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Chemical composition and insecticidal effect of methanol extract of *Capparis spinosa* L. fruits on *Tribolium confusum* Jacquelin du Val, 1863 and *Sitophilus oryzae* (L., 1763) adults

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Abstract: *Tribolium confusum* and *Sitophilus oryzae* are stored product pests found worldwide. Environmental damages, human health issues and the emergence of resistance are driving scientists to seek alternatives to synthetic insecticides for its control. Under this scenario, plant secondary metabolites are being increasingly studied as bioinsecticides because they are renewable, natural, biodegradable, non-persistent in the environment and safe to non-target organism and humans. In this study, the chemical composition and lethal effects of methanol extract of *Capparis spinosa* fruits on *Tribolium confusum* and *Sitophilus oryzae* adults were studied. The LC_{50} of extract on *T. confusum* and *S. oryzae* in contact method were 14.7 and 10.5 mg cm^{-2}, respectively, whereas in the dip method, the LC_{50} value determined 41.3 and 34.3 mg ml^{-1} for *T. confusum* and *S. oryzae*, respectively. The most important identified compounds were the thymol (22.5 %), methyl sulfonyl heptyl isothiocyanate (13.3 %), butyl isothiocyanate (8.1 %), γ-terpinene (6.2%) and iso propyl isothiocyanate (5.8 %). The results confirmed the potential of the *C. spinosa* extract in controlling stored-product insects.

Key words: *Capparis spinosa*; GC-Mass; insecticidal plant extract; stored-product pest; erpenoides

Kemijska sestava in insekticidni učinki metanolnih izvlečkov plodov kaprovca *Capparis spinose* L. na odrasle osebke malega mokarja (*Tribolium confusum* Jacquelin du Val, 1863) in riževega žužka (*Sitophilus oryzae* (L., 1763))

Izvleček: Mali mokar (*Tribolium confusum*) in rižev žužek (*Sitophilus oryzae*) sta po vsem svetu razširjena skladiščna škodljivca. Okoljska škoda, škodljivi vplivi na zdravje ljudi in pojav odpornosti na insecticide vodijo raziskovalce k iskanju alternativ sintetičnim insekticidom za uravnavanje teh škodljivcev. V tem pogledu se povečuje preučevanje sekundarnih metabolitov rastlin, ker so obnovljivi, naravni, razgradljivi, neobstojni, okoljsko varni za neciljne organizme in človeka. V raziskavi so bili preučevani letalni učinki metanolnih izvlečkov plodov kaprovca (*Capparis spinose*) na odrasle osebke malega mokarja (*Tribolium confusum*) in riževega žužka (*Sitophilus oryzae*). LC_{50} vrednosti izvlečkov za malega mokarja in riževega žužka sta bili pri kontaktni metodi 14,7 in 10,5 mg cm^{-2}, pri metodi potapljanja pa 41,3 in 34,3 mg ml^{-1}. Najpomembnejše sestavine, določene v izvlečkih plodov kaprovca so bile timol (22,5 %), metil sulfonil heptil izotiocianat (13,3 %), butil izotiocianat (8,1 %), γ-terpinen (6,2 %) in izopropil izotiocianat (5,8 %). Rezultati so potrdili potencialno uporabo izvlečkov plodov kaprovca za uravnavanje škodljivih skladiščnih žuželk.

Ključne besede: *Capparis spinosa*; masni plinski kromatograf; insekticidni rastlinski izvlečki; skladiščni škodljivci; terpenoidi

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1 INTRODUCTION

Food production and preservation has always been a major concern of human beings. Likewise, the increasing trend of human population has made people experience numerous problems such as hunger and environmental pollution. In fact, over 600 species of beetles, 70 species of butterflies, and about 355 species of mites, which infest stored agricultural products, reduce the quantity and quality of these warehoused crops. As much as 50 %–60 % cereal yields can be lost during the storage stage due to the lack of technical possibilities for their proper harvesting and storage (Kumar and Kalita, 2017). In Iran, in rural areas, mainly due to the traditional and unsuitable conditions of warehousing, this rate arrives to 80 % (Forghani and Marouf, 2015).

