

EFFECT OF SOME MEDICINAL PLANT OILS ON BIOCHEMICAL ACTIVITIES OF *Sitophilus zeamais* (Motschulsky).

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ABSTRACT

The toxic and biochemical effects of six medicinal plant oils (*Peppermint, Marjorana, Lavander, Sweet basil, Spearmint and Artemisia*) on *Sitophilus zeamais* were investigated in the laboratory. Marjorana hortensis oil was more toxic to the insect than the other tested oils. The LC50 treatment of all test oils caused depletion in total proteins, lipids and carbohydrates. Disturbance (increase or decrease) were found in amylase, transaminases (GOT & GPT) and acetylcholine esterase enzymes activities of the treated weevils

Keywords : *Sitophilus zeamais*, medicinal Plant oils, biochemical effects.

INTRODUCTION

Certain insect pests cause heavy losses to stored grain, although food grains are commonly protected by insecticide or fumigants, such practices may pose health risk. Toxic residues and the selection of insecticides resistant pest strains are additional problems associated with use of insecticides in grain protection.

Thus, there is an urgent need to develop safe alternatives that have the potential to replace the toxic fumigants, yet are simple and convenient to use many spices, herbs and their extracts are known to possess insecticidal activity, which are frequently present in the essential oil fraction (Brattsten, 1988, Schmidt *et al.*, 1991 and Shaaya *et al.*, 1991) most of the essential oil constituents are monoterpenoids, which are secondary plant chemicals and considered to be of little metabolic importance (Shaaya *et al.*, 1997).

The toxicity of a large number of essential oils and their constituents have been evaluated against number of stored product insects such as *Pogostemon heyneanus*, *Ocimum basilicum* and *Eucalyptus* showed insecticidal activity against *S. oryzae*, *Stegobium paniceum*, *T. castaneum* and *Callosobruchus chinensis* (Desphande *et al.*, 1974, Desphande and Tipnis, 1977). Toxic effects of terpenoids, d-limonene, linalool and terpinene were observed on Coleoptera damaging post-harvest products (Karra and Coats, 1988). Fumigant toxic activity and reproductive inhibition induced by a number of essential oils against the bean weevil *Aconthoscelides obtectus* (say) and the moth *Sitotroga cerealella* (Regnault-Roger and Hamroui, 1995).

Therefore the present investigation was undertaken to evaluate the toxic effect of six essential oils derived from medicinal plants against the adult of *Sitophilus zeamais*. The effect of these oils at LC50 doses on the activities of several enzymes (GOT & GPT, Amylase and acetyl choline esterase) and levels of the main metabolic of total proteins, lipids and carbohydrates in the total body homogenates of insect were determined.

MATERIALS AND METHODS

1- Medicinal plant oils

Five essential oils extracted from plants belong to family Labiatae: Peppermint (*Mentha piperita*), Spearmint (*Mentha viridis*), Marjorana (*Marjorana hortensis*), Sweet basil (*Ocimum basilicum*), Lavander (*Lavandula officinalis*) and another oil extracted from *Artemisia herba alba* plant (Family: Compositae) were tested on adult of *S. zeamais*.

2- Preparation of essential oils

Samples of six medical plants were hydro-distilled for 3 hrs. using a cleavenger type apparatus. The oils were separated and dried over anhydrous sodium sulphate and kept in deep freezer (Momen *et al.* 2001).

3-Bioassay

3.1- Tested insects

The tested insects were laboratory strain of *Sitophilus zeamais* which was reared on zea grains under rearing room conditions away from insecticidal contamination at 28C° with 60 ± 10 % R.H. Experiments were conducted under the same conditions.

3.2- Insecticidal activity of tested oils

Seven grams of zea grains in Petri-dishes were mixed with 0.05, 0.025, 0.018, 0.010, 0.008 and 0.005 gram of tested oils, which were dissolving in 1ml acetone. Control were treated with acetone only. Every treatment was replicated three times. Twenty adults insects were introduced into each dish and the number of dead insects was recorded after 7 days. Percentage of insect mortality was calculated for each dish using the formula proposed by Abbot (1925). Percentage of mortality after 7 days was transformed into probits and the values obtained were regressed on logarithm of the concentrations of the essential oils to obtain the lethal concentration for 50% of the tested insects.

