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OIL AND POWDER OF SPEARMINT AS AN ALTERNATIVE TO *SITOPHILUS ORYZAE* CHEMICAL CONTROL OF WHEAT GRAINS

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Abstract: Stored product pests such as *Sitophilus oryzae* are a major concern. Alternative and safe control methods for such pests are needed. Therefore, powder and essential oil of *Mentha viridis* plant were tested under laboratory conditions for their ability to protect wheat grains against the insect *S. oryzae*. The insect was reared and tested on whole wheat grains. The emergence and adult mortality of the insect *S. oryzae* were tested. The efficacy of these plant products was evaluated and compared with malathion. The standard compound for controlling *S. oryzae* has been malathion. The effect of botanical products and malathion on the germination of wheat grains was also evaluated. The chemical components of spearmint oil were also identified using GC-MS analysis. The results showed that, the oil and powder of *M. viridis* were effective against *S. oryzae* with the respect to adults mortality. Also, the oil and powdered products of *M. viridis* significantly reduced the emergence of *S. oryzae* compared to the control treatment. Spearmint oil and powder do not significantly affect wheat grains germination relative to the control treatment. The efficacy of spearmint against the tested insect was due to the presence of a mixture of bioactive compounds. The results suggested that, spearmint oil and powder are promising as alternatives to chemical control used against *S. oryzae* in wheat grains. Also, these spearmint products submit a solution of resistance development by insect due to the presence of a lot of bioactive components rather than the single insecticide.

Key words: oil, powder, malathion, wheat, spearmint, stored

INTRODUCTION

Control of stored-product insect populations is primarily dependent upon continued applications of liquid and gaseous insecticides (White and Leesch 1995). Although these methods are effective, their repeated use for several decades has had its consequences. Repeated applications of liquid and gaseous insecticides has disrupted natural enemies' biological control system and led to outbreaks of insect pests, widespread development of resistance, undesirable effects on non-target organisms, and environmental and human health concerns (Subramanyam and Hagstrum 1995; White and Leesch 1995).

These problems have highlighted the need for the development of new types of selective insect-control alternatives. Plants may provide potential alternatives to currently used insect-control agents because plants constitute considered a rich source of bioactive chemicals (Wink 1993). Since these bioactive chemicals are often active against a limited number of species including specific targeted insects, are often biodegradable to non-toxic products, and are potentially suitable for use in integrated pest management, they could lead to the development of new classes of safer insect-control agents. Much effort has, therefore, been focused on plant-derived materials for potentially useful products as commercial insect-control agents. Little work has been done to manage stored-product insects by using aromatic medicinal plants, despite their excellent pharmacological actions (Tang and Eisenbrand 1992; Namba 1993; Kim 1996).

Essential oils are among the best-known substances tested against insects. These compounds may act as fumigants (Rice and Coats 1994; Regnault-Roger and Hamraoui 1995; Shaaya *et al*. 1997), contact insecticides (Saxena *et al*. 1992; Weaver *et al*. 1994a; Schmidt and Streloke 1994), repellents (Ndungu *et al*. 1995; Plarre *et al*. 1997), and antifeedants (Harwood *et al*. 1990). Essential oil compounds may affect some biological parameters such as growth rate, life span and reproduction (Saxena *et al*. 1992; Regnault-Roger and Hamraoui 1994; Pascual-Villalobos 1996). Most of these substances were tested against insects attacking stored products in order to establish new control practices with lower mammalian toxicity and low persistence in the environment. In fact, management of stored product pests, using substances of natural origin, is nowadays the subject of much research.

The use of plant materials can lead to the identification of new bio-insecticides for the benefit of tropical agriculture. Therefore, the present study attempted to evaluate the efficiency of spearmint oil and powder compared to the recommended compound malathion, against *S. oryzae.* with the respect to adult mortality and progeny reduction, to identify the chemical components of spearmint that may be responsible for its insecticidal activity against *S. oryzae*. Finally, this study investigated the effect of spearmint oil and powder on the germination of wheat grains.