Confused flour beetle (*Tribolium confusum* Jacquelin du Val, 1863) and rice weevil *Sithophilus oryzae* (L., 1763) are regarded two of the most important pests of stored cereals around the world, which not only causes significant losses due to feeding, but also, they infect the stored crop with their fecal pellets and larval shells, and reduces the crop's quality greatly (Forghani and Marouf, 2015).

In recent years, a great number of researchers have been attracted to the ways of pest management by using herbal essential oils and plant extracts. The plant extracts have useful insecticide properties such as less effect on natural enemies, no plant-burning, minimal lethality on the vertebrates and rapid degradation in the environment and can be partly replaced by artificial insecticides (Wink, 2018).

Capparis spinosa L. is a plant of the family Cappridaceae, a flowering bush with two bases branching from the base and broadly exfoliated on the ground. The leaves are plain and light green, and flowers are reddish-white with a large number of long flags and the fruit is oval and meaty and bright green, gradually reddish. It grows in different regions of Iran with different soils and has a high tolerance to heavy soil texture (Ahmadi and Saeidi, 2018).

Based on our knowledge, few studies have been reported previously relating to the activity of *C. spinosa* extract against insect pests. However, insecticidal activities of other species of *Capparis* genus were reported in various studies (Hussein et al., 2006; El-Shershaby, 2010; Upadhyay, 2012; Ladhari et al., 2013).

In this research, chemical composition and insecticidal effect of methanol extract of *Capparis spinosa* on two species of storage product pests (confused flour beetle and rice weevils) was investigated.

2 MATERIALS AND METHODS

2.1 INSECTS CULTURES

The two species of insects were obtained from the infected storages located in Zabol, Iran and maintained in the dark in incubators (27 ±1 °C; relative humidity = 65 ± 5%) for propagation. The larvae of *T. confusum* were picked up and reared in glass containers (0.5 l) containing wheat flour mixed with yeast (10:1, w/w). *S. oryzae* were reared on cracked rice seeds. Insects used in all of the experiments were one week old adults.

2.2 PLANT MATERIAL AND EXTRACTIONS

For the extraction of secondary metabolites, fresh fruits of *C. spinosa* were collected from the central part of Hirmand (61° 32' E and 31° 0' N), Sistan and Baluchistan province, Iran, during July and August, 2013. The fruits were dried naturally and grounded into powder. The powder was weighed and extraction was also done by soaking the powdered fruits in methanol solvent (ratio 1:10 w/v) and shaking the mixture on a shaker (350 rpm) for 24 hours at 25 °C.

After the completion of extraction, the liquid extract was filtered through Whatman No.1 filter paper fitted in a Buchner funnel using suction. The extracted plant substances were stored in airtight containers covered with aluminum foils in refrigerator at 4 °C. To determine the dry mass of pure extract at one milliliter of solution, the small parts of solution (5 ml) were collected in three replicate, separately and allowed to dry completely at 100 °C in oven (Ghaemi et al., 2006).

2.3 INVESTIGATION OF LETHAL EFFECT BY CONTACT METHOD (RESIDUAL TOXICITY)

For determination contact toxicity, each experimental unit consisted of a 6 cm Petri dish (with an area of 28.26 cm^2). In order to evaluate the lethal effect of the plant extract on both insect pests, different volumes ranging between 1.5 to 4.8 ml of the plant extract with the concentration of 117 mg ml^{-1} (equivalent to 6.21, 8.69, 11.8, 13.66, 14.91, 16.15, and 19.87 mg cm^{-2}) were uniformly poured by a 1 ml sampler into a Petri dish. In the control samples, various volumes of 1.5 to 4.8 ml of methanol were also poured through a Petri dish. The Petri dish cap was left open for 30 minutes to let the solvent evaporate. Then, ten 1-7- day- old adult insects were put in each dish and the Petri dish was covered by selephon nylon. The Petri's patch was placed in the incubator at 27 ± 1 °C and relative humidity of 65 ± 5 °C in darkness for 24 h (Akbar et al., 2022; Tavares et al., 2021; Seremet et al., 2018).

