

SOME BIOCHEMICAL EFFECTS OF NATURAL MINT OIL ON SOME SPECIES OF STORED GRAIN PESTS

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ABSTRACT

The present work aims to evaluate the efficacy of the natural mint oil on some biochemical constituents of *Sitophilus oryzae* (L.), *Sitophilus granarius* (L.) and *Rhizopertha dominica* (F.).

The studied characters were adult mortality (%) and the effect of oil on total content of proteins, lipids, carbohydrates, GOT, GPT and Ach E.

Results revealed that insect mortality (%) was increased with increasing concentration and the exposure period depending on the species. Mint oil was more toxic to *S. granarius* (LC₅₀ = 4.7 ml/kg) than *S. oryzae* (LC₅₀ = 6.2 ml/kg) and *R. dominica* (LC₅₀ = 10.5 ml/kg).

Total protein was significantly reduced in the treated insects compared to control.

Lipids content was found increased in *S. oryzae* and *S. granarius*, while was found decreased in *R. dominica* compared to control.

Reduction (%) in carbohydrates content was significantly decreased in both homogenate of *S. granarius* and *R. dominica*, while increased in *S. oryzae* compared to untreated adults. A significant reduction (%) in GOT, GPT and Ach E. compared to untreated insects.

The present study showed that mint oil have direct toxic effects on the mortality of studied insects and indirect effects on the studied biochemical constituents as total proteins, lipids, carbohydrates, GOT, GPT and Ach E.

Further studies are required to evaluate effect of this oil on other insects of stored grains as well as its effect when combined with other control factors.

INTRODUCTION

The efficiency of insecticides against stored product insects was studied by many investigators. Unfortunately, the side effects of using insecticides is causing concern among users and environment protection authorities all over the world. Also, synthetic insecticides are causing serious problem, such as harmful residues in the chain of food, risk of hazards and pollution of the environment, thus disruption of biological balance and destruction of the natural enemies of certain insect pest resistance.

Hence, a modern trend is tending again towards the old age practice of using indigenous plant as a source of natural insecticides. Every achievement to this respect would be of great value, especially to the developing countries, in solving their problems of crop and stored food protection by a possible cheap production of natural insecticides.

One of the possible alternatives to synthetic pesticides is the screening of plant in search for alternative pest control agents such as plant extracts, leaves, flowers, seeds, plant oils . . . etc.

The bioactivity of several plant oils as pest control against stored product pests was studied by many investigators (Messina and Renwick, 1983; Khaire et al., 1992; Pacheco et al., 1995 and Mahgoub et al., 1998).

Composition of natural mint oil:

Natural mint oil contains, chitinous compound (carvon), mentol, menthon consist of sinod and kadenin and limonene.

The present work is mainly concerned with some biochemical studies of natural mint oil on three species of stored product pests, rice weevil, *Sitophilus oryzae* (L.), grain weevil, *Sitophilus granarius* (L.) and lesser grain borer, *Rizopertha dominica* (F.).

MATERIALS AND METHODS

Insects used:

Three species of stored products insects, namely; the rice weevil- *S. oryzae* (L.), the granary weevil- *S. granarius* (L.) and the lesser grain borer- *R. dominica* (F.) reared under laboratory conditions at $28 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ R.H. Adults of 1-2 weeks old were used to carry out the present work. Experiments were conducted under the same conditions.

Test oil:

Natural mint oil was obtained from local market.

Toxicity test:

Toxicity of oil was determined by adding one ml of natural mint oil to ten ml petroleum ether. The appropriate amount of oil to give the required concentrations was mixed thoroughly with 10 grams wheat grains in glass vials, and left for two hours to evaporate the solvent.

25 adults of *S. oryzae* were kindly introduced into the vials then covered with muslin fixed with a rubber band.

Each concentrations was represented with three vials (replicates). Similar three vials contained untreated grains were used as a check and kept at the same previous conditions. The same technique was done for both *S. granarius* and *R. dominica*.