3.3-Biochemical studies

About 100 mg of the control and LC50 treated adult of *S. zeamais* in each replicate were crushed in 5ml of 0.15M NaCl solution (at 4C°) with the help of a motor driven teflon-glass homogenizer was centrifuged at 4.900 g for 10 min. at (4C°). the supernatant thus obtained was used for the estimation of Acetylcholine esterase, transaminases (Gop and Gpt) and Amylase enzymes activities. The main metabolites (total proteins, total lipids, and total carbohydrates) were determined in total homogenate.

3.3.1- Determination of Acetylcholine esterase (Ach.E)

Acetylcholine esterase (Ach-E) was measured according to the method described by Simpson *et al.*; (1964) using acetylcholine bromide (Ach-Br).

3.3.2- Determination of Transaminase activities (GOT and GPT)

The level of both transaminases (GOT and GPT) were determined colorimetrically according to Reitman and Franke (1959).

3.3.3- Determination of Amylase activity

The method was based on the digestion of starch by amylase according to the method described by Ishaaya and Swiriski (1976). The free aldehydic group of glucose formed after starch digestion was determined using 3,5 dinitro salicylic acid reagent.

3.3.4- Determination of the main metabolites

The main metabolites: total proteins, lipids and carbohydrates were determined in the total body homogenate, according to Bradford (1976), Singh and Sinha (1977) and Knight *et al.*, (1972) respectively.

4-Statistical analysis

The data were subjected to statistical analysis according to the equation of Dixon and Massay (1957).

RESULTS AND DISCUSSION

1- Toxicological effects of plant oils

Marjorana hortensis had superior toxic effect (LC50 : 10.5mg/ml) against *S. zeamais* adults followed by *Mentha viridis* (12.96mg/ml), *Artemisia herba alba* (15.41mg/ml), *Lavandula officinalis* (17.24mg/ml), *Ocimum basilicum* (19.46mg/ml), while *Mentha piperita* oil was the lowest effective one (25.49mg/ml).

2- Effect of plant oils on total proteins, lipids and carbohydrates of body homogenate:

Total proteins

Effect of plant essential oils on total proteins, lipids and carbohydrates of *S. zeamais* adults is shown in table (1). All plant oils adversely affected protein levels on the adult of *S. zeamais*. The percentage of decrease in protein levels comparing with the untreated check were 21.84, 21.32, 17.32, 16.36, 11.84 and 9.92% for LC50 treatment of *Ocimum basilicum*, *Mentha piperita*, *Marjorana hortensis*, *Mentha viridis*, *Artemisia herba alba* and *Lavandula officinalis* oils respectively.

The above results showed that, LC50 of the tested essential oils caused highly significant decrease in total protein level of tested insect. These results clearly indicated that the highest percentage of reduction in protein level were 21.84 and 21.32% for *Ocimum basilicum* and *Mentha piperita*, respectively.

Data in table (1) indicated that the order of protein reduction were *Ocimum basilicum* > *Mentha piperita* > *Marjorana hortensis* > *Mentha viridis* > *Artemisia herba alba* > *Lavandula officinalis*.

From the obtained data it can be concluded that, treatment of adult of *S. zeamais* with the tested essential oils resulted in considerable inhibition of protein synthesis. This decreasing in total protein due to treatment with the essential oils may be attributed to that, the protein of treated insects make protein binding complex with such foreign compounds as previously recorded by Mostafa (1993).

Similar results have been also reached by El-Kordy *et al.*, (1994). Also Huang *et al.* (2004) found that azadirachtin significantly influenced protein level.

Total lipids

Data in table (1) showed that the highest reduction of lipid percentages was 23.61% for *Artemisia herba alba* followed by *Mentha viridis* (21.25%), *Mentha piperita* (16.14%) and *Margorana hortensis* (14.88). However

Ocimum basilicum and *Lavandula officinalis* caused increasing in total lipid of *S. zeamais* (15.51% and 3.91%) compare with the check, respectively.

Total carbohydrates

Feeding the adult of *S. zeamais* on zea grain treated with the LC50 of the tested oils caused significant decrease in the level of carbohydrates as shown in table (1). The highest percentage of reduction of carbohydrates was (65.82%) by *Artemisia herba alba* followed by *Mentha piperita* (64.08%), *Marjorana hortensis* (58.52%), *Lavandula officinalis* (50.68%), *Mentha virids* (23.70%) and *Ocimum basilicum* (23.15%) comparing with the control.

The depletion of carbohydrate in the total body homogenate however may be due to their increased utilization in response to the hyperactivity caused by essential oils treatment. Hence the results of the present study are in accordance with the finding of Singh (1986).

