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Bioactivity of Marjoram Oil and Powder against the Rice Weevil *Sitophilus oryzae*

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ABSTRACT

The rice weevil *Sitophilus oryzae* is one of the most important pests of stored products which attacks grains causing weight loss and reduced nutrition values. Laboratory experiments were conducted to evaluate the insecticidal activity, as well as the repellent effect of marjoram *Origanum majorana,* oil and powder, against adults of *S. oryzae* compared to malathion insecticide. The chemical composition of marjoram oil was identified by GC-MS analysis. The results indicated that fumigant toxicity of marjoram oil was the most effective against adults of *S. oryzae.* Based on the contact toxicity 7days post-treatment at the highest concentration marjoram oil with LC_{50} values of 0.533, the mortality was achieved 90.0% and reduction in adult F_1 progeny reaching maximums of 81.2%. Also, the mortality in fumigant toxicity of marjoram oil was achieved 88.3% and reduction in adult F_1progeny reaching 86.9%. In addition, all treatments decreased the wheat grain weight loss with increasing the concentration compared to control. The repellent effect of marjoram oil was more effective than its powder especially at the highest concentrations. With regard to the results of the current study it could be suggested that marjoram oil and powder may have potential to be used as an alternative to insecticides in an integrated pest management program for protection stored grains

INTRODUCTION

Storage of grains is part of the post-harvest system through which food material passes on its way from field to consumer. It is generally accepted that 5–15% of the total weight of all cereals, oil seeds, and pulses is lost after harvest [1]. Stored products represent good media for most species of stored product insects. *S. oryzae* (L.) (Rice weevil, Coleoptera: Curculionidae) is one of the most important pests of stored products in the world. Feeding by larvae and adult can reduce weight by as much as 75% [2]. Controlling stored product insect populations is primarily depended upon continued applications of insecticides. However, the implications of these are serious problems of toxic residues, health and environmental hazards, development of insect strain resistant to insecticides and increasing cost of application [3].

Plants products may provide potential alternatives to insect control because its constitutes are considered a rich source of bioactive chemicals, hence they could lead to the development of new classes of safer insect control agents. Also, many natural products are repellent to insects or attractive or antifeedant and oviposition inhibitor [4-8]. Furthermore, many plant powder oils have been explored for their insecticidal properties against stored grain pests [6,9-12]. Marjoram (O. majorana L. Lamiaceae) is an aromatic plant, rich in phenols, flavonoids and terpenoids [13-15]. The plant has been used as a flavouring and herbal spice from time immemorial. Medicinally it is used in cure various human ailments.

Therefore, the purpose of this investigation was to evaluate the insecticidal properties of O. majorana oil and powder compared to malathion insecticide against S. oryzae with respect to adult mortality, progeny reduction, repellency, weight loss and germination of wheat grains. The constituents of the essential oil was also determined by gas chromatography-mass spectrometry (GC/MS) analysis.

MATERIALS AND METHODS

Tested Insect

Rice weevil, (*Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) adults were reared free of insecticidal contamination at 28 ± 2°C, 70 ± 5 R.H. at the laboratory. of Stored Product Pests Research Department, Plant Protection Research Institute, Sakha

Agricultural Research Station. The culture insects were emplaced in glass jars (1000 g) containing 500 g of sterilized wheat grain and 400-500 of *S. oryzae* adults. The mouth of the jars was covered with muslin cloth. Adult insects were left for two weeks for egg laying in the jar and kept again at the untreated conditions in the rearing laboratory under the same conditions. The newly emerging adults (1-2 weeks-old) of *S. oryzae* were used for the current experiment.

The Plant Product

The fresh leaves of marjoram (*Origanum majorana*) (L.) were collected from local market and were dried at room temperature (25-28°C). The dried parts were powdered mechanically by using an electrical blender, then sieved through 300 mesh size. The resulting fine powders were maintained in tightly closed dry bags until used for the experimental work. The marjoram oil was collected from Hashem Brother Company for essential oils and aromatic products.

