.

Rod *Beauveria* velja za enega od najpomembnejših taksonov entomopatogenih gliv, predvsem zaradi njegove kozmopolitske razširjenosti, enostavne identifikacije, širokega spektra gostiteljev in enostavne izolacije (Goettel in sod., 1990). V kulturi na petrijevki *Beauveria* (Slika 2) navadno proizvede bel micelij in konidije, nekateri izolati pa se lahko pozneje obarvajo tudi rumenkasto. Najbolj izrazita morfološka značilnost rodu so simpodialni skupki pogosto cikcakasto rastočih konidiogenih celic, ki tvorijo enocelične, hialinske, holoblastične konidije (Rehner, 2005).

*Beauveria bassiana* (Bals.-Criv.) Vuill. je najbolj razširjena vrsta tega rodu z velikim številom gostiteljskih žuželčjih vrst (Zimmermann, 2007). V poskusih zatiranja je gliva povzročila 65-80 % smrtnost ogrcev tretje larvalne stopnje pahljačnika *Cyclocephala signaticollis* Burmeister, 1847 (*B. bassiana* sev Bb 53; Berón in Diaz, 2005), mlinarja *Polyphylla fullo* (Linnaeus, 1758) (*B. bassiana* sev PPRI 5339; Erler in Ates, 2015), ter odraslih osebkov japonskega hrošča (*B. bassiana* sev INRS 236; Giroux in sod., 2015). Ni pa se izkazala za uspešno pri zatiranju avstralskega avtohtonega pahljačnika *Adoryphorus coulonii* (Burmeister, 1847) (Rath in sod., 1995) ter pri pahljačniku vrste *Phyllophaga anxia* (LeConte, 1850) (Poprawski in Yule, 1991). *B. bassiana* sev ATCC 74040 je tudi aktivna učinkovina bioinsekticida Naturalis®, ki uspešno zatira predvsem prve larvalne stopnje vrste *Oryctes agamemnon* subsp. *arabicus* Fairmaire, 1896 (Ibrahim, 2017).

Spekter gostiteljev vrste *Beauveria brongniartii* (Sacc.) Petch je precej ožji v primerjavi z vrsto *B. bassiana* in uspešno deluje predvsem proti talnim hroščem družin Scarabaeidae in Curculionidae. Gliva *B. brongniartii* (sevi Bt96, Bt106, Bt107, Bt110, Bt112, Bt113 in Bt114) se je izkazala za uspešno pri zatiranju pahljačnika *Hoplochelus marginalis* (Fairmaire, 1889), ki je pomemben škodljivec sladkornega trsa (Neuvéglise in sod., 1994), ter pri zatiranju pahljačnika *Holotrichia serrata* (Fabricius, 1781) na plantažah betlovih palm (Ranganathaiah in sod., 1973). Na območju severozahodnega dela Italije (Dolci in sod., 2006), prav tako v Švici (Keller, 2000; Enkerli in sod., 2004) in Avstriji (Mayerhofer in sod., 2015) pa so bili sevi *B. brongniartii* F, 1871-1875 in BIPESCO 2 izolirani iz poljskega majskega hrošča uspešni pri zatiranju ogrcev omenjene vrste v sadovnjakih, na njivah in travnikih. Zastopanost poljskega majskega hrošča pomembno vpliva na preživetje glive *B. brongniartii* v tleh, saj zmanjšanje količine glive v odsotnosti gostitelja potrjuje visoko specifičnosti glive. Hkrati je razmnoževanje glive brez gostitelja malo verjetno (Kessler in sod., 2004). *B. brongniartii* sev IMBST 95.031 je tudi aktivna učinkovina bioinsekticida Melocont Pilzgerste®, ki je bil uspešno uporabljen za zatiranje poljskega majskega hrošča na Idrijskem (Poženel, 2007), Łabanowska in Bednarek (2011) sta poročala o visoki učinkovitosti tega sredstva proti pahljačnikom v sadovnjakih, Laengle in sod. (2005) pa v krompirju. Obratno pa v gozdnih ekosistemih rezultati pogosto niso bili zadovoljivi (Sierpińska in sod., 2015; Niemczyk in sod., 2019). Izolati teh gliv so namreč v večini zgledov asociirani s kmetijskimi zemljišči, kar lahko vpliva na njihovo sposobnost preživetja in obstojnost v različnih habitatih, kot so na primer naravna gozdna tla.


