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Allelopathic effect of medicinal plant *Cannabis sativa* L. on *Lactuca sativa* L. seed germination

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ABSTRACT

In order to examine allelopathic effect of *Cannabis sativa* L. on germination capability and seedling growth of *Lactuca sativa* L., a study was performed in laboratory conditions. Treatments were set up in randomised block design in four replications for each of four concentration ranges of 25, 50, 75 and 100 % of aqueous extract made of shoot parts and 4 identical extract concentrations made of root of cannabis. Control variant was lettuce seed treated by distilled water. During the studies shoot and seminal root length of lettuce seedlings were measured after treatments with different concentrations of extracts made of root and shoot parts of cannabis, and the obtained values were compared with the control. The obtained results suggest that the extract from the shoot parts of cannabis in high concentrations of 75 and 100% had inhibiting effect to the germination indices while the extract from the root had no statistically significant effect on germination of lettuce seeds. Extract made of root part of cannabis showed also stimulatory effect to shoot and seminal root length of lettuce seedlings in extract concentrations of 50, 75 and 100 %.

Key words: allelopathy, lettuce, aqueous extract, cannabis, seed germination indices

IZVLEČEK

ALELOPATIČEN UČINEK KONOPLJE (*Cannabis sativa* L.) NA KALITEV SEMEN VRTNE SOLATE (*Lactuca sativa* L.)

V laboratorijskih razmerah je bil preučevan alelopatičen učinek navadne konoplje (*Cannabis sativa* L.) na kalitev semen in rast kalic vrtne solate (*Lactuca sativa* L.). Obravnavanja so bila izvedena kot naključni bločni poskus v štirih ponovitvah z vsako od štirih koncentracij, 25, 50, 75 in100 %, vodnega izvlečka poganjkov in korenin konoplje. V kontrolnem poskusu so bila semena vrtne solate tretirana na enak način z distilirano vodo. Poleg indeksa kalitve sta bili v poskusi merjeni še dolžina poganjkov in semenskih korenin kaleče solate. Rezultati so pokazali, da je imel izvleček poganjkov konoplje v večjih koncentracijah, 75 in 100 %, inhibitorni učinek na kalitveni indeks, medtemko izvlečki iz korenin niso imeli statistično značilnega vpliva na iste merjene parametre. Izvleček iz korenin konoplje je imel stimulativni učinek na dolžino poganjkov in semenskih korenin vrtne solate pri koncentracijah 50, 75 in 100 %.

Ključne besede: alelopatija, vrtna solata, vodni izvleček, konoplja, kalitveni indeks

1 INTRODUCTION

Hundreds of medicinal plant species are being used in modern medicines. They have been used as remedy for different diseases e.g. fever, malaria, cough, flu, asthma, colds, chest diseases, skin itch, acne, headache, jaundice, nausea, ulcer, tumors, typhus, stomach pain, heart attack, chills, inflammation, herpes, hepatitis, swelling, etc (Ishaque and Shahni, 1998). Most of them have been collected from wild sources. There is an increasing demand for medicinal plants-based