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MATERIALS AND METHODS

Insect rearing

S. oryzae (Egyptian strain) was obtained from the Department of Stored Products Pest Control, Research Institute of Plant Protection, Sakha Kafr-El-Shiekh. This strain was continuously reared free of insecticidal contamination for several years at 30±2°C and 70±5 relative humidity (RH). The cultures were maintained under the same conditions in the Pesticide Department, Faculty of Agriculture, Kafr-El-Shiekh University, Egypt and 200–400 adults from the pervious culture were added in 1,000 ml glass jars containing 400 gram of wheat as a culture medium. The mouth of the jars were covered with muslin cloth. Then, 7–14 d old adults were used for experimental work.

The stored product

Wheat grains were used to culture *S. oryzae* and to evaluate the efficacy of spearmint oil and powder against the same insect as well. Wheat grains were stored in airtight tins until required for experiments. The experiments were carried out in a room kept at a constant temperature of 25°C and 70% relative humidity (RH).

The plant products

Powdered and oil products of spearmint plant leaves were obtained from a local supermarket.

Effect of spearmint plant (oil and powdered) and malathion on the emergence of *S. oryzae*

Spearmint oil, at concentration levels of 100, 200 and 300 ppm was used to evaluate its efficiency against *S. oryzae*. A concentration levels of 0.5, 1 and 1.5% w/w (powder/wheat grains) were used to evaluate the efficiency of spearmint powder against *S. oryzae*. Malathion was used as recommended compound against *S. oryzae* at concentration levels of 0.3, 0.6 and 1.2 ppm. Each concentration was applied in three replicates, and in each replicate there were 20 gm of wheat grains. For, malathion and spearmint oil, the treatment was carried out by adding 1 ml of each concentration to the wheat grains, mixing well and then spreading the treated wheat grains on top of plastic sheets to dry for 90 min before using them in the experiment. However, for spearmint powder, the treatment was carried out by mixing the powder with wheat grains at the selected concentration levels. The wheat grains were shaken thoroughly to ensure uniform coverage by the different treatments. The control treatment was carried out using water only, and was replicated three times. After that, 10 adults males and females of *S. oryzae* were transferred to the treated wheat grains which putted in a 85x45 mm plastic jar. and kept at 30±2°C and 70±5 RH. relative humidity, according to the method described by Kestenholz *et al*. (2007). The insects which emerged from the hatched eggs were recorded after 6 weeks. These insects were used to calculate the reduction percentages in *S. oryzae* progeny from the use of spearmint oil and powder as well as malathion, compared to the control as shown in equation:

$$\%\ \text{reduction} = \frac{\text{MNEC} - \text{MNET}}{\text{MNEC}} \times 100$$

MNEC – mean No. of those which emerged in the control
MNET – No. of those which emerged in the treatment

Efficiency of spearmint plant (oil and powder) and malathion on *S. oryzae* by mean mortality

Wheat grains were treated with spearmint oil, powder and malathion to evaluate their effects on the adults mortality of *S. oryzae,* at concentration levels mentioned before. The treatment of wheat grains with spearmint oil, powder and malathion was carried out, as mentioned before. Each treatment was applied in three replicates, and each replicate consisted of 20 gm wheat grains infested with 10 *S. oryzae* adults. The wheat grains were shaken thoroughly to ensure uniform coverage of each treatment and then kept under 30±2°C and 70±5 RH relative humidity in a 85x45 mm plastic jar, according to the method described by Kestenholz *et al*. (2007). The control treatment was carried out using water only, and replicated three times. The glass jars were covered with cotton cloth held on with rubber bands. The number of dead insects in each jar was counted after one and two weeks of treatment. The percentage of insect mortality was calculated and then corrected using the Abbott formula (Abbott 1925).

Chemical composition of spearmint oil

GC/MS analysis was conducted on a HP 6 890 GC system coupled with a 5,973 network mass selective detector with a capillary column of HP-5MS (60 m x 0.25 mm, film thickness 0.25 m). The oven temperature program was turned on at 40°C, held for 1 min then raised up to 230°C at a rate of 3°C/min held for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min, with a split ratio equal to 1/50. The detector and injector temperatures were 250 and 230°C, respectively. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library, and National Institute of Standards and Technology (NIST) (Mahboubi and Haghi 2008). The samples were analyzed in the central laboratory for pesticides, Agriculture Research Centre, Egypt.