Chemical Composition of Marjoram Oil

The constituents of essential oil of marjoram (*Origanum majorana*) was analyzed by gas chromatography-mass spectrometry (GC/MS) using HP5890 system with HP column (60-meter × 0.25 millimeter, 0.25 μm film thickness). Detector was Flame Ionization (FID). The mobile phase was nitrogen and hydrogen was the stationary phase. Initial temperature was 60°C and maximum temperature was 250°C. The injector temperature was 240°C according to Renjie et al. [16]. Identification of the oil constituents was achieved by library search on a Wiley 275 L GC-MS data base and by comparing the retention indices and mass fragmentation patterns. All steps of sample preparation, extraction and analysis procedure were carried out in the Analysis Laboratory of Hashem Brothers for Essential Oils and Aromatic Products, Abdel Moneim Riad St., Giza, Egypt.

Contact Toxicity and Progeny Assessment

Wheat grains treatment was carried out to evaluate the efficiency of marjoram oil and powder against adults of *S. oryzae*. The considerable concentrations were: 1.0%, 2.0%, 3.0% and 4% w/w for marjoram oil, 0.5%, 1.5%, 3.0% and 5.0% w/w for marjoram powders. Malathion insecticide at the rate of 0.04, 0.06, 0.08 and 0.1% w/w was used as positive controls. Each concentration of marjoram oil was dissolved in acetone. The oil treatment was carried out by adding 1 mL from each concentration separately above the surface of twenty gm. of wheat grains using a micropipette, mixing well and then left until the solvent evaporated before using them in experiment. The marjoram powder and malathion dust treatments were carried out by mixing each concentration separately with twenty g wheat grains. The untreated grains were served as a control. Each concentration and control was replicated three times. Twenty of newly emerged adults unsexed of S. oryzae (1-2 week-old) were transferred to each jar, covered with muslin cloth and kept under laboratory conditions according to El-Lakwah et al. [17]. Mortality counts were recorded after 3 and 7 days. All results were corrected using Abbott's formula [18]. The alive adults were allowed to complete their life cycle and discarded after twenty days. The newly adults emergence were recorded, it were used to calculate the reduction in *S. oryzae* progeny compared to the control by the following equation:

$$\% \text{ reduction of progeny} = \frac{MEC - MET}{MEC} \times 100$$

MEC: Mean number of adults emerged in control.

MET: Mean number of adults emerged in treatment

Fumigant Toxicity and Progeny Assessment

The fumigant toxicity of marjoram oil against was tested as previously described by Wang et al. [19]. One ml of each concentrations of the oils were 34.7, 69.4, 138.8 and 277.6 μL/L air prepared in acetone on filter papers Whatman No.1, 5 cm diameter pieces. (28.26 cm^2). After complete dryness at room temperature, each filter paper was adhered under surface of the jar cap. Each jar (170 cm^3) contain 10 gm wheat grains and ten unsexed adults of S. oryzae (1-2 weeks old). Three replicates for each treatment and control. The control was treated with acetone only. The treatments were kept in an incubator set 28 ± 1°C and 70 ± 5 R.H. Mortality was recorded 3 and 7 days post-treatment. Mortality counts were recorded according to Abbott's formula after 3 and 7 days. The newly adult emergence was used to calculate the reduction in progeny as mentioned before.