Mortality counts were recorded after 1, 3 and 7 days and percentages of corrected mortality were calculated by Abbott's formula (1925). These three days after treatment percentages were statistically computed according to Finny (1952) to produce LC_{50} and LC_{95} and toxicity slope for each insect. Percentages of reduction in the total of main metabolites were calculated according to the following equation:

$$\% \text{ Reduction in total content} = \frac{\text{Total content in untreated} - \text{total content in treatment}}{\text{Total content in untreated}} \times 100$$

Main metabolites:

Determination of the main components:

The main metabolites (total protein, lipids and carbohydrates) were determined in the total body (mg/ml) homogenates.

Total proteins:

Total proteins were determined by the method of Bradford (1976).

Total lipids:

Total lipids were estimated according to Knight et al. (1972).

Total carbohydrates:

Total carbohydrates were determined by the method described by Singh and Sinha (1977).

Determination of transaminases activity:

The level of both glutamic oxaloacetic and glutamic pyruvic transaminases (GOT and GPT) international unit / minute/ml (Iu/ml) was determined colorimetrically according to Reitman and Frankel (1957).

Esterases:

Acetylcholine esterase (Ach E.).

Acetylcholine esterase (Ach E.) was measured according to the method described by Simpson *et al.* (1964), using Acetyl choline bromide (Ach Br/min./ml) Mg acetyl choline bromid/minute/ml as substrate.

Statistical analysis:

Differences between means of the different treatments were statistically analyzed using ANOVA and Duncan tests.

RESULTS AND DISCUSSION

1- Toxicity of the various concentrations of natural mint oil on some stored product insects:

Table (1) demonstrates the effect of various tested natural mint oil on *S. oryzae*, *S. granarius* and *R. dominica*. Results revealed that, insects mortalities were increased with increasing concentration and exposure time. After three days from treatment, mortality ranged from 20-96%, 20-93% and 24-94% at the different tested concentrations of natural mint oil for *S. oryzae*, *S. granarius* and *R. dominica* respectively. These values were increased to 64-100%, 100% and 38-100% after 7 days from the initial treatment, for the *S. oryzae*, *S. granarius* and *R. dominica*, respectively.

Table (1): Effect of natural mint oil on mortality percent \pm S.E of *S. oryzae* (L.), *S. granarius* (L.) and *R. dominica* (F.) adults under the same previous conditions.

Species	Concentrations (ml/kg)	% Adult cumulative mortality after indicated period (days)		
		1	3	7
<i>S. oryzae</i>	4	0.0	20 \pm 2.3	64 \pm 3.1
	5	0.0	21 \pm 1.8	100
	6	1.3 \pm 0.3	39 \pm 0.7	100
	7	2.7 \pm 0.9	42 \pm 3.1	100
	8	14 \pm 1.2	76 \pm 1.5	100
	9	49 \pm 2.4	96 \pm 1.5	100
<i>S. granarius</i>	3.5	0.0	20 \pm 1.2	100
	4.0	0.0	25 \pm 1.5	100
	4.5	12 \pm 1.2	36 \pm 2.1	100
	5.0	28 \pm 2.0	51 \pm 2.9	100
	5.5	32 \pm 0.6	70 \pm 3.2	100
	6.0	46 \pm 2.3	93 \pm 1.2	100
<i>R. dominica</i>	9	8 \pm 1.2	24 \pm 1.5	38 \pm 2.7
	10	4 \pm 0.6	37 \pm 1.7	56 \pm 1.5
	11	22 \pm 0.3	68 \pm 1.5	100
	12	26 \pm 1.7	88 \pm 3.5	100
	13	74 \pm 2.0	94 \pm 1.2	100

S.E. = Standard error.

In this respect, Mahgoub *et al.* (1998) reported that the *Petroselinum sativum* oil toxic for *S. oryzae*, where LC_{25} , LC_{50} and LC_{95} levels were 1.2, 2.3 and 13.5 ml oil per kg wheat grain respectively.

A comparison based on LC_{50} values of tested oil given in Table (2) shows that (4.7 ml/kg) of the oil applied wheat grains as surface treatment resulted in 50% mortality to exposed *S. granarius* adults after 3 days, while larger amount (6.2 ml/kg wheat grains) of the oil gave the same mortality (50%) to exposed *S. oryzae* (L.) adults. While in case of *R. dominica* (F.) the amount of oil was (10.5ml / kg wheat grains) of the oil to gave the same mortality. The obtained results revealed clearly that, the adults of *S. granarius* were the least susceptible species to the natural mint oil followed by *S. oryzae* (L.) and *R. dominica* (F.) adults.

