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GC-MS analysis and insecticidal effect of methanol extract of *Pistacia khinjuk* Stocks leaves

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Abstract: Regarding the significance of environmental issues, the use of herbal compounds is proposed as an alternative method for methyl bromide and phosphine. In this research, the insecticide effect of MeOH extract of *Pistacia khinjuk* leaves on *Tribolium confusum* (Jacquelin du Val, 1863) and *Oryzaephilus surinamensis* (Linnaeus, 1758) was studied. The chemical compounds of the extract were also identified by the GC-Mass device. The contact toxicity test was performed on 1 to 7-day-old insects. LC_{50} values of 9.32 and 5.47 mg ml^{-1} were calculated for *T. confusum* and *O. surinamensis*, respectively. More than 39 compounds were identified in MeOH extract of *P. khinjuk*, with 5-ethoxy-4-phenyl-2-isopropylphenol (29.02 %), phenyl ethyl alcohol (10.78 %), benzyl alcohol (7.8 %) and 1, 2-benzenediol (6.67 %) as main compounds. In addition, there were the known insecticide compounds such as spathulenol (2.07 %), myrcene (2.03 %), ρ-cymene (1.67 %), apiol (1.61 %), borneol (0.79 %), and pulegone (0.44 %) in the plant extract that confirmed the potential of the *P. khinjuk* extract in controlling stored-product insects.

Key words: wild pistachio; insecticide; flour beetle; saw toothed grain beetle; herbal extract

GC-MS analiza in insekticidni učinki metanolnih izvlečkov listov divje pistacije (*Pistacia khinjuk* Stocks)

Izvleček: Glede na okoljske izzive se uporaba spojin iz zelišč priporoča kot nadomestna metoda za metilbromid in fosfin. V raziskavi so bili preučevani insekticidni učinki metanolnih izvlečkov listov divje pistacije (*Pistacia khinjuk* Stocks) na dve vrsti škodljivih hroščev*Tribolium confusum* (Jacquelin du Val, 1863) in *Oryzaephilus surinamensis* (Linnaeus, 1758)). Spojine v izvlečkih so bile analizirane z GS-MS metodo. Preiskus kontaktne strupenosti je bil izveden na 1 do 7-dni starih hroščih. LC_{50} vrednosti sta bili za malega mokarja 9.32 in za zobatega žitnika 5.47 mg ml^{-1}. V metanolnih izvlečkih listov divje pistacije je bilo določenih več kot 39 spojin. Med njimi so imele glavne sestavine naslednje deleže: 5-etoksi-4-fenil-2-isopropilfenol (29,02 %), feniletilalkohol (10,78 %), benzilalkohol (7,8 %) in 1, 2-benzendiol (6,67 %). Med spojinami, določenih v izvlečkih, so bili tudi znani insekticidi kot so spatulenol (2,07 %), mircen (2,03 %), ρ-cimen (1,67 %), apiol (1,61 %), borneol (0,79 %), in pulegon (0,44 %). Vse to potrjuje, da bi lahko bili izvlečki iz listov divje pistacije uporabni za zaščito shranjenih pridelkov pred žuželkami.

Ključne besede: divja pistacija; insekticidi; mali mokar; zobati žitnik; rastlinski izvlečki

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1 INTRODUCTION

Proper monitoring of storage pests is one of the most important storage factors of foods such as cereals and legumes. Such pests are among the most crucial challenges of these products in stock after harvest until their consumption date. An average of 10 to 40 percent of the world's stored crops is annually destroyed, resulting in significant economic losses (Kumar and Kalita, 2017).

The confused flour beetle (*Tribolium confusum* (Jaqcquelin du Val, 1863), Coleoptera, Tenebrionidae) is one of the most important cereal pests around the world, which not only causes significant losses due to feeding, but also, contaminates the crop with its feces and larvae shells due to its rapidly increasing population; thus, reducing the crop quality (Forghani and Marouf, 2015).