Repellency Test

The repellent effect of marjoram oil, powder and malathion against *S. oryzae* adults was conducted according to Helen [20]. The apparatus consists of a metallic ring (6 cm diameter x 1 cm height) was placed at the center of Petri-dish (12 cm diameter × 2.5 cm height). For marjoram oil, each concentration was dissolved in 1 ml acetone then it was applied on ten grams of grains and mixed well inside the metallic ring. However, each concentration of marjoram powder and malathion, was mixed well with ten grams wheat grains inside the ring. The control treatment was carried out using acetone only. The treatments and control were replicated three times. Twenty unsexed adults (1-2-week-old) of *S. oryzae* were released separately at the center of the ring. The Petri-dishes were covered and were kept at 28 ± 1°C and 70 ± 5 R.H. percentage repellency (PR) values were estimated after 6, 12, 24, 48 and 72 hours according to the following equation:

$$\% \, Repellency \ (PR) = \frac{No. of \ adlts \ outside \ ring}{Total \ no \ of \ adults \ used} \times 100$$

Weight loss in wheat grains

The weight loss of wheat grains due to infestation with *S. oryzae* was determined by Harris and Lindblad three months post-treatment by sieving the insect from the wheat grains [21]. Three replicates were done for each treatment and control. The weight loss of wheat grains was calculated as dry weight loss according to the following equation:

$$\% \, weihgt \ loss = \frac{Initial \ dry \ weight \ of \ grains - dry \ gains \ weight \ after \ 3 \ months}{initial \ dry \ weight \ of \ grains} \times 100$$

Germination Test

Germination test was carried out by Qi and Burkholder, sixty undamaged wheat grains of each treatment three months post-treatment were divided into three replicates, placed in Petri dishes containing cotton layer (instead of filter paper) soaked with tap water and covered with tissue [22]. Grains germination percentage were recorded after four days.

Statistical Analysis

The data were analyzed statistically by using SPSS data processing software (1995) and the different means were compared by Duncan's multiple range test.

RESULTS

Chemical Components of Marjoram Oil

From GC-MS analysis, a total of 11 components from the essential oil *O majorana* were counted 87.8% of the total oil. The major oil constituent oil were Terpinen-4-ol (26.12%), followed by γ-Terpinene (12.96%), *Cis*-3-hexanol (10.12), *α*-terpinene (8.74%), Linolool(8.0%), sabinene (5.58%), *β*-phellandrene (4.11%), α-Terpineol (3.6%), *β*-Caryophellene (3.2%) and Linalyl acetate (2.75%) and *p*-cymene (2.62%).

Contact Toxicity and Progeny Assessment

Results obtained in **Table 1** showed the toxicity of malathion and *O majorana* (oil and powder) against *S. oryzae* adults 3 and 7 days post treatment. The results indicated that, malathion was the most effective treatment against the tested insect followed by oil and powder of marjoram with LC_{50} values of (0.05, 0.646 and 1.14) and (0.036, 0.533and 0.86) after 3, 7daysof treatment, respectively. Marjoram oil was more effective than the powder against *S. oryzae* weevil at all concentration levels. The LC_{50} values of the tested materials were negatively correlated with the time of exposure under all treatments. LC_{50} values after 3 days were higher than this after 7 days in the all treatments. The mortality percentages of *S. oryzae* 3 and 7 days post-treatment were demonstrated in **Table 2**. The mortality percentages increased with increasing the concentration of all treatments and exposure time. The results showed that malathion resulted in the highest mortality compared to marjoram oil and powder. The highest mortality values were recorded 3, 7 days post-treatment for malathion (93.3, 100.0%) at 0.1%w/w followed by marjoram oil (80.0, 90.0%) at the 4.0%w/w, marjoram powder (75.0, 86.7%) at 5.0%w/w. Also, data in obtained that the reduction in adult F_1 progeny was significantly increased with increasing concentrations in all treatments compared to control.

At the higher concentration, malathion was the most effective in suppressing adult emergence followed by marjoram oil and powder. The reduction percentages at the highest concentration were100, 81.2% and 75.7% respectively.

Also, the results in **Table 2** demonstrated that all treatments significantly reduced wheat grains weight loss that decreased with increasing the concentrations. The lowest weight loss was found with the highest concentrations. The percentages of wheat grains weight loss at the highest concentration were: 1.3, 2.3, and 3.4% for malathion, marjoram oil and powder resp. compared to 35.0 for the control.