Table (2): Lethal concentrations (LC_{50} and LC_{95}) (ml/kg) of natural mint oil after 3 days from treatment for *S. oryzae*, *S. granarius* and *R. dominica* adults.

Species	LC_{50} (ml/kg)	LC_{95} (ml/kg)	Slope
<i>S. oryzae</i>	6.2	11.1	6.38
<i>S. granarius</i>	4.7	6.4	12.07
<i>R. dominica</i>	10.5	12.4	22.02

These results agree with Taheya *et al.* (1995) with *Nigella sativa* seeds which affected on *S. oryzae* (L.) and *C. maculatus* (F.). When LC_{50} values were worked out, *C. maculatus* adults were more sensitive to the extract (LC_{50} = 3.53 ml/kg cowpea seeds), than *S. oryzae* adults (LC_{50} = 5.89 ml/kg wheat grains).

2- Effect of mint oil on some biochemical components:

The total content of proteins, lipids and carbohydrates of *S. oryzae*, *S. granarius* and *R. dominica* feeding for 48 hr. on wheat grains treated with mint oil at LC_{50} are tabulated in Table (3) and illustrated in Figs 1a, 1b and 1c.

2.a. Effects on total proteins:

The total protein content (mg/ml) for adult homogenate of *S. oryzae*, *S. granarius* and *R. dominica* were 15.2, 16.1 and 19.8 for treated insects while the values were 18.9, 21.9 and 22.4 (mg/ml) for untreated adults respectively. These data showed significant differences in comparison to control which a significant reduction (%) occurred in treated adults (19.58, 26.48 and 11.61) compared with untreated species of *S. oryzae*, *S. granarius* and *R. dominica*.

2.b. Effects on total lipids:

The obtained results showed that the total lipids content of the treated adults homognate were 22.2, 26.5 and 12.9 for *S. oryzae*, *S. granarius* and *R. dominia* respectively, while the values were 16.2, 18.7 and 15.1% in the untreated insects respectively.

Calculation of reduction (%) of the total lipids estimated for each insect showed a highly significant differences with significant increased values of -37.03 and -41.7 for treated *S. oryzae* and *S. granarius*, while a significant decreased value (14.6%) for treated *R. dominica* compared to the untreated insects.

These results agree with Beudoin *et al.* (1968) using mature larvae of *Tribolium confusum*. They found that the percent of the total lipids of the whole body extract of mature larvae ranged between 15.1 and 19.6%.

2.c. Effects on total carbohydrates:

Values of the estimated total carbohydrates of the treated adults showed significant differences and were 10.9, 7.9 and 7.3 mg/ml compared to the untreated insects (8.3, 11.4 and 11.8%) of the three tested insects respectively.

The calculated values of reduction (%) showed an increased significant value (-31.33) for treated *S. oryzae*, while a decreased values of 30.7 and 38.14 for treated *S. granarius* and *R. dominica* compared to untreated insects.

3- Effects of mint oil on some insect enzymes:

The total content of GOT, GPT and acetylcholinesterase of *S. oryzae*, *S. granarius* and *R. dominica* feeding for 48 hr. on wheat grains treated with mint oil at LC_{50} are tabulated in Table (4) and illustrated in Figs. 1a, 1b, 1c and Fig. (2).

3.a. Effects on GOT:

The data in Table (4) and Figs. (1a, 1b and 1c) showed significant variations in the total content of GOT and its percentage reduction compared to the control of *S. oryzae*, *S. granarius*. While, no significant differences in the total content of GOT for treated and untreated for *R. dominica* adults.

The values ranged from 5.8 to 3.5 (lu/ml) for treated *S. granarius* and *S. oryzae* while its values in these untreated insects were 6.6 to 6.1 (lu/ml) respectively. The reduction in GOT ranged from 42.62 to 5.0% for *S. oryzae* and *R. dominica* respectively.

3.b. Effects on GPT:

The data in Table (4) showed significant differences in the total content of GPT and its percentage reduction compared to the untreated of *S. oryzae*, *S. granarius* and *R. dominica* respectively.

The values ranged from 16.8 to 5.1 (lu/ml) for treated *S. granarius* and *R. dominica*. While its values in these untreated insects were 22.3 and 7.3 respectively, thus showing a reduced effect on the GPT content.