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drugs and pharmaceuticals in the world market. However, pharmaceutically active compounds can also behave as allelochemicals. These allelopathic compounds can also be used as natural herbicides and other pesticides (Einhelling, 1995).The term "allelopathy" was proposed for expressing the harmful, stimulatory, enhanced and beneficial effects that one plant species has on another through the formation of chemical retardants escaping into the environment (Molisch, 1937). Allelochemicals are plant metabolites present in plants as end products and by-products. These chemicals are present in different parts of plants like stem, leaves, roots, flowers, inflorescence, fruits and seeds. Out of these plant parts, leaves seem to be the most consistent producers of these allelochemicals. These allelochemicals are often released from the plants by volatilization, leaching, exudation and decomposition from plant residues (Molisch, 1937). Major allelochemicals found in plants with documented allelopathic activity are phenolic compounds (Chon et al., 2002) that have stimulatory effects on seed germination and seedling growth of plants. This is generally accepted in the literature that phenolic compounds at low concentrations are stimulatory to germination and plant growth (Hegab et al., 2008; Ghareib et al., 2010), but higher concentrations resulted in a sharp germination reduction. The well-known terpenoids include menthol, camphor, thujone and the cannabinoids are found in *Cannabis sativa* (Ameh et al., 2010). Allelopathics are often due to synergistic activity of allelochemicals rather than to single compounds. The additive or synergistic effects become significant even at low concentrations of the extracts (Einhelliing, 1995). The concept of allelopathy was further supported and developed by Bonner (1950), Grummer and Beyer (1960), Evenari (1961), Whittaker (1970), Pitman and Duke (1978) and Fischer *et al*. (1978). According to Lavabre (1991), allelopathic effects are controversial and still poorly understood. In allelopathy, a major tool for research is bioassay which controls laboratory conditions, high sensitivity gives reproducible results, and takes relative short time to perform. There are many ways to evaluate the herbal aqueous extracts of allelopathic activities. These are hydroponic culture methods, Ratoon screening method, Plant box method (Lee *et al*., 2003), a plastic tray with 6 holes (Fujii *et al*., 2004), Dish pack method - a new bioassay for volatile allelopathy (Fujii *et al*., 2005), Sandwich method (Fujii *et al*., 2003) and Filter paper (Barbosal *et al*., 2008). The filter paper is a suitable method because it can tolerate the moderate temperature during incubation (25 °C) in the laboratory. The aqueous extracts remain stable for longer period of time. Millipore filter paper is used to make the method sterile. The reason for the use of filter paper in techniques is that, it is easily available and free from contamination. It is easily handled and a good media for germination, it has high flow rate for movement of extracts and porosity (Gill et al., 2009). Allelopathic effect of medicinal species against other plants is well studied (Wahab *et al*., 1967; Rice, 1971; Han *et al*., 2008 and Li *et al*., 2009). *Cannabis sativa* is known for centuries as an effective weeds suppressing crop plant in Iran. Weeding role of this plant is mainly attributed to its high competitiveness for water, food and light, as it overshadows the soil quickly after the initial growth phase and therefore suppresses weed growth (Ranalli, 1999). In general, crop rotation and related practices should aim to give the competitive advantage to the crops in rotation against weeds. Only few studies concerned the rotation effects of *Cannabis sativa*, as an annual crop, fits well into crop rotation and can serve to the control of pests, as it is not related to conventional food crop species (Ranalli, 1999), hence the main purpose of the present study is to evaluate the allelopathic activity of medicinal plant, *Cannabis sativa* L. ssp. *sativa* on lettuce (*Lactuca sativa* L.).

2 MATERIALS AND METHODS

2.1 Plant material and extraction procedure

In the period 2013-2014, at farms near Mashhad, parts of *C. sativa* L. ssp. *sativa* ('TN-96-35') were collected. Plant material was divided into shoot, i.e. stem plus leaf and root. Shoot and root parts were pulverized, after which separate extracts were made from each of these parts of of *C. sativa* L. Ten g of fresh shoot or root parts were mixed with 100 ml distilled water in a blender. The homogenate was filtered through tissue paper after 10 minutes and the filtrate was centrifuged at 3000 × g for 20 min and the supernatant was used as stock solution. Extract of shoot and and root part of *C. sativa* was made in a range of concentrations of 25, 50, 75 and 100 % of stock solution

2.2 Seed germination test

Filter paper in Petri dishes, of 150 x 25 mm in size was moistened by 5 ml of the obtained extracts, and tested lettuce seed was germinated on it. Control was moistened by distilled water. Lettuce seed (*Lactuca sativa*'Longifolia') surface was sterilized before adding of extracts according to Elemar and Filho (2005). Each concentration of *C. sativa* extracts was made in four replications. Samples were laid in thermostatic device at 22 ± 2°C for 7 days. Laboratory tests were set up in randomized block design in four replications. Each Petri dish contained 25 lettuce seeds, i.e. 100 seeds per treatment. Germination tests were performed according to the rule issued by the International Seed Testing Association. The number of germinated seeds was noted daily for 7 days. Seeds were considered as germinated when their seminal root reached at least 1 mm length. In this study, we used following germination parameters: Germination percentage (GP, %), Germination rate (GR), Relative germination percentage (RGP), Mean germination time (MGT), Germination index (GI) and Weighted germination index (WGI). Final percentage germination (GP) for each treatment was calculated after seven days. The germination index (GI) is based on number of seeds that germinated and the germination rate. These parameters were also calculated from the formulas proposed by (Figueroa and Armesto, 2001; Bu et al., 2007; Wu and Du 2007).

$$GP = 100 \times GN / SN \quad (1)$$

GN is the total number of germinated seeds, SN is the total number of seeds tested

$$RGP = GP\ treatment / GP\ control \times 100 \quad (2)$$

$$GI = (\Sigma(N-i) \times G_i) \times 100)/(N \times GN) \quad (3)$$

GI is a synthetic measure designed to reflect the synthetical germination ability including germination rate and germination numbers. Where i is the number of days since the day of sowing and Gi is the number of seeds germinated on day i.