The saw-toothed grain beetle (*Oryzaephilus surinamensis* (Linnaeus, 1758), Coleoptera, Silvanidae) is economically one of the most significant storage pests that targets all herbal products such as wheat, rice, corn, barley, flour, bran, pasta, bread, biscuits, oilseed, and dried fruits, and caused heavy losses (Forghani and Marouf, 2015).

For chemical control of the stored crops in the warehouses, it is a common way to use fumigant insecticides. Methyl bromide (CH_3Br) and phosphine (PH_3) are two compounds of chemical pesticides that are mainly used for pest control. However, both are limited mainly because of their high toxicity for humans. Besides, methyl bromide is considered an ozone depleting chemical. Meanwhile, high resistance of the warehouse pests to PH_3 has been reported in many countries (Bell and Wilson, 1995).

Despite their importance, insecticides also have negative impact on human health, so, herbal insecticides can be a good alternative for artificial ones, especially due to their unique properties such as weaker impact on natural enemies, lack of phytotoxicity, minimum toxicity level on vertebrates, and rapid degradation in the environment, and so far, a great number of studies have been carried out on the insecticidal properties of plant products (Rojht et al., 2012; Campolo et al., 2018).

Pistacia khinjuk Stocks is a plant species in the family Anacardiaceae, native to Egypt, western Asia and parts of the Himalayas. The tree grows up to 10 metres (Rhodes and Maxted, 2016). In Iran this species is wide ranging, occurring through the Makran Zone, Zagros Mountains and the Sanandaj-Sirjan Zone (Ghaemmaghami et al., 2009).

Literatures are available about composition and properties of *P. khinjuk* essential oil. The most substance found in essential oil of *P. khinjuk* aerial part (collected from Kermanshah province, Iran) was γ-terpinene (81 %), follwed by β-pinene (3.9 %) and α-terpinolene (2.4 %) (Tahvilian et al., 2017). Major constituents of essential oil from the leaves of *P. khinjuk* (collected from Rafsanjan, Iran) were myrcene (18.7 %), α-eudesmol (12.3 %), β-eudesmol (9.3 %), 1,7-di-epi-β-cedrene (7.3 %), bicyclogermacrene (5.6 %) and δ-eudesmol (4.9 %) (Abolghasemi et al., 2018). But limited studies are available on *P. khinjuk* leaves extract. By extraction of phenolic components of *Pistacia khinjuk,* seven phenolic compounds, mainly flavonoids and galloylated compounds were isolated from the aqueous methanol extract (Esmat et al., 2012).

A few reports have been received about the insecticidal activity of *Pistacia khinjuk* on insect pests. It has been reported that the essential oil of the tested plant had insecticidal activity against eggs and nymphs of Pistachio Psyllid, *Agonoscena pistaciae* (Burckhardt & Lauterer, 1989) (Hemiptera, Psyllidae), the key pest of cultivated pistachios (Abolghasemi et al., 2018). Also, similar studies on the lethal effect of the extracts of other species of pistachios such as *Pistacia lentiscus* Linnaeus have been conducted on the red flour beetle, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) (Pascual-Villalobos and Robledo, 1998) and the essential oils of *P. terebinthus* Linnaeus, *P. lentiscus and P. vera* Linnaeus on the adults of *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Curculionidae) (Aslan et al., 2004).

The objective of the present study was to determine the chemical composition of *P. khinjuk* leaf extract and also to evaluate the insecticidal activity of extract on two important stored product pests, *Tribolium confusum* and *Oryzaephilus surinamensis* (Linnaeus, 1758).

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS

The sample leaves of *Pistacia khinjuk* were collected from their natural habitats in Kermanshah province, Iran (34° 32' N, 47° 07' E; alt. 1374 m), in the period ranging from October to November, 2012. Sampled leaves were air-dried and grounded into powder. The powder was weighed and extraction was also done by soaking the powdered leaves in methanol solvent (ratio 1:5 w/v) and shaking the mixture on a shaker (150 rpm) for 24 hours at 25 °C. The liquid extract was filtered through Whatman No.1 filter paper fitted in a Buchner funnel using suction. For concentrate, the solvent was evaporated from the solution using the rotary evaporator. The extracted plant substances were stored in airtight containers covered with aluminum foils in refrigerator at 4 °C until it was used.