The number of the dead insects was calculated after 24 hours based on the movement of the legs and tentacles. In case of observation of mortality in the control, the percentage of the lethality in different concentrations of the extract was corrected by Abbott's formula (Abbott, 1925).

2.4 INVESTIGATION OF LETHAL EFFECT BY INSECT-DIP METHOD (TOPICAL TOXICITY)

To evaluate the toxicity of metanol extract in dip method, different concentrations of extracts, including 26.5, 29.5, 35.4, 41.3, 44.2, 47.2 and 59 mg ml^{-1} were prepared, and the insects were immersed in each concentration for 10 seconds. Then, the insects were placed on the filter paper for 5 minutes to dry the bodies. The insects of each replicate were transferred into a 6 cm Petri dish on a filter paper and the Petri dish was closed. In the control, immersion was performed in the methanol solvent for 10 seconds. The number of dead insects was determined after 24 hours based on the movement of the legs and tentacles (Akbar et al., 2022; Tavares et al., 2021; Liu et al., 2016).

In both bioassay method, experiment was conducted on ten 1-7- day- old adult insects in a completely random design with four replicates for each concentration. To determine the 50 % lethal concentration (LC_{50}), five concentrations causing between 20 % and 80 % mortality were used mainly based on the preliminary test results of the contact toxicity of the extract. The experiment was carried out in the same way as the above test, and the number of live and dead insects was counted. The LC_{50} values were calculated using Probit analysis (IBM SPSS V21.0).

2.5 GAS CHROMATOGRAPHY–MASS SPECTROSCOPY OF EXTRACT

Gas chromatography–mass spectroscopy (GC–MS) analyses of metanol extract were carried out on a Hewlett-Packard 5890 gas chromatograph coupled to a HP 5970 mass-selective detector (MSD) using a fused silica ultra performance cross linked methyl silicone column (50 m × 0.2 mm i.d.; 0.25 µm film thickness) at 4 °C/min ramp rate. Helium was the carrier gas at 1 ml/min flow rate. Mass spectra were recorded over 40–500 amu range at 1 scan/s with ionization energy 70 eV and ion source temperature 250 °C. Constituent's identification was made by comparison of their mass spectra with those stored in NIST and Wiley libraries or with mass spectra from the literature (Ravan et al., 2019; Rojht et al., 2012).

2.6 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 $p < 0.05$.

3 RESULTS AND DISCUSSION

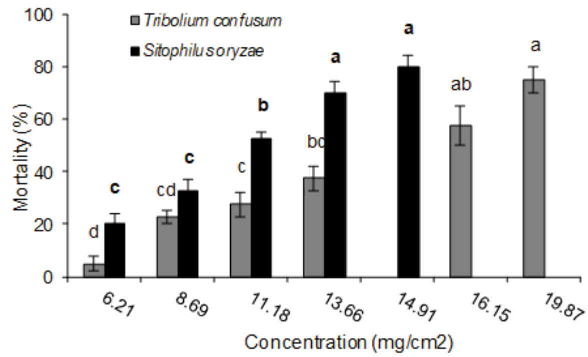
3.1 INSECTICIDIAL ACTIVITIES

The results showed that by increasing the concentration of metanol extract of plant, the mortality rate of the tested insects increased significantly in both bioassay methods (Figure 1, $F_{5, 23} = 27$, $p < 0.001$ for *T. confusum* and $F_{4, 19} = 39.6$, $p < 0.001$ for *S. oryzae* in contact method and $F_{5, 23} = 32.6$, $p < 0.001$ for *T. confusum* and $F_{5, 23} = 108$, $p < 0.001$ for *S. oryzae* in insect-dip method).