A weighted germination index (WGI) as described by Bu et al. (2007) was calculated with maximum weight given to the seeds germinating early and less to those germinating late

$$WGI = [N \times n_1+(N-1) \times n_2+(N-Z) \times n_3+\ldots]/ N \times N' \quad (4)$$

where n1, n2, …, n60 are the number of seeds that germinated on first, second, and subsequent days until the 60th day, respectively; N is total days of experiment; N′ is the total number of seeds placed in incubation

$$GR = \Sigma\ G_i / \Sigma\ n_i\, G_i \quad (5)$$

Where i is the number of days since the day of sowing and Gi is the number of seeds germinated on day i

Vigor index = germination % × seedling length (root + shoot). (6)

After an incubation period of 7 days, shoot and seminal root length of seedlings were measured using a ruler.

2.3 Data analysis

Significant differences for all statistical tests were evaluated at the level of $p \leq 0.05$ with ANOVA. All data analyses were conducted using SPSS for Windows, Version 13.0.

3 RESULTS

Based upon conducted studies, data on effect of extracts made of shoot and root parts *of C. sativa* to germination indices and seedling growth of *L.sativa* were obtained. Extracts made from shoot parts in concentrations of 75 and 100 % showed significant effects on germination indices and seedling growth of lettuce including: germination percent, relative germination percent, germination rate, mean germination time, weighted germination index and seminal root length, while all concentrations of shoot extracts of *C. sativa* showed no significant effect on germination index, vigor index and shoot length. Measured values of lettuce seed germination treated by extract of the shoot parts of *C. sativa* confirmed that extract showed allelopathic – inhibitory effect to the germination indices and growth of seminal root in comparison to the obtained control values.

The obtained results for extract concentration of 75 % made of shoot parts of *C. sativa* showed that germination percent and relative germination percent were 90 % which was less in comparison to the value of control of 100 %; for the medium extract concentration of 100 % determined germination percent was 76.67 %. For mean germination time, at higher concentrations were also established lower values. For extract concentration of 75 %, MGT was 4.8 days, whereas in the highest concentration of 100 %, MGT of lettuce seeds was the lowest, i. e. 4.53 in comparison to control variant that was 5 days. For concentration of 100 % of extract made of shoot parts of *C. sativa*, the average value of WGI was 0.84, and control was 1, for concentration of extract of 75 %, the established value of WGI was 0.93,while control was 8.09. Concentration of 75 % and 100 % resulted in GR of 0.45 and 0.38 $Nday^{-1}$, respectively, with control value of 0.5 $Nday^{-1}$. For variant of 75 % of extract from the shoot parts of *C. sativa*, lettuce seminal root length was 2.3 cm. while control was 3.3 mm, the application of highest concentration of extract made of shoot part of 100 % resulted in average seminal root length of 2.1 cm. Duncan test showed statistically significant difference ($P \leq 0.05$) between the most germination indices of lettuce seed only in the case of the extract made of the shoot parts of *C. sativa* in concentration of 75 and 100 % (Table 1).

Table 1: Statistical data (mean ± standard error) on effects of the shoot parts of *Canabis sativa* L. to germination of lettuce seeds

Shoot extract (%)	GP(%)	GR(Nd^{-1})	RGP	MGT(day)	GI	WGI	VI	Shoot length(cm)	Seminal root length(cm)
0	100± 0	0.5± 0	100± 0	5± 0	33.3± 0	1 ±0	506.67± 110	2.1 ±0.65	3.3± 0.26
25	96.67± 5.7	0.48± 0.02	96.67 ±5.7	4.93± 0.11	32.2± 1.9	0.97± 0.04	575.6± 89	2.86± 0.7	3.06± 0.11
50	90± 10	0.45± 0.05	90± 10	4.8± 0.2	29.9± 3.3	0.93± 0.07	457.3± 57.9	2.4± 0.45	2.7± 0.46
75	*90± 0	*0.45± 0	*90± 0	* 4.8 ±0	33.3± 0	*0.93± 0	483± 99	2.8± 0.36	*2.3 ±0.6
100	*76.67± 15.2	*0.38± 0.07	*76.67 ± 15.2	*4.53 ±0.3	26.6± 6.6	*0.84± 0.1	361± 58.9	2.6± 0.26	*2.1± 0.32

*Marked differences are significant at $p<0.05$

Bioassay revealed that for extract of the root of *C. sativa* there were no statistically significant deviations between values of lettuce seed GP, RGP, MGT, GI, VI from control values. For variant of 25, 75 and 100 % of extract from the root parts of *C.sativa*, WGI of lettuce seeds was 0.97, while control was 1. The application of highest concentration of extract made of root part of 100 % resulted in average GR of 0.46 $Nday^{-1}$, whereas the average GR in control was 0.5 $Nday^{-1}$. Length of lettuce seminal root in treatment with the 75 and 100 % extracts made of the root part of *C. sativa* confirmed stimulatory effect in comparison to the control variant. Extract made of root part of *C. sativa* also did show stimulatory effect to shoot length in extract concentrations of 50 % (Table 2).