**Slika 2:** *Beauveria bassiana* na PDA gojišču (foto: Špela Modic)
**Figure 2:** *Beauveria bassiana* on PDA medium (photo: Špela Modic)

### *3.2 METARHIZIUM* SPP.

*Metarhizium* je izredno raznolik rod in ta pestrost je rezultat različnih habitatov, podnebnih razmer ter gostiteljskih rastlinskih in žuželčjih vrst, na katerih se ta rod pojavlja (Brunner-Mendoza in sod., 2019). V kulturi *Metarhizium* navadno proizvede temno zelene, svetlo zelene, rumenkaste ali rjavkaste kolonije z belim robom. Konidiji so hialinski in tvorijo verigi podobno tvorbo. Konidiji se razlikujejo po velikosti in obliki in znotraj rodu predstavljajo pomemben morfološki znak (Sinha in sod., 2016).

Konidiji vrste *Metarhizium anisopliae* (Metschn.) Sorokīn so v tleh zelo stabilni, njihovo število pa se lahko več let ohrani brez večjih izgub (Rath in sod., 1995). Moslim in sod. (1999) ter Gopal in sod. (2006) so poročali, da je uporaba glive *M. anisopliae* (seva Bp in MO pri Moslim. in sod. (1999)) znatno zmanjšala populacijo odraslih hroščev, zlasti pa ličink vrste *Oryctes rhinoceros* (Linnaeus, 1758), ki ji glavni vir hrane predstavljajo cve-

tovi kokosove in oljne palme. Prav tako je vrsta *M. anisopliae* uspešna pri zatiranju pahljačnika *Protaetia brevitarsis* (Lewis, 1879) (sev JEF-314; Kim in sod., 2020), japonskega hrošča (sev INRS 705; Giroux in sod., 2015) ter pahljačnikov rodu *Phyllophaga.* Pri slednjih sta Poprawski in Yule (1991) testirala različne poti vnosa patogena v gostitelja, pri čemer se je izkazalo, da so ličinke relativno odporne na oralno okužbo in bolj dovzetne na okužbo z glivami, apliciranih v tla, kar sovpada z naravnimi potmi kolonizacije entomopatogenih gliv, in sicer preko kutikule žuželčjega gostitelja.

Gliva *M. anisopliae* sev KTU-27 je pokazala visoko insekticidno aktivnost tudi proti poljskemu majskemu hrošču v regijah Turčije, kjer gojijo lešnike (Sevim in sod., 2010), nasprotno pa Putnoky-Csicsó in sod. (2020) poročajo o neučinkovitosti glive *M. anisopliae* sev NCAIM 362 za zatiranje poljskega majskega hrošča v sladkem krompirju. Nizko stopnjo virulence so izolati *M. anisopliae* pokazali tudi v primeru zatiranja pahljačnika *C. signaticollis* (sev Ma 8; Berón in Diaz, 2005) in junijskega hrošča (*Amphimallon solstitiale* (Linnaeus, 1758)) (sev MaF; Fătu in sod., 2018). *M. anisopliae* je tudi aktivna učinkovina bioinsekticidov, kot sta BioGreen® in BioCane® (sev FI-1045). Slednji se uporablja za zatiranje pahljačnika *Dermolepida albohirtum* (Waterhouse, 1875), z njegovo uporabo pa so Logan in sod. (2000) uspešno zatrli 50-60 % populacije v šestih mesecih. *D. albohirtum* je pomemben škodljivec korenin sladkornega trsa, saj na letni ravni povzroči škodo v vrednosti 10 milijonov dolarjev (Sallam in sod., 2007). BioGreen pa je v Avstraliji registriran za zatiranje avtohtonega pahljačnika *A. coulonii* (Burmeister, 1847), katerega ličinke so pomembni škodljivci korenin poljščin in trav ter ostalih rastlin na travnikih in pašnikih (Bullard in sod., 1993).

### *3.3 CORDYCEPS* SPP.

Rod *Cordyceps* spada v isto družino parazitskih gliv kot rod *Beauveria*. Mnoge vrste rodu *Cordyceps* lahko rastejo na umetnih gojiščih, nekatere pa lahko izoliramo le iz tal. Izolati so navadno vrstno specifični, saj vsak parazitira le eno vrsto ali skupino sorodnih vrst gostitelja (Dworecka-Kaszak, 2014). Skupaj te glive okužujejo mnogo vrst žuželk, najpogosteje predstavnike redov Lepidoptera in Coleoptera. Pahljačniki so gostitelji vsaj sedmih vrst iz rodu *Cordyceps,* med katerimi jih večina parazitira ličinke in le ena vrsta odrasle hrošče (Willis, 1959; Shrestha in sod., 2016). *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (včasih *Isaria fumosorosea* Wizqwe) je tudi aktivna učinkovina bioinsekticida PreFeRal®, ki povzroči do 50 % smrtnost prve larvalne stopnje vrste *O. agamemnon* subsp. *arabicus* (Faimaire), pomembnega škodljivca kokosove, cikas in datljeve palme (Ibrahim, 2017).