The 50 % lethal concentration (LC_{50}) in contact method was determined as 10.5 and 14.7 mg cm^{-2} for *S. oryzae* and *T. confusum*, respectively. LC_{50} was calculated as 34.3 and 41.3 mg ml^{-1}, 24 hours after dipping in the metanol extract of *C. spinosa*, for *S. oryzae* and *T. confusum*, respectively (Table 1). Due to the non-overlapping of the 95 % confidence level of LC_{50}, the toxicity of metanol extract of *C. spinosa* on *S. oryzae* was significantly more than that of *T. confusum* (Table 1).

No study has been reported concerning the contact toxicity of *Capparis spinosa* extract on confused flour beetle and rice weevil. However, insecticidal activities of other species of *Capparis* genus were reported in various studies. The essential oil of another species *Capparis* genus, *Capparis decidua* (Forssk.) Edgew., had an insecticide and repellency effect on *Sitophilus oryzae* and *Rhyzopertha dominica* (Fabricius, 1792) (Upadhyay 2012). Besides, the lethality and repellency of *Capparis aegyptia* Lam. on *Tetranychus urticae* Koch, 1836 (Acari, Tetranychidae) was reported (Hussein et al. 2006).

Moreover, the biological and toxic effects of the leaf extract of *Capparis aegyptia* on *Agrotis segetum* (Denis and Schiffermüller, 1775) (Lepidoptera: Noctuidae) and *A. ipsilon* (Hufnagel, 1766) was studied and according to



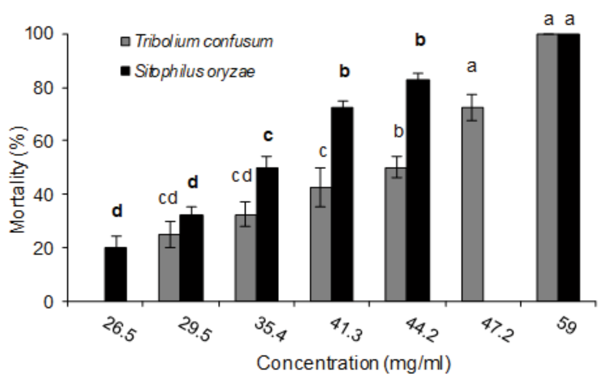


Figure 1: Mortality percentage (Mean ± SE) of *T. confusum* and *S. oryzae* exposed to metanol extract of *C. spinosa* in contact (above) and dip method (below) bioassay
Means with the different letters above the columns for each pest species are significantly different ($p < 0.05$) (Tukey post-hoc test after analysis of variance)

the results, its extract reduced the fertility of adult female insects by 50 % (El-Shershaby 2010).

The metanol extracts of *C. spinosa* leaf in the concentration of 10000 ppm caused 46 % mortality of the third instar larvae of *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera, Noctuidae) after three days of treatment. This rate increased by 100 % after a 7- day treatment. The anti-nutritional index of the metanol extract of this plant in the same concentration on larvae was 49.7 % (Ladhari et al. 2013).

3.2 CHEMICAL COMPOSITION OF PLANT EXTRACT

Gas chromatography-mass spectrometry analysis indicated that there were 33 compounds in the metanol extract of *C. spinosa* fruits, comprising 98.6 % of the total mass, among which the most notably were thymol (22.5 %), methyl sulfonyl heptyl isothiocyanate (13.3 %), butyl isothiocyanate (8.1 %), γ-terpinene (6.22 %) and isopropyl isothiocyanate (5.8 %) (Table 2).

The present result was similar to the other literature reported previously. The main components of *C. spinosa* leaf oil were thymol (26.4 %), isopropyl isothiocyanate (11 %), 2- hexenol (10.2 %) and butyl isothiocyanate (6.3 %). The volatile oils of the fruits composed mainly of isopropyl isothiocyanate (52.2 %) and methyl isothiocyanate (41.6 %) (Afsharypuor et al., 1998).