Also Saleem, et al; (1998), found that the adult of *Tribolium eastaneum* which treated with the LC25 of Ripcord showed utilization of carbohydrates, proteins and lipids in the given order, perhaps to produce extra energy to combat insecticidal stress.

Table (1): The homogenate contents of the treated adult of *S. zeamais* with the plant essential oils and the rate of change.

Plant essential oil	Homogenate contents					
	Total protein	% of change	Total lipids	% of change	Total carbohydrate	% of change
<i>Mentha piperita</i>	10.63 ± 0.36	-21.32	14.6 ± 1.23	-16.14	3.94 ± 0.42	-64.08
<i>Mentha virids</i>	11.30 ± 0.41	-16.36	13.71 ± 1.81	-21.25	8.37 ± 0.17	-23.70
<i>Marjorana hortensis</i>	11.17 ± 0.44	-17.32	14.82 ± 1.72	-14.88	4.547 ± 0.53	-58.52
<i>Ocimum basilicum</i>	10.56 ± 0.38	-21.84	20.11 ± 1.16	+15.51	8.43 ± 0.24	-23.15
<i>Lavandula officinails</i>	12.17 ± 0.21	-9.92	18.09 ± 1.11	+3.91	5.41 ± 0.33	-50.68
<i>Artemisea herba alba</i>	11.91 ± 0.50	-11.84	13.3 ± 1.50	-23.61	3.75 ± 0.67	-65.82
Check	13.51 ± 0.56	-	17.41 ± 1.40	-	10.967 ± 0.61	

(-) decrease (+) increase

3- Effect of the plant oils on enzymatic activities in *S. zeamais*

The effect of LC50 of six tested essential oils on Amylase, GOT, GPT and ACh.E are shown in table (2).

Amylase activity:

The results indicated that, Amylase activity was reduced with all tested plant oils against *S. zeamais* comparing with control. The highest reduction in Amylase activity was present in *Mentha piperita* treatment (57.33%) followed by *Artemesia herba alba* (50.69%), *Lavandula officinalis* (50.31%), *Ocimum basilicum* (39.62%), *Mentha virids* (22.19%) and *Marjcrana hortensis* (22.03%) treatment.

Glutamic oxaloacetic Transaminase (GOT)

The effect of the LC50 of the essential oils on the activities of glutamic oxaloacetic Transaminase (GOT) of the adult of *S. zeamais* is shown in table (2).

Table (2): Activities GOT, GPT transaminases, Amylase and Acetylcholine-esterase enzymes of *S. zeamais* adults treated with the LC₅₀ of plant essential oils.

Enzymes Plant essential oil	GOT		GPT		amylase		Acetyl choline esterase	
	Activity	% of change	Activity	% of change	Activity	% of change	Activity	% of change
<i>Mentha piperita</i>	18.07± 0.07	-24.18	3.08± 0.18	-5.23	572.87± 16.73	-57.33	1292.90± 78.70	+16.69
<i>Mentha virids</i>	27.44± 0.04	+15.15	3.40± 0.21	+4.62	1044.75± 29.68	-22.19	1180.84± 32.64	+6.6
<i>Marjorana hortensis</i>	21.48± 0.35	-9.87	3.18± 1.00	-2.15	1046.85± 11.34	-22.03	1006.06± 48.97	-9.2
<i>Ocimum basilicum</i>	20.39± 0.19	-14.44	3.30± 0.03	+1.54	810.73± 10.63	-39.62	1280.79± 35.66	+15.59
<i>Lavandula officinalis</i>	18.96± 0.16	-20.44	3.43± 0.09	+5.54	667.22± 20.58	-50.31	1144.16± 43.40	+3.26
<i>Artemisea herba alba</i>	25.03± 0.12	+5.04	3.57± 0.13	+9.85	662.04± 10.63	-50.69	1213.96± 20.06	+9.56
Check	23.83± 0.38	-----	3.25± 0.16	-----	1342.66± 36.4	-----	1108.03± 79.01	---

(-) decrease (+) increase

Data clearly indicated that *Mentha virids* and *Artemesia herba alba* increased the activity of GOT (15.15% and 5.04%) compared with the check, while there was a significant reduction in the GOT activity for *Mentha Piperita* (24.17%), *Lavandula officinalis* (20.44%), *Ocimum basilicum* (14.44%) and *Marjorana hortensis* (9.86%)

Glutamic pyruvic Transaminase (GPT)

Data in table (2) clearly indicated that, there was increased in Glutamic pyruvic Transaminase (GPT) activity for *Artemesia herba alba* (9.85%), *Lavandula officinalis* (5.54%), *Mentha virids* (4.62%) and *Ocimum basilicum* (1.54%) treatment, while *Mentha Piperita* and *Marjorana hortensis* caused a significant reduction in (GPT) activity (5.23% and 2.15%) compared with the check .