Effect of the tested products on the germination of wheat grains

The effect of spearmint oil, powder and malathion on the germination of wheat grains was evaluated. Wheat grains were treated with spearmint oil, powder and malathion at the different concentration levels mentioned before. After one month of treatment with the tested products, 20 wheat grains were transferred to cotton bed saturated with water in Petri dishes. Germination percentages were then recorded by counting the number of germinated relative to the total wheat grains.

Statistical treatment

Data were analyzed statistically by the analysis of variance test and the different means were compared by Duncan's multiple range test.

RESULTS

Effect of spearmint plant (oil and powder) and malathion on the emergence of *S. oryzae*

The number of emerged *S. oryzae* adults were significantly decreased with all treatments (mint oil, powder and malathion) compared to the control, as shown in table 1. Increasing the concentration level of all tested treatments (concentration dependent) reduced the emerging of *S. oryzae* even more. Among the tested treatments, malathion and spearmint oil were the most effective treatment on progeny of *S. oryzae,* followed by spearmint powder with the reduction percentages 78.8, 86 and 82.5%, respectively. Spearmint oil was more effective than spearmint powder against the progeny of the *S. oryzae* weevil, at all concentration levels.

Efficiency of spearmint plant (oil and powder) and malathion on *S. oryzae* determined by mortality values

The mortality percentages of *S. oryzae* beetle after treatment with malathion as well as powder and oil of the spearmint plant were shown in table 2. The results indicated that, malathion was the most effective treatment against the tested insect followed by oil and powder of spearmint plant with LC_{50} values of 1.27, 239 and 304 after one week and 0.74, 158 and 204 ppm after two weeks of treatment, respectively. Spearmint oil was more effective than spearmint powder against the *S. oryzae* beetle at all concentration levels. The LC_{50} values of the tested treatments against *S. oryzae* adults were positively correlated with the time of exposure under all treatments, since the LC_{50} values in the first week were higher than the second week in all treatments. The LC_{50} values of spearmint oil were higher than that of spearmint powder after both one and two weeks treatments.

Chemical components of spearmint oil

The GC-MS analysis of the spearmint oil yielded thirteen main volatile compounds representing 96% of the oil content. The compounds were identified as alpha pinene, beta pinene, cineole, limonene, methone, menthal, pulegone, bronyl acetate, menthyl, caryophyllene, eicosane, heneicosane and docosane, as shown in table 3. The identified compounds belonged to eldyhydes, esters, alcohols and fatty acids. The GC-MS separated compounds were identified from the recorded mass spectra, by comparison with the mass spectra from the Wiley library.

Effect of the tested treatments on the germination of wheat grains

The germination percentages of wheat grains after one month of treatment with malathion, powder, and oil of spearmint plant were shown in table 4. The results indicated a slight effect in the germination of wheat grains in all treatments compared to the control. Spearmint oil

Table 1. Effect of *M. viridis* oil, powder and malathion on the emergence of *S. oryzae*

Treatment	Concentration level	No. of emerged adults after 6 weeks of treatment	Reduction [%]
Mentha viridis oil	100 ppm	85 c	58.7 c
	200 ppm	42 f	79.6 d
	300 ppm	28 h	86 e
Mentha viridis dust	0.5%	110 b	46.6 d
	1%	52 d	74.7 d
	1.5%	36 g	82.5 d
Malathion	0.3 ppm	47 e	77 d
	0.6 ppm	36 g	82.5 d
	1.2 ppm	25 i	87.8 e
Control	0.00	206 a	0.00 a

Table 2. Effect of *M. viridis* oil, powder and malathion on adult mortality of *S. oryzae* after one and two weeks of treatment

Treatment	LC_{50}	Upper	Lower
After one week			
Malathion	1.278	1.65	0.99
Spearmint oil	239	334	195
Spearmint powder	304	402	234
After two weeks			
Malathion	0.7418	1.69	0.19
Spearmint oil	158	204	113
Spearmint powder	204	248	160