### *3.4 OPHIOCORDYCEPS* SPP.

Večina vrst tega rodu je temno pigmentiranih, glavnino njihovih gostiteljev pa predstavljajo ličinke hroščev v tleh ali v razpadajočem lesu, vendar obstajajo izjeme, saj nekatere vrste parazitirajo odrasle stadije mravelj in os (Kornsakulkarn in sod., 2018; Lin in sod., 2020). Anamorfne oblike družine Ophiocordycipitaceae so načeloma počasi rastoče glive, ki jih je pogosto težko gojiti v laboratoriju (Dworecka-Kaszak, 2014). Pahljačniki predstavljajo gostitelja vsaj 18 vrstam *Ophiocordyceps* spp., večina med njimi so naravni patogeni ličink (Shrestha in sod., 2016).

### *3.5 AKANTHOMYCES* SPP.

Vrste rodu *Akanthomyces* (včasih *Lecanicillium*) so pogosto mikrobno sredstvo za biotično zatiranje in se v veliki meri uporabljajo proti sesajočim škodljivim žuželkam, kot so listne uši, ščitkarji, kaparji in drugi (Reddy, 2020). Vsaj 15 pripravkov na osnovi *Akanthomyces* spp. je bilo ali pa so v postopku komercializacije, med katerimi sta tudi bioinsekticida Vertalec® z aktivno učinkovino *Akanthomyces longisporum* B. Huang, S.B. Wang, M.Z. Fan & Z.Z. Li, sev HRI 1.72 (včasih *Lecanicillium longisporum* (Petch) Zare & W. Gams) in Mycotal® z aktivno učinkovino *Akanthomyces muscarium* (Petch) Spatafora, Kepler & B. Shrestha, sev Ve6 (včasih *Lecanicillium muscarium* (Petch) Zare & W. Gams). Bioinsekticida se nista izkazala za uspešna pri zatiranju odraslih osebkov japonskega hrošča, prav tako sta imela le minoren vpliv na njihove ličinke (Giroux in sod., 2015). Tudi *Akanthomyces lecanii* (Zimm.) Spatafora, Kepler & B. Shrestha (včasih *Lecanicillium lecanii* (Zimm.) Zare & W. Gams) se pri zatiranju vrste *O. agamemnon* ni izkazal za uspešnega, saj je povzročil manj kot 30 % smrtnost ličink tretje larvalne stopnje (Saleem in Ibrahim, 2019).

### *3.6 FUSARIUM* SPP.

Glive rodu *Fusarium* naseljujejo različne ekološke niše. Lahko so endofiti, saprofiti ter rastlinski in živalski patogeni. Vrste so občasno izolirane iz mrtvih ali živih žuželk in veljajo za oportunistične patogene žuželk. Visoko smrtnost povzročajo predvsem pri škržatkih, kaparjih in dvokrilcih (Teetor-Barsch in Roberts, 1983). Zaradi uspešnega obstanka v tleh kot saprofiti, vrstne specifič-

nosti do gostitelja in enostavnega gojenja v laboratoriju, je rod *Fusarium* kot agens za biotično zatiranje učinkovit, vendar pa so lahko določeni izolati zelo škodljivi patogeni rastlin (Sharma in Marques, 2018). *Fusarium* sp. velja za naravnega patogena pahljačnika vrste *Costelytra zealandica* (White, 1846) in se je v laboratorijskih poskusih izkazal za učinkovitega pri zatiranju te vrste (Goh in sod., 1991).

### *3.7 PENICILLIUM* SPP.

Glavni pomen predstavnikov rodu *Penicillium* v naravi je razgradnja organskih materialov. Povzroča rastlinsko gnilobo, ob čemer lahko sprošča širok spekter mikotoksinov (Visagie in sod., 2014). *Penicillium* je lahko patogen za členonožce, velja pa za oportunističnega sekundarnega patogena ali saprofita, ki nima vrstno specifičnih gostiteljev (Sosa-Gómez in sod., 2010). Rod je poznan po proizvajanju toksičnih sekundarnih metabolitov, ki lahko predstavljajo strategijo za hitro zastrupitev gostiteljske žuželke za poznejše saprofitsko prehranjevanje (Mora in sod., 2018).