3.c. Effects on (AchE.):

Data in Table (4) and Fig. (2) showed that, calculated values of total AchE. and its reduction (%) in treated adults were significantly varied compared to untreated adults of *S. oryzae*, *S. granarius* and *R. dominica*. Values were 1121, 1273 and 428 (Ach Br / min./ml) while its values in untreated adults were 1416, 1511 and 593 respectively. These results showed a reduced effect of the mint oil on the Ach E. by reducing its values. This result agree with Salwa, M.S. Ahmed (1992) when *S. oryzae* adults were treated with LC_{50} of *Mentha longifolia* extract, complete inhibition of AchE was noticed. Therefore, LC_{25} of this extract showed the highest potency in enzyme inhibition in *S. oryzae* adults. In case of *C. maculatus* adults, treating with LC_{50} of plant extracts caused an obvious decrease in AchE. level.

Table (3): Total proteins, lipids and carbohydrates content (mg/kg homogenate) of *S. oryzae*, *S. granarius* and *R. dominica* feeding for 48 hr. on wheat grains treated with natural mint oil.

Biochemical parameters	<i>Sitophilus oryzae</i> LC ₅₀ = 6.2 ml/kg			<i>Sitophilus granarius</i> LC ₅₀ = 4.7 ml/kg			<i>Rhizopertha dominica</i> LC ₅₀ = 10.5 ml/kg		
	Total content (mg/ml) of insect homogenate ± S.E.			Total content (mg/ml) of insect homogenate ± S.E.			Total content (mg/ml) of insect homogenate ± S.E.		
	T	C	Reduction(%)	T	C	Reduction(%)	T	C	Reduction(%)
Proteins	15.2± 0.5b	18.9± 0.4a	19.58	16.1± 0.5b	21.9± 0.3a	26.48	19.8± 0.2b	22.4± 0.5a	11.61
LSD 5%	1.68		—	1.55	—	—	1.46	—	—
Lipids	22.2± 0.6a	16.2± 0.6b	37.03	26.5± 0.4a	18.7± 0.5b	41.70	12.9± 0.4b	15.1± 0.3a	14.60
LSD 5%	2.21		—	1.79	—	—	1.62	—	—
Carbohydrates	10.9± 0.4a	8.3± 0.4b	31.33	7.9± 0.4b	11.4± 0.4a	30.70	7.3± 0.6b	11.8± 0.3a	38.14
LSD 5%	1.50		—	1.60	—	—	1.85	—	—
T = Treated insects	C = Untreated insects			Means within a column followed by a different letter are significant differences.					

Table (4): Total GOT*, GPT* and acetylcholinesterase** of *S. oryzae*, *S. granarius* and *R. dominica* after 48 hr feeding on wheat grains treated with mint oil.

Biochemical parameters	<i>Sitophilus oryzae</i> LC ₅₀ = 6.2 ml/kg			<i>Sitophilus granarius</i> LC ₅₀ = 4.7 ml/kg			<i>Rhizopertha dominica</i> LC ₅₀ = 10.5 ml/kg		
	Total content of insect homogenate ± S.E.			Total content of insect homogenate ± S.E.			Total content of insect homogenate ± S.E.		
	T	C	Reduction(%)	T	C	Reduction(%)	T	C	Reduction(%)
GOT	3.5± 0.2b	6.1± 0.4a	42.62	5.8± 0.3b	6.6± 0.3a	12.12	3.8± 0.2a	4.0± 0.1a	5.0
LSD 5%	1.3		—	1.2	—	—	0.5	—	—
GPT	10.4± 0.6b	18.5± 0.5a	43.78	16.8± 0.6b	22.3± 1.0a	24.66	5.1± 0.4b	7.3± 0.4a	30.14
LSD 5%	2.0		—	3.23	—	—	1.41	—	—
AChE.	1121± 47.1b	1416± 37.8a	20.83	1273± 59.8b	1511± 55.9a	15.75	428± 23.1b	593± 4.0a	27.82
LSD 5%	167.0		—	226.4	—	—	64.88	—	—
** = Ach Br/ min / ml. Means within a column followed by a different letter are significant differences.									