Table 3. The main constituents of spearmint oil

Identified compounds	Retention time	Area [%]	Main fragments ions
1-Alpha pinene	9.64	8.95	93-77
2-Beta pinene	11.6	2.59	93-69-77
3-1,8 Cineole	14.75	17.32	93-68
4-Limonene	14.80	18.20	108-81
5-Menthone	23.01	16.11	112-69-139
6-Menthal	23.5	21.81	112-69-139
7-Pulegone	28.31	1.04	81-152-67
8-IsobBronyl acetate	31.21	5.16	136-121-95
9-Menthyl acetate	31.81	5.79	95-138-81
10-Caryophyllene	39.10	0.41	133-161-93
11-Eicosane	70.85	1.04	57-71-85
12-Heneicosane	75.15	0.61	57-71-85

Table 4. Effect of *M. viridis* oil, powder and malathion on germination of Wheat grains after one month of treatment.

Treatments	Concentration level	Germination [%]
Mentha viridis oil	100 ppm	90 d
	200 ppm	85 e
	300 ppm	72.5 f
Mentha viridis dust	0.5%	100 a
	1%	97.5 b
	1.5%	95 c
Malathion	0.3 ppm	98.5 ab
	0.6 ppm	96.25 cb
	1.2 ppm	95 c
Control	0.00	100 a

was the highest treatment that reduced the germination percentage of wheat grains followed by spearmint powder and malathion, respectively. The inhibition in the germination percentage of wheat grains was positively correlated with the concentration level of all the tested treatments since there was significant difference among the concentration levels of each treatment.

DISCUSSION

The results of the present study implied that the tested spearmint oil and powder were effective against *S. oryzae* in stored wheat with respect to progeny and adult mortality. The efficacy of spearmint oil and powder against the *S. oryzae* weevil in stored wheat, with respect to progeny and adult morality, have been reported by many researchers (Varma and Dubey 2001; Papachristos and Stamopoulos 2002).

The study has also shown that spearmint oil was significantly more effective against *S. oryzae* with respect to adult mortality and emergence, than spearmint powder. It has been reported that one of the main mechanisms of plant oil action is their ability to penetrate the chorion of bruchid eggs via the micropyle and cause the death of developing embryos through sphyxiation (Credland 1992). This may be one of the reasons why spearmint oil caused considerably higher reductions of adult emergence than spearmint powder. Also, the higher efficacy of spearmint oil than powder against *S. oryzae* may be due to the oil's higher ability to penetrate into the insect's body. Reduction of adults emergence may have been achieved through a combination of high mortality of eggs and larvae immediately after eclosion and contact with spearmint oil (Lale and Abdulrahman 1999).

Thus, the tested spearmint oil and powder, especially the oil, showed high efficacy against *S. oryzae* in stored wheat, with respect to progeny and mortality of the adults, that was the same as or near the recommended chemical compound malathion. This suggests that these botanical products may be used as alternatives to chemical control of such stored product pests as *S. oryzae*. This approach can contribute to reducing the amount of applied pesticides and subsequently minimize hazards to the environment and human health. This approach also overcomes the problem of chemical pesticide resistance development by the insect.

The identified compounds by GC-MS in this study agree with those identified in other studies (Franzios *et al*. 1997; Gherman *et al*. 2000; Chauhan *et al*. 2009). Among the identified compounds, Alpha pinene (8.95%), 1,8 Cineole (17.32%), Limonene (18.2%), Menthal (21.81%) were detected in higher percentages compared to other detected compounds. These compounds may be responsible for the insecticidal activity of spearmint oil and powder recorded against the tested insecticide in this study (Franzios *et al*. 1997; Gherman *et al*. 2000; Lee *et al*. 2000). Although, the insecticidal activity of spearmint oil and powder is attributed mainly to its major compounds mentioned earlier, the synergistic or antagonistic effect of one compound in the mixture has to be considered.

Each of the components has its own contribution to the biological activity of the extract. For example, pulegone was detected in a low percentage but it is known to possess diverse biological properties, such as insect repellant (Gordon *et al*. 1982).