### *3.8 ASPERGILLUS* SPP.

Rod *Aspergillus*, prav tako kot rod *Penicillium*, uvrščamo v red Eurotiales. Glive izločajo širok spekter encimov, ki jim omogočajo izkoriščanje različnih virov hranil, zaradi česar lahko delujejo bodisi kot saprofiti bodisi kot oportuni patogeni, čeprav pogosto z nizko virulenco. *Aspergillus* spp. na splošno veljajo za oportunistične patogene, ki za kolonizacijo potrebujejo poškodovane ali kako drugače oslabljene gostitelje, obrambo gostitelja pa premagajo predvsem z izločanjem toksičnih sekundarnih metabolitov (St. Leger in sod., 2000). *Aspergillus* sp., *Penicillium* sp. in *Fusarium* sp. veljajo za naravne patogene pahljačnika *P. anxia* (LeConte, 1850). Najdeni so bili v asociaciji le z ogrci omenjene vrste, na splošno pa je njihova izolacija redka. *Fusarium* sp. in *Penicillium* sp. sta povzročila 50-60 % smrtnost ogrcev, *Aspergillus* sp. pa le 30 % smrtnost (Poprawski in Yule, 1991). Prav tako veljajo za naravnega patogena pahljačnika *Brahmina coriacea* (Hope, 1831). *Penicillium* je bil redko izoliran iz ogrcev *B. coriacea* in tudi v laboratorjiskih poskusih je povzročil manj kot 40 % smrtnost. Nasprotno pa so bili *Aspergillus* spp. in *Fusarium* spp. pogost vzrok naravnih okužb, pri čemer so določene vrste povzročile 60-80 % smrtnost (Sharma in sod., 2012).

## 4 VRSTNA SPECIFIČNOST ENTOMOPATOGENIH GLIV

Čeprav je raznolikost gliv, povezanih s pahljačniki, velika, v mnogih zgledih slednji kažejo znake odpornosti na določene izolate gliv. Eden izmed možnih razlogov je dolg proces koevolucije ogrcev in mikroorganizmov v tleh, kjer povečanju patogenosti mikroorganizma sledi razvoj odpornosti gostitelja. Talne žuželke, ki se torej razvijajo v mikrobno bogatem mediju, bodo občutljive le na izredno specializirane izolate, ki bodo sposobni izkoristiti določeno fiziološko ali vedenjsko pomanjkljivost svojih gostiteljev (Jackson, 1999). Entomopatogene glive so torej lahko vrstno zelo specifične, zato je za uspešno implementacijo takšnih biotičnih agensov odvisno tudi morfološko poznavanje vrst škodljivcev, ki so zastopani na nekem območju. Odrasle hrošče najhitreje prepoznamo po značilnih pahljačastih tipalkah, saj so zadnji trije segmenti stransko razširjeni (lamelasti). Tipalke so devetčlenaste, zadek pa je sestavljen iz šestih dobro vidnih trebušnih ploščic. Epipleuron (del zunanjega roba pokrovk) je ozek in ne doseže vrha pokrovk (Jessop, 1986). Vrstno določevanje odraslih hroščev je enostavnejše od določevanja ličink. Ličinke hroščev pahljačnikov so morfološko bolj podobne ličinkam družine Lucanidae kot pa Passalidae. Od predstavnikov slednje se razlikujejo po tri-, štiri- in lahko tudi petčlenastih antenah, štiričlenastih maksilarnih palpih in podobi telesa v obliki črke C. Od ličink iz družine Lucanidae pa se razlikujejo po prečni, oglati ali Y obliki analne odprtine brez ovalnih rež (ang. oval lobes) ob straneh (Carlson, 1991). Ličinke pahljačnikov dosežejo dolžino od 10 do 125 mm. Telo je pogosto obarvano belo ali rumenkasto, glava pa je v odtenkih rdeče in rjave barve. Pomemben določevalni znak znotraj družine je specifičen vzorec dlačic ali ščetin, ki so prisotne na spodnji strani končnega trebušnega segmenta, imenovan raster (Gibb, 2015).

## 5 ZAKLJUČEK

Pahljačniki so izjemno pestra skupina hroščev, predvsem zaradi svoje kozmopolitske razširjenosti, poseljevanja različnih habitatov ter prehranjevalnih navad, zaradi katerih so mnoge vrste prepoznane kot gospodarsko pomembni škodljivci. Imajo kriptičen način življenja, saj večina njihovih ličink, imenovanih ogrci, živi v tleh in je zato preučevanje in zatiranje le-teh oteženo. Ogrce, predvsem njihove začetne larvalne stopnje, bi bilo potrebno obravnavati v programih biotičnega varstva, z namenom zmanjšanja škode, ki jo povzročijo na kmetijskih, gozdnih in travnatih zemljiščih. Entomopatogene glive, predvsem iz rodov *Metarhizium* in *Beauveria*, ima-

jo potencial za ohranjanje okoljsko uravnotežene ravni populacije škodljivcev, vendar so močno virulentni izolati relativno redki in v večini primerov specifični za vrsto gostitelja. Zaradi tega je pomembno, da poznamo vrstno sestavo pahljačnikov na ogroženih zemljiščih.

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