* = IU/ml

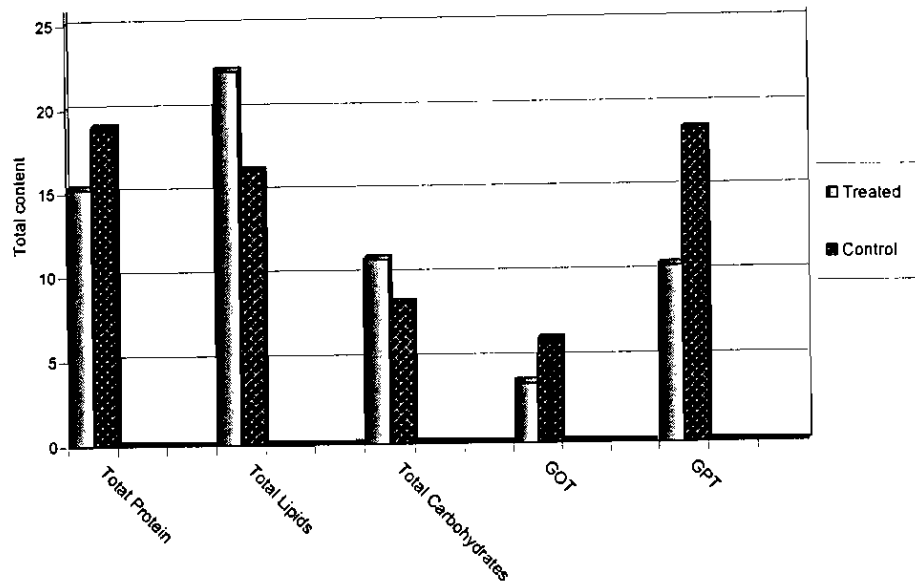


Fig.(1a): Total protein, lipids, carbohydrates (mg/ml) Homogenate and (GOT-GPT) IU/ml for *S. oryzae* L. post 48 hrs. feeding on wheat grains treated with LC_{50} (ml/kg) of natural mint oil.

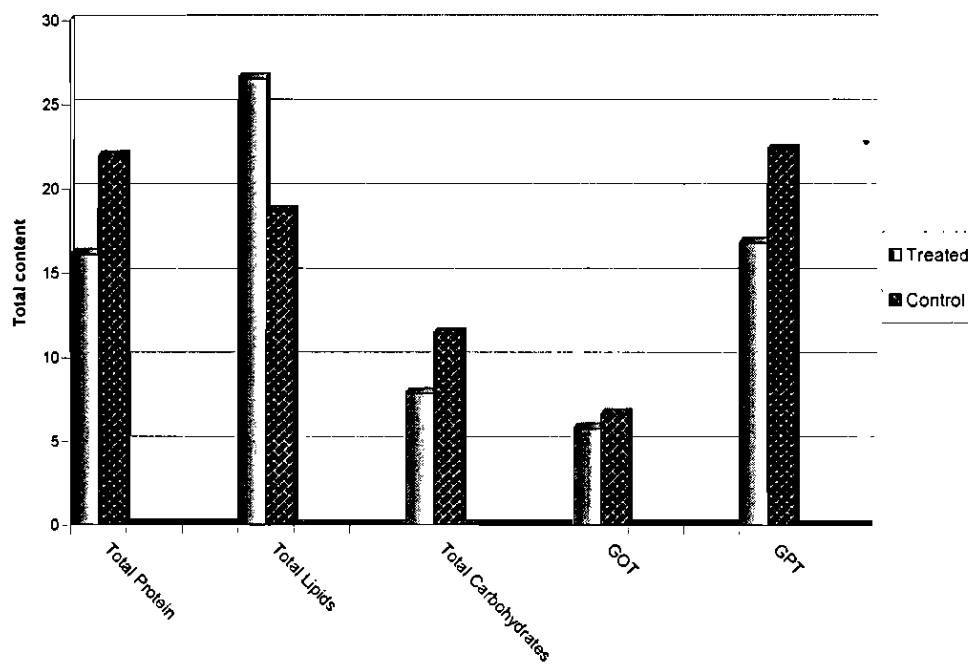


Fig.(1b): Total protein, lipids, carbohydrates (mg/ml) Homogenate and (GOT-GPT) IU/ml for *S. granarius* L. post 48 hrs. feeding on wheat grains treated with LC_{50} (ml/kg) of natural mint oil.