The mode of action of bioactive natural monoterpinoids (hydrocarbons, alcohols and ketones) from spearmint oils may be due to inhibition of acetylcholinestearase (Miyazawa *et al*. 1997; Lee *et al*. 2000). Lee *et al*. (2000) reported that, 1,8-cineole was the most potent inhibitor of eel AChE among the monoterpenes tested. This inhibition may be a mode of action for essential oil and monoterpene fumigation toxicity against stored grain insect pests as well. Also, the insecticidal mode of action of the compounds in spearmint may be largely attributable to fumigant action. The compounds may prove toxic when penetrating the insect body via the respiratory system (Shaaya *et al*. 1997; Park *et al*. 2003).

The essential oils as pest control agents have two positive things in their favor: the first, is that their natural origin makes them safer for people and the environment, and the second, is that they are considered low risk for resistance development by stored product insects. It is believed that it is difficult for *S. oryzae* to develop resistance to such a mixture of components with, apparently, different mechanisms of pesticidal activity. This study presents the first step in the investigation of the use of these effective botanical extracts for control of different pests as an alternative to chemical control. The use of botanical extracts to control pests will help to reduce environmental pollution and the adverse effect on human health resulting from pesticide use.

CONCLUSIONS

The insecticidal activity of spearmint products against *S. oryzae* indicate the potential for using spearmint as a natural source of insecticidal material. Insecticidal activity was confirmed in spearmint oil and powder, although the results showed that oil and powder of spearmint varied in their effectiveness against the *S. oryzae* insect. The ability of using botanical products as alternatives to the chemical control of *S. oryzae* is possible. This approach can help reduce the amount of insecticides applied, and subsequently minimize its hazards to the environment and human health. Work which identify a new insecticidal compounds should continue on other invasive species. Also, field trials should be conducted with promising extracts or compounds to evaluate its efficiency under natural conditions. Further research is needed in order to obtain information regarding the practical effectiveness and lack of side effects of essential oils, in protecting stored products.

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POLISH SUMMARY

OLEJEK I PROSZEK ROŚLINNY Z MIĘTY JAKO ALTERNATYWA CHEMICZNEGO ZWALCZANIA *SITOPHILUS ORYZAE* NA ZIARNIE PSZENICY

Szkodniki magazynowe takie jak *Sitophilus oryzae* są obiektem szczególnego zainteresowania. Wzrasta zapotrzebowanie na wykorzystywanie alternatywnych bezpieczniejszych metod zwalczania szkodników magazynowych. Przedmiotem badań była ocena przydatności olejku eterycznego oraz proszku roślinnego z mięty zielonej *Mentha viridis* pod kątem przydatności do ochrony ziarna pszenicy przed wołkiem ryżowym *S. oryzae*. Badania wykonano w warunkach laboratoryjnych. Hodowlę szkodnika prowadzono na całkowitych ziarniakach pszenicy. Przy ocenie skuteczności testowanych komponentów pod uwagę brano wyląg potomstwa i śmiertelność dorosłych osobników. Jako standardowy preparat zastosowano malathion. Przedmiotem oceny był również wpływ zastosowanych preparatów pochodzenia botanicznego oraz insektycydu standardowego malathion na kiełkowanie ziarna przenicy. Chemiczny skład olejku eterycznego z mięty określono przy pomocy techniki GC-MS. Wyniki prowadzonych testów wykazały, że zarówno olejek eteryczny jak sproszkowany materiał roślinny *M. viridis* okazały się skuteczne biorąc pod uwagę śmiertelność osobników dorosłych. Zastosowany olejek i proszek z mięty *M. viridis* istotnie ograniczały wyląg potomstwa w porównaniu do kontroli i nie wpływały istotnie na kiełkowanie ziarna pszenicy. Skuteczność testowanych komponentów z mięty przeciwko szkodnikowi była warunkowana obecnością składników aktywnych biologicznie. Przedstawione wyniki badań dowodzą, że wykorzystany olejek eteryczny i proszek roślinny z mięty można uznać za obiecującą alternatywę dla chemicznego zwalczania *S. oryzae* na ziarnie pszenicy. Testowane preparaty z mięty dzięki zawartości rożnych składników aktywnych biologicznie mogą okazać się bardzo pomocne przy rozwiązywaniu problemu uodparniania się szkodnika na chemiczne środki ochrony roślin.