Table 1. Toxicity of *Origanum majorana* (oil and powder) against adults of rice weevil, *Sitophilus oryzae*, 3 and 7days post-treatment.

Treatment	Days after treatment	LC_{50} %w/w	Confidential limits		Slop value (S.V.)
			Upper	Lower	
Malathion	3 days	0.05	0.0533	0.0467	2.9
	7 days	0.036	0.0512	0.0272	3.1
Marjoram oil	3 days	0.646	0.704	0.479	1.4
	7 days	0.533	0.619	0.446	1.7
Marjoram powder	3 days	1.14	2.424	1.02	1.1
	7 days	0.86	2.62	0.512	1.9

Fumigant Toxicity and Progeny Assessment

Results obtained in **Table 3** showed the fumigant toxicity of marjoram oil against *S. oryzae* adults 3 and 7 days post treatment. The results obtained that, the LC_{50} values were negatively correlated with the time of exposure, it 130.1 and 72.8 µL/L air 3and 7daysof treatment, resp. The mortality of *S. oryzae* 3 and 7 days post-treatment were demonstrated in **Table 4**. The mortality, the reduction in adult F_1 progeny were increased with increasing the concentration and exposure time. The highest mortality values 88.3%was recorded 7 days post- treatment at the highest concentration. At the higher concentration, The reduction in F_1 progeny was 86.9%.

Also, the results in **Table 4** demonstrated that marjoram oil reduced the weight loss that decreased with increasing the concentrations. The lowest loss of grains weight was found with the highest concentrations. The percentage of wheat grains weight loss at the highest concentration was 3.1% compared to 35.0% for the control.

Repellent Effect

Data in **Table 5** show the repellent effect of marjoram oil, powder and malathion against adults of *S. oryzae* at different exposure periods, 6, 12, 24, 48 and 72 hour post-treatment. The results obtained that, all treatments exhibited repellent activity. The repellency percentages increased with increasing the concentrations and exposure periods. In addition, the repellent effect of marjoram oil was more effective than marjoram powder and malathion. At the highest concentration, repellency was ranged between 90-100% for marjoram oil, 75-90% for marjoram powder and 27-36% for malathion.

The Effect on Grain Germination

The effect of malathion, marjoram oil and powder on wheat grains germination three months post treatment was shown in **Table 6**. The results indicated that, malathion has no effect on the germination (99-100%) of wheat grains three months pos-treatment. A slight effect in the germination (97-100%) in marjoram powder compared to the control. Marjoram oil was higher than its powder in reducing the germination of wheat grains (70-90%).

Table 2. Biological activity of Origanum majorana (oil and powder) against adults of rice weevil, Sitophilus oryzae, 3 and 7days post-treatment.

Treatments	concentration %w/w	% mortality after		Mean no. of adult emergence	% Reduction	% wheat grains weight loss
		3days	7days			
Malathion	0.04	53.3	66.7	86.0^g	68.8^e	6.1^d
	0.06	70.0	80.0	52.0^i	81.2^c	3.2^e
	0.08	86.7	95.0	22.0^j	92.0^b	2.1^{ef}
	0.1	93.3	100.0	0.0^k	100.0^i	1.3^f
Marjoram oil	1.0	50.0	56.7	137.0^c	50.4^i	19.0^b
	2.0	66.7	73.3	106.0^e	61.4^g	11.0^c
	3.0	70.0	83.3	73.0^h	73.6^d	5.1^d
	4.0	80.0	90.0	52.0^i	81.2^c	3.4^e
Marjoram powder	0.5	53.3	66.7	141.0^d	48.9^f	17.0^b
	1.5	63.3	76.6	110.0^f	60.1^d	11.0^c
	3.0	70.0	80.0	80.0^i	70.3^b	7.1^d
	5.0	75.0	86.7	67.0^j	75.7^a	2.3^e
Control	0.0	0.0	0.0	276.0^a	0.0	35.0^a

Different superscripted letters indicate values significantly than respective control (P≤ 0.05).
%Reduction of adult progeny, % loss weight three months post-treatment .