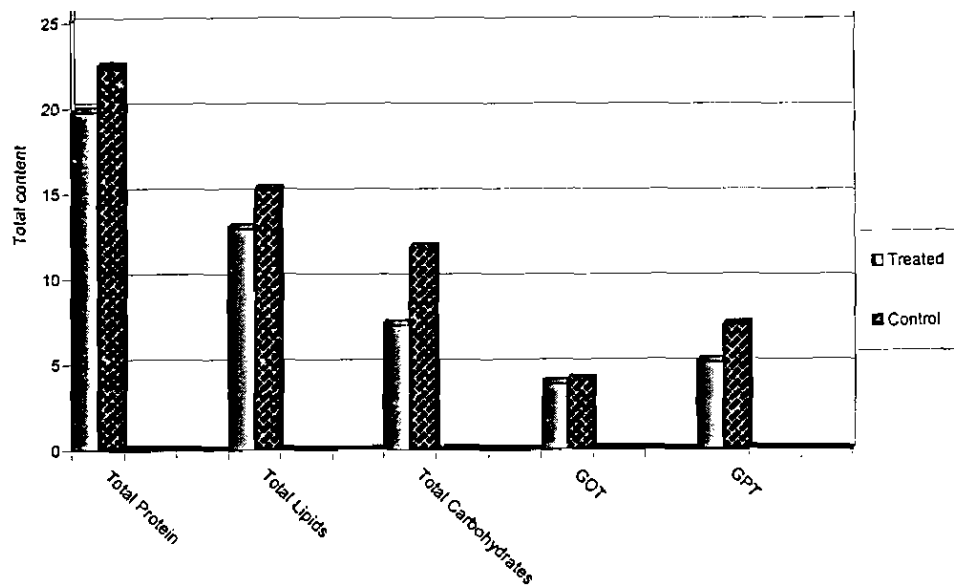


Fig.(1c): Total protein, lipids, carbohydrates (mg/ml) Homogenate and (GOT-GPT) IU/ml for *R. dominica* F. post 48 hrs. feeding on wheat grains treated with LC_{50} (ml/kg) of natural mint oil.

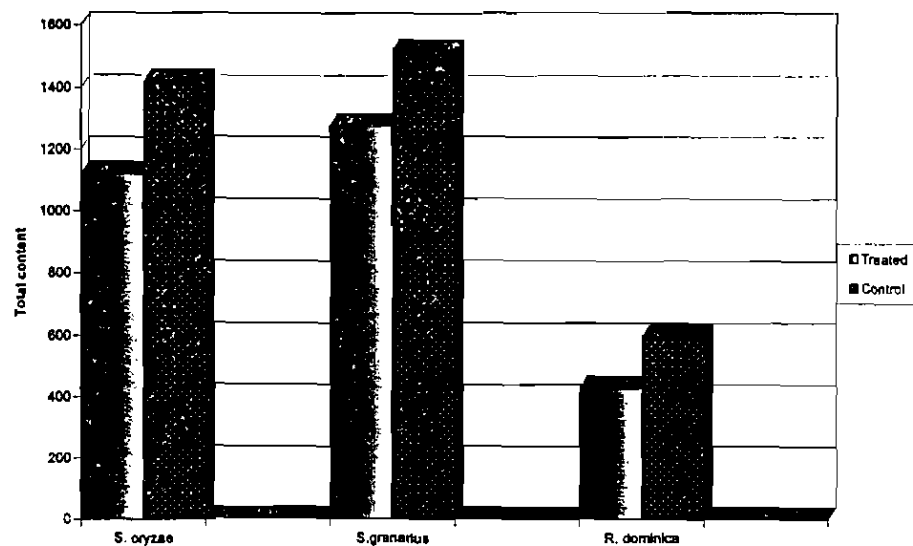


Fig.(2): Total acetyl cholinesterase (AchBr/min/ml) Homogenate of *S. oryzae* L., *S. granarius* L. and *R. dominica* F. post 48 hrs. feeding on wheat grains treated with LC_{50} (ml/kg) of natural mint oil.

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تأثير المعاملة بزيت النعناع الطبيعي