Acetylcholine esterase activity (Ach.E)

The effect of six essential oils on Ach.E activity in *S. zeamais* are shown in table (2). Data clearly indicated that all tested oils increased Ach.E activity except *Marjorana hortensis* which reduced the enzyme activity (9.2%) compared with the check. The order of increased Ach.E activity were *Mentha piperita* (16.69%), *Ocimum basilicum* (15.59%), *Artemesia herba alba* (9.56%), *Mentha virids* (6.6%) and *Lavandula officinalis* (3.26%) compared with the check.

The increased activity of Ach.E in the adult of *S. zeamais* after treatment with essential oils indicated that :

- 1-Higher activity of enzymes due to decreased body weight.
- 2- Increased levels of particular enzymes to defend against insecticide stress conditions. These results agree with Saleem *et al.*, (1998).

The previous results indicated that, the essential oils caused a significant decrease in the activity of Amylase digestive enzyme in the adult of *S. zeamais*. The reduction in Amylase activity as a result of the treatment with the plant extracts was reported also with Abo-El-Ghar *et al.*, (1996) who found that the ethanol extracts of *Melia azed-archetin* and *Vinca rosea* decreased Amylase activity in the haemolymph of 6th instar *Agrotis ipsilon* (Hufugel) larvae.

As mentioned for GOT, the results indicated that the tested essential oils caused a reduction in GOT activity except *Mentha virids* and *Artemesia herba alba* which caused increased in the activity of enzyme.

The effect of plant extracts against activity of GOT enzyme were also reviewed by Mostafa (1993) who found that the plant extract caused a significant increased in GOT activity, while GPT showed a reduction in the enzyme activity in larvae of *Spodoptera littoralis*.

As for GPT activity, our results indicated that all tested essential oils caused increasing in GPT activity except *Mentha piperita* and *Marjorana hortensis* which caused reduction in GPT enzyme activity. These results agree with Azmi *et al* (1998) who found that, the neem extract caused decreasing in GPT activity (57.48%) and almost no effect on GPT was observed in the adults of *Sitophilus oryzae*. Also Tabassum *et al* (1998) indicated that the neem formulation caused decreasing in GPT and GOT activities in adults of *Alphitobius diaperinus* (52.48 and 12.15% respectively). Also, Aly (1999) found that the formulation of plants extraction caused a great increasing in GPT and GOT activity.

These results clearly indicated that, the tested essential oils effected on GOT, GPT, Amylase and Ach.E activity of the adult of *S. zeamais*, and these oils caused a reduction in some enzymes activities, such as GOT and Amylase, whereas it caused increased in GPT and Ach.E activities comparing with the check.

The effect of essential oils was depended on the type of the compound and the species of insect. Also, the main compounds of these oils were γ -terpine and sabinent β -pinene (Amer *et al.*, 2001).

From the present results , it can be concluded that the effect of LC 50 treatments of six medicinal plant oils on *S. zeamais* caused depletion in the main metabolites and disturbances in some enzymes activities of the treated weevils .

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تأثير زيوت بعض النباتات الطبية على الأنشطة البيوكيميائية لسوسة الذرة سينوفيليس زياميز

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تم اختبار التأثيرات السمية والبيوكيميائية لسوسة زيوت مستخلصة من نباتات النعناع البلدى والبردقوش والافندر والريحان والنعناع الفلفلى والشيخ على سوسة الذرة في المعمل. وكان الزيت المستخرج من نبات البردقوش هو أكفاء الزيوت المختبرة. وقد أحدثت المعاملة بالتركيز النصفى المميت (LC50) لجميع الزيوت انخفاض في المحتوى الكلى للبروتين والليبيدات والكربوهيدرات وكان أيضاً لهذه الجرعة تأثير غير مترن (زيادة أو نقص) على نشاط أنزيمات الاميليز والترانس أمينيز (GOT & GPT) والاستيل كولين أستريز في الحشرات المعاملة.