Existence of the glucosinolates degradation products as isothiocyanate derivatives in metanol extract of *C. spinosa* fruits, are of pharmacological interest because they have insecticidal effect and so contribute to the plant's overall defense mechanism (Bohinc et al., 2014; Gupta et al., 1999).

In addition, investigation of the plant extract showed the presence of terpenoid compounds such as thymol (22.5 %), γ-terpinene (6.2 %), carvacrol (3.1 %), caryophyllene (2.3 %), linalool (1.5 %) α-thujene (1.3 %), β-pinene and 1,8-cineole (1.1 %) in extract of *C. spinosa* fruits that have been well documented as active fumigants, repellents, and insecticides toward stored-product insects (Rojht et al., 2012; Papachristos et al., 2004).

4 CONCLUSIONS

The metanol extracts of *C. spinosa* fruits showed insecticidal activities against *S. oryzae* and *T. confusum* adults. The main components of plant extract have insecticidal properties such as isothiocyanate derivatives and terpenoid compounds. However, further tests are needed

Table 1: LC_{50} values of methanol extract of *C. spinosa* against *T. confusum* and *S. oryzae* after 24 h

Bioassay method	Insect	LC_{50}	95 % CL	P-value	Slope ± SE	x^2(df)
Contact	*T. confusum*	14.7*	(13.4-16.6)	0.67	4.24 ± 0.61	2.33 (4)
	S. oryzae	10.5	(9.5-11.6)	0.49	4.56 ± 0.73	2.73 (3)
Dipping	*T. confusum*	41.3	(38.3-45.8)	0.34	5.47 ± 1.29	3.34 (3)
	S. oryzae	34.3	(32.3-36.4)	0.92	7.68 ± 1.18	0.48 (3)

* Ten individuals per replicate, four replicates per concentration, 5 or 6 concentrations per assay, total number of tested insects were 200 or 240 adults for every test; LC: lethal concentration (mg cm^{-2} in Contact method and mg ml^{-1} in insect-dip method), CL: confidence limits

to develop a formulation and to improve the potency and stability of these potential insecticides for practical use.

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Table 2: Chemical constituents of methanol extract of *Capparis spinosa* fruits

Peak No	Compounds	Rate (%)	Kovats index
1	Methyl isothiocyanate	2.7	741
2	Isopropyl isothiocyanate	5.8	822
3	2-Hexeneal	1.5	858
4	Benzyl aldehyde	1.1	869
5	Methyl sulfonyl heptyl isothiocyanate	13.3	902
6	Methyl furan	2	919
7	Butyl isothiocyanate	8.1	936
8	α-Thujene	1.3	957
9	Comphene	0.9	971
10	β-pinene	1.1	988
11	3-octanone	3.4	1011
12	α-Terpinene	1.5	1020
13	Ocymene	1	1051
14	1,8-cineole	1.1	1080
15	γ-Terpinene	6.2	1106
16	Linalool	1.5	1128
17	Cis-sabinene hydrate	1.8	1175
18	n-Dodecane	2.6	1220
19	Comphor	1	1248
20	Para-menta-6,8,dien-2-ol-acetate	1.3	1270
21	Carvone	1.4	1311
22	Thymol	22.5	1345
23	Caryophyllene	2.3	1382
24	n-tetradecane	1	1460
25	α -farnesene	0.9	1481
26	Carvacrol	3.1	1495
27	Cadinol	1	1520
28	Geranyl acetone	0.8	1588
29	Hexadecanoic acid	2.5	1707
30	Frulic acid	0.8	1731
31	Octa decanoic acid	1.2	1815
32	n-Eicosane	1.1	1878
33	Beta-sesquiphellandrene	0.8	1897
	Total	98.6	

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