Table 2: Statistical data on effects of the root parts of *Canabis sativa* L. to germination of lettuce seeds

Root extract(%)	GP(%)	GR(Nd^{-1})	RGP	MGT(day)	GI	WGI	VI	Shoot length(cm)	Seminal root length(cm)
0	100± 0	0.5± 0	100± 0	5± 0	33.3± 0	1± 0	506.67± 110	2.1± 0.65	3.3± 0.26
25	96.67 ±5.7	0.48± 0.02	100± 0	4.93± 0.11	32.2± 1.9	*0.97± 0.04	382.6± 105	2.1 ±0.55	3.3± 0.47
50	96.67 ±5.7	0.5 ±0	100± 0	5± 0	33.3± 0	1± 0	322± 42.3	2.1± 0.52	2.9± 0.3
75	96.67 ±5.7	0.48 ±0.02	96.67 5.7±	4.93± 0.11	32.2 ± 1.9	*0.97± 0.04	314± 16.9	*3.36± 0.46	*4.3 ±0.85
100	93.33± 5.7	*0.46 ±0.02	96.67 5.7±	5.26± 0.64	31.1 ± 1.9	*0.97± 0.04	197.3± 62	2.6± 0.57	*4.3± 0.51

*Marked differences are significant at $p<0.05$

4 DISCUSION

In the study, allelopathic effect of the medicinal plant, *C. sativa* was established for germination indices and early growth of lettuce. Extracts made of shoot parts of *C. sativa* showed inhibitory effects to lettuce seed germination indices except from GI and VI. Extract made of the shoot parts, in concentration of 75 and 100 % significantly affected mentioned factors, while the lower two concentrations did not show a significant effect. Extracts made of shoot parts of *C. sativa* did not show statistically significant effect to shoot growth in any concentration. Extracts made of shoot part of *C. sativa* in concentrations of 75 and 100 % showed statistically significant effect to the growth of seminal root, while concentrations of 25 and 50 % had no statistically significant effect.

The seeds imbibed with shoot extracts of *C. sativa* delayed and inhibited germination in comparison to control. The inhibitory effect of different concentrations of shoot extract of *C. sativa* on seed germination might be due to imbalance in metabolism regulated by various enzyme activities (Oyun, 2006). The structure of plasma membrane might have become denatured by the phytotoxins present in shoot extract of *C. sativa* when the seeds were soaked in extract. Positive correlation was observed between the increase in the concentration of extract and the inhibition of germination percentage. Allelochemicals are known to inhibit the metabolic processes i.e. cellular respiratory ability of the target plant and energy transfer which is responsible for ATP synthesis (Demos *et al*., 1974). The phytotoxins present in shoot extracts of *C. sativa* might have arrested the protease, α - amylase activity resulting in inhibition of protein and starch breakdown which reduces the germination process.

Extract made of root part of *C. sativa* had stimulatory effect to shoot and seminal root length in extract concentrations of 50, 75 and 100 %. The similar phenomenon was observed by other authors (Sun *et al*., 2006) analyzing the allelopathic effect of *Solidago canadensis* L. on rape. Anjum *et al.* (2010) performed laboratory experiments to examine the allelopathic potential of some medicinal plants on the germination and growth of lettuce. Using sandwich method, *Albezzia lebbeck* (L.) Benth. and *Broussonetia papyrifera* (L.) Vent. have strong inhibitory effects on the seminal root and hypocotyls growth of lettuce. However, stimulatory effects were recorded with *C. sativa* and *Parthenium hysterophorus* L. at 5mg leaves concentrations (Anjum *et al.*, 2010).

Extract from the shoot parts of *C. sativa* has a higher inhibitory effect to lettuce germination and seedling growth than extract from roots. Contents of biologically active compounds in shoot and root parts of *C. sativa* are probably different both qualitatively and quantitatively.

5 CONCLUSIONS

Higher content of allelochemicals present in extract of shoot parts of *C. sativa* may be responsible for the inhibition of metabolic activities of lettuce in the laboratory bioassay tests

The study revealed reduced germination capability of lettuce seeds after treatment by extract made of the shoot parts of *C. sativa* which was supported by calculated statistical significance that indicated existence of allelopathic effect. Extract made from the roots of *C. sativa* did not have allelopathic effect to the growth of lettuce and seed germination.

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