Table 3. Fumigant toxicity of *Origanum majorana* oil against adults of rice weevil, *Sitophilus oryzae*, 3 and 7days post-treatment.

Materials	Days after treatment	LC_{50} µL/L air	Confidence limits		Slop value (S.V)
Marjoram oil *Origanum majorana*	3 days	130.1	170.0	90.2	2.3
	7days	72.8	1126.2	315.8	2.7

Table 4. Efficacy of *Origanum majorana* oil on biology adults of rice weevil, *Sitophilus oryzae*, following 3and 7 days exposed to fumigated wheat grains.

Oils	Conc. µL/L air	% Mortality		Mean no. of adults emergence	% reduction	% loss of wheat grains weight
		3 days	7 days			
Marjoram *Origanum majorana*	34.7	41.3	50.0	121.0^d	56.2^f	23.0^b
	69.4	53.3	66.7	88.0^g	68.1^e	12.6^c
	138.8	70.0	81.3	61.0^j	77.9^c	6.1^d
	277.6	75.0	88.3	36.0^m	86.9^a	3.1^e
Control	0.0	0.0	0.0	276.0^a	0.0	35.0^a

Different superscripted letters indicate values significantly than respective control (P≤ 0.05).
%Reduction of adult progeny, % loss weight three months post-treatment.

Table 5. Repellent effect of *Origanum majorana* (oil and powder) against *Sitophilus oryzae* at different exposure times

Treatments	Conc. %w/w	% repellency after indicated hours post treatment				
		6	12	24	48	72
Malathion	0.04	6.6^{j}	13.0^{i}	6.6^{i}	12.0^{j}	6.6^{j}
	0.06	20.0^{i}	17.0^{h}	20.0^{h}	18.0^{i}	16.0^{i}
	0.08	24.0^{h}	25.0^{g}	27.0^{g}	30.0^{h}	30.0^{g}
	0.1	36.0^{g}	36.0^{f}	32.0^{f}	33.0^{g}	27.0^{h}
Marjoram oil	1.0	70.0^{d}	60.0^{f}	60.0^{e}	61.0^{e}	45.0^{g}
	2.0	75.0^{c}	65.0^{g}	60.0^{e}	60.0^{e}	55.0^{e}
	3.0	100.0^{a}	90.0^{b}	90.0^{b}	85.0^{c}	80.0^{c}
	4.0	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	90.0^{b}
Marjoram powder	0.5	60.0^{f}	65.0^{d}	65.0^{d}	50.0^{f}	45.0^{f}
	1.5	66.0^{e}	66.0^{d}	65.0^{d}	65.0^{d}	50.0^{e}
	3.0	75.0^{c}	70.0^{c}	75.0^{c}	60.0^{e}	60.0^{d}
	5.0	85.0^{b}	90.0^{b}	90.0^{b}	75.0^{c}	75.0^{c}
Control	--	3.3^{h}	--	--	3.7^{j}	--

^{a}Values followed by the same letter, within the same column, are not significantly different

Table 6. Effect of malathion and *Origanum majorana* (oil and powder) on germination wheat grains three months post-treatment.

Treatments	concentration %w/w	Germination %
Malathion	0.04	100^{a}
	0.06	100^{a}
	0.08	100^{a}
	0.1	99^{a}
Marjoram oil	1.0	90.0^{c}
	2.0	80.0^{d}
	3.0	75.0^{e}
	4.0	70.0^{f}
Marjoram powder	0.5	100^{a}
	1.5	98.0^{b}
	3.0	97.0^{b}
	5.0	98.0^{b}
Control	0	100.0^{a}

DISCUSSION

The results revealed that marjoram oil and powder had insecticidal activity against the adults of *S. oryzae*. Fumigant toxicity of marjoram oil was more effective than contact toxicity. Marjoram oil exhibits contact toxicity more than its powder. Regarding to the contact toxicity of marjoram oil against the adults of *S. oryzae*, showed that essential oils produced contact toxicity through the insect cuticle and produced fumigant toxicity through the respiratory and digestive systems [23,24].