To determine the dry mass of pure extract at one milliliter of solution, the small parts of solution (5 ml) were separately collected in three replicates, and allowed to dry completely at 100 °C in oven.

2.2 INSECT BREEDING

The tested warehouse pests including the saw-toothed grain beetle (*Oryzaephilus surinamensis*) and flour beetle (*Tribolium confusum*) were gathered from the contaminated sites and were reared in an incubator at 27 ± 1 ° C and 65 ± 5 % RH and darkness on wheat flour (wheat flour with bread yeast: 10:1). The adult 1 to 7- day- old insects were used for bioassay experiments.

2.3 BIOASSAY TEST

This experiment was carried out in a completely random design with four replications with the control. Each experimental unit consisted of an 8 cm diameter petri dish. One milliliter of different concentrations of the extract (2, 4, 8, 20, 30, and 50 mg ml^{-1}) was poured by a sampler over the filter paper located on the bottom of the petri dish. For the control treatment, a filter paper impregnated with methanol was used. The petri dish was left open for 1 hour for the solvent to evaporate. Then, 10 insects were placed inside each petri dish and were capped. The petri dishes were placed in an incubator in dark condition, at 25 °C and 70 ± 5 % RH, and the number of the dead insects was counted after 24 hours.

2.4 IDENTIFICATION OF CHEMICAL COMPOUNDS

GC-MS analysis of methanolic extract of *P. khinjuk* was performed in an Agilent-7890 gas chromatograph with 5975C Mass Spectrometer and a flame ionization detector which was operated in EI mode at 70 eV with a mass range of 40–400 m/z. Injector temperature was set at 260 °C. The HP-5MS capillary column (15 m x 0.25 mm id x 0.25 µm) was used for the analysis. Helium was the carrier gas at 1 ml min^{-1} flow rate. After analysis and identification of the peaks, the active ingredients were identified using the Willy library.

2.5 STATISTICAL ANALYSIS OF DATA

Data were analyzed by SPSS software (IBM SPSS V21.0). Normality of raw data was surveyed using Non-parametric One-Sample Kolmogorov-Smirnov test. Statistical analysis of the mortality data was performed by one-way analysis of variance (ANOVA) with a post-hoc Tukey test at $p< 0.05$. The effective lethal concentration of 50 % (LC_{50}) was determined using linear probit analysis in $SPSS_{21}$ software.

3 RESULTS AND DISCUSSION

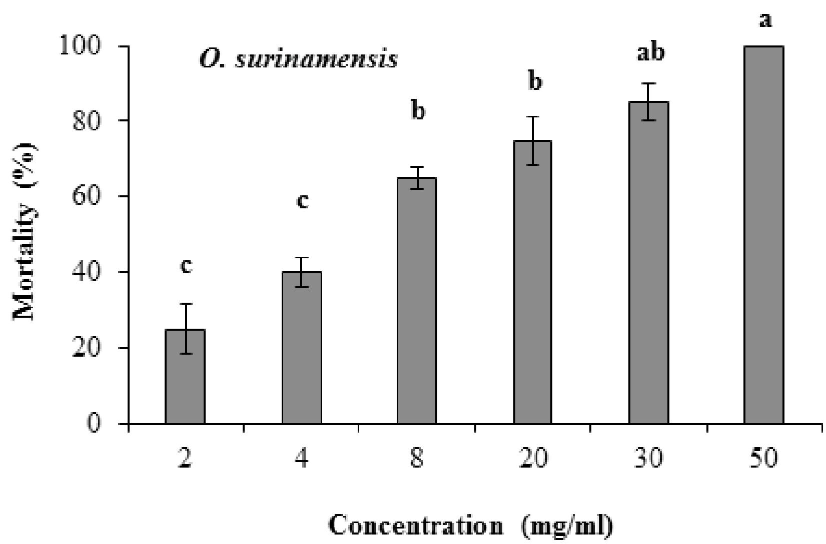
3.1 CONTACT TOXICITY OF MEOH EXTRACT

The results of the experiments showed that the mortality rate of the tested insects increased significantly at 1 % level (Figure 1, $F_{5, 23} = 35.5$, $p < 0.001$ for *O. surinamensis*; $F_{5, 23} = 27.1$, $p < 0.001$ for *T. confusum*). The results of the experiment revealed that there was no significant difference between the mortality rate of two species of the beetles exposed to the leaf extract of *Pistacia khinjuk* for 24 h (Independent sample t-test, t = -1.34, df = 46, p = 0.187).

The LC_{50} value for *T. confusum* calculated 9.32 mg ml^{-1} and the LC_{50} value for *O. surinamensis* calculated 5.47 mg ml^{-1}, 24 hours after application of the extract. As it can be seen in the Table 1, with respect to the overlapping range of 95 % confidence limits of LC_{50}, the lethal effect of *P. khinjuk* leaf extract on *T. confusum* had no significant difference with the *O. surinamensis* (Table 1).

Literature review of published surveys revealed that so far, there has been no report on the contact toxicity of wild pistachio (*Pistacia kinjuk*) extract neither on *T. confusum* nor on *O. surinamensis*, but there were a great number of studies on the extract lethality of similar species of this plant genus on other stored product pests. One example is the lethal effect of *Pistacia lentiscus* extract on the *Tribolium castaneum* pest (Pascual-Villalobos and Robledo, 1998). In this study, combination of esthetic *P. lentiscus* extract with the insect's diet (0.05 %) showed a rate of over 50 % of lethality compared to the control within 2-24 hours after exposure to the extract. The extract of *P. lentiscus*, *P. terebinthus*, and *P. vera* at concentration of 8 µl l^{-1} caused 50, 70 and 85 % mortality of the adult insects of *Sitophilus granarius* after 24 hours, respectively. The essential oils of these plants caused 100 % mortality of the adult insects 96 hours after application of the extract. (Aslan et al., 2004).

However, limited research has been reported the insecticidal activity of *Pistacia khinjuk* on insect pests. It has been reported that the essential oil of *P. khinjuk* leaves had insecticidal activity on Pistachio Psyllid, *Agonoscena pistaciae* (Hemiptera, Psyllidae). Both essential oil of the tested plant and myrcene caused approximately



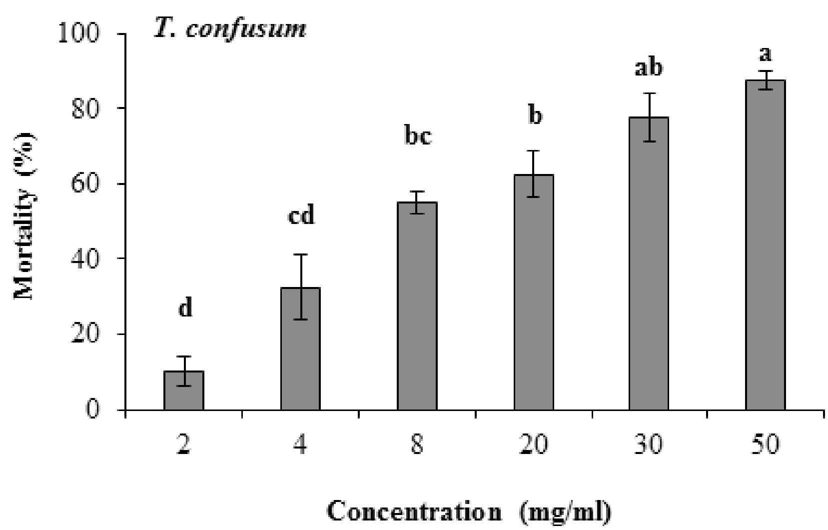


Figure 1: Mortality percentage (Mean ± SE) of *Oryzaephilus surinamensis* and *Tribolium confusum* exposed to MeOH extract of *Pistacia khinjuk* leaves
Means with the different letters above the columns are significantly different ($p < 0.05$) (Tukey post-hoc test after analysis of variance).

equal mortalities of 30-45 % on eggs and 65-90 % on nymphs of the insect (Abolghasemi et al., 2018).

3.2 IDENTIFICATION OF CHEMICAL COMPOUNDS OF PLANT EXTRACT

Based on the results of GC-mass in the extract of *Pistacia khinjuk*, 39 compounds were identified (Figure 2, Table 2). The main compounds of this plant were 5-ethoxy-4-phenyl-2-isopropylphenol (29.02 %), phenyl ethyl alcohol (phenethyl alcohol) (10.78 %), benzyl alcohol (7.8 %), 1,2-benzenesediol (6.67 %), spathulenol (2.07 %), and myrcene (2.03 %) (Table 2).

Existence of the isopropylphenol derivatives as thymol analogs and the main compound in MeOH extract

Table 1: LC_{50} values of MeOH extract of *Pistacia khinjuk* leaves against *Tribolium confusum* and *Oryzaephilus surinamensis* after 24 h

Insects	No.*	95 % CL	χ^2(df)	Probability	LC_{50}	Slope ± SE
T. confusum	240	(6.99-12.16)	3.39(4)	0.494	9.32	1.53 ± 0.195
O. surinamensis	240	(3.98-7.11)	4.76(4)	0.312	5.47	1.64 ± 0.207

* Ten individuals per replicate, four replicates per concentration, 6 concentrations per assay, LC: lethal concentration (mg ml^{-1}), CL: confidence limits

of *P. khinjuk* leaves, are of pharmacological interest because they have insecticidal effect and so contribute to the plant's overall defense mechanism (Park et al., 2017). By using the spray bioassay against adults of *Pochazia shantungensis* Chou and Lu?? (Hemiptera, Ricaniidae), the LC_{50} values of 2-isopropylphenol, 3-isopropylphenol, and 4-isopropylphenol were 85.77, 104.65, and 122.36 mg l^{-1}, respectively (Park et al., 2017).

Phenethyl alcohol and benzyl alcohol, the simplest of the aromatic alcohols, were the next main compounds in the *P. khinjuk* leaves extract. These two compounds are probably the most prestigious aroma chemical in the world of perfumery. Benzyl alcohol used in insect repellant solutions and ointments and as a stabilizer in insecticidal formulations by the agriculture industry (Mookherjee and Wilson, 2000). In insect control, phenethyl alcohol has been considered as a mosquito repellent (Roadhouse, 1953), and its acetate has been used as an ingredient in Japanese beetle bait (Langford and Gilbert, 1949).

In addition, investigation of the plant extract showed the presence of terpenoid compounds such as spathulenol (2.07 %), myrcene (2.03 %), ρ-cymene (1.67 %), apiol (1.61 %), borneol (0.79 %), and pulegone (0.44 %) in extract of *P. khinjuk* that have been well documented as active fumigants, repellents, and insecticides toward stored-product insects (Isman, 2006; Abolghasemi et al., 2018). Availability of terpenoid compounds in essential oil of *P. khinjuk* was previously reported by other researchers (Tahvilian et al., 2017; Abolghasemi et al., 2018).

4 CONCLUSIONS

At present, environmental immunity of a pesticide is of utmost importance. Due to the low risk of plant compounds and their low durability in nature and environmental impacts and their ease of use, these can be a suitable substitute for chemical pesticides in pest management and reduce using insecticides and chemical pesticides. The MeOH extract of *P. khinjuk* leaves showed insecticidal activities against *T. confusum* and *O. surinamensis*. The main components of plant extract were active insecticide compounds including isopropylphenol derivatives as thymol analogs and terpenoid compounds

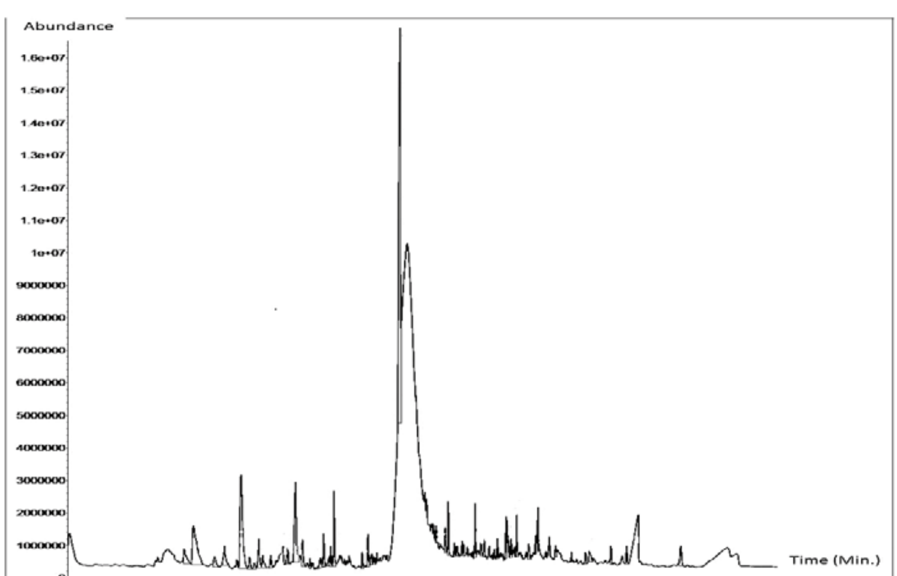


Figure 2: Chromatogram of methanol extract of *Pistacia khinjuk* leaves.

Table 2: Chemical composition of MeOH extract of *Pistacia khinjuk* leaves

Compounds	Composition (%)	Kovats Index
ρ-Cymene	1.67	992
Benzyl alcohol	7.80	1004
Benzene, (2-methyl-1-propenyl)-	1.78	1044
Phenyl ethylalcohol	10.78	1066
Pyranone	1.98	1090
Borneol	0.79	1108
N-methylpyrrole-2-carboxylic acid	0.75	1131
1,2-Benzenediol	6.67	1142
Benzofuran, 2,3-dihydro-	1.50	1152
Pulegone	0.44	1163
Benzeneacetic acid	0.74	1179
5,4'-dimethoxy-2-methylbibenzyl	1.75	1186
Vitispirane	0.89	1193
L-α-bornyl acetate	3.21	1198
4-hydroxy-1-methylproline	0.67	1241
Phenol, 2,6-dimethoxy-	1.66	1250
5-ethoxy-4-phenyl-2-isopropylphenol	29.02	1300
Pyrazole-5-carboxylic acid	0.55	1362
5-methyl-2,3-dihydro-1H-benzazep	0.99	1378
Myrcene	2.03	1383
1,2,3-benzenetriol	0.51	1398
Elemol	0.45	1406
Elemicin	0.51	1409
Spathulenol	2.07	1429
Allyltetramethoxybenzene	0.73	1439
4-(2,4,6-trimethylphenyl)-butan-	0.78	1445
1-(2,3,6-trimethylphenyl)-3-buten-2-one	0.44	1453
Selinenol	0.94	1467
β-selinenol	1.78	1482
Apiol	1.61	1500
3,5-dimethoxybenzoic acid	0.89	1522
Phenol, 2-ethyl-	0.68	1532
2-pyridinamine, 3-methyl-	1.46	1540
Azulon	0.58	1573
Benzoic acid, 2-phenylethyl ester	0.46	1633
Pentadecanoic acid	0.98	1640
Methyl palmitate	0.83	1686
Methyl octyl ether	0.87	1719
Methyl linolenate	1.46	1836
Others	6.29	-

such as spathulenol, myrcene, ρ-cymene, apiol, borneol and pulegone. However, further tests are needed to develop a formulation and to improve the potency and stability of these potential insecticides for practical use.

5 ACKNOWLEDGMENTS

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