The surface protectant for wheat grains was correlated with the oil and powder concentrations and exposure periods. Previous researchers studied the insecticidal activity of various plant oils and powders against s. oryzae, on the mortality, reduction in F_1 progeny, fumigant toxicity oils [25-27].

The reduction in adult emergence by fumigant toxicity, it could be due to larval mortality or even reduction in the hatching of the eggs. About the development from eggs to adults, these results confirm by Credland explain the reduction of emergence species of *Callosobruchus* by the ovicidal action of the oils. Oils occlude the funnel, which permit gas exchange with outside creating asphyxiation of the eggs [28]. Daniel and Smith showed that eggs of *Callosobruchus maculatus* had an increase in oxygen uptake between the first to 7th days after oviposition [29]. If oils reduce the oxygen uptake, eggs will starve. Also, the reduction of total emergence of *C. maculatus* by the ovicidal action of oils. Occluding the funnel by which there is gas exchange between eggs and the outside is a physical action [30].

In addition the current results obtained that, plant oil and powder had repellent effect on S. oryzae as reported by Liu et al. [8,31].

Repellency may be related to the chemical substances which cause the insect to make oriented movement away from the source of the substances [32]. Further, the reduction of wheat grain germination by oil was also reported [33,34]. The reductions of germination of treated seeds can be explained by the problem of water absorption by seeds. Water and oil are not miscible. Seeds` coat is covered of oil after treatment thus they cannot absorb enough water which is necessary for the germination. This situation can create also gas exchange problems [30].

The main components of *O. majorana* oil in current study were Terpinen-4-ol, γ-Terpinene, α-terpinene, Linolool, sabinene and β-phellandrene, it is agree with those identified in other studies [35]. Other compounds, such as flavonoid glycosides, tannins, phenolic acids, di-terpenoids and triterpenoids were identified [36]. Raina and Negi reported the identified compounds belonged to monoterpene-hydrocarbons, aldehydes, alcohols and other compounds. The constituents such as linalool, the high toxicity of linalool was reported against the rice weevil S. oryzae and Rhyzopertha dominica [37].

In this study the potent insecticidal activity of O. majorana oil could be attributed to the major constituents individually or combined may have synergistic effect and responsible for the insecticidal activity of marjoram against *S. oryzae* that can penetrate into insects rapidly and interfere with their physiological functions. Although major components of essential oils might be responsible for insecticidal activity, minor compounds also may have activity or show synergistic effect together with major compounds [38].

Furthermore, many plant extracts and essential oils are composed of alkanes, alcohols, aldehydes and terpenoids, especially monoterpenoids, and exhibit fumigant activity they may be toxic by penetrating the insect body via the respiratory system [39]. Previous laboratory evaluations of monoterpenoids on various insect pests have established their biological activity as ovicides, fumigants, and contact toxicants [40]. Additionally, monoterpenoid compounds have been considered as potential pest control agents because they are acutely toxic to insects and possess repellent.

Since the bioactive chemicals are often active against a limited number of species including specific targeted insects, are often biodegradable to non-toxic products, are potentially suitable for use in integrated pest management, and less detrimental environment [41].

CONCLUSION

The efficiency of the plant products as natural insecticides using contact and fumigant toxicity of marjoram against *S. oryzae*, may be help to reduce the negative impact of insecticides because of their low toxicity, no development of resistant of insects and biodegradable. Also many plants locally available which are being traditionally used by some farmers are less costly and easily available. So, the present finding suggests application of marjoram oil and powder as protectants for stored grains as alternatives to the chemical control. Furthermore, research is needed in order to obtain information regarding the practical effectiveness and side effects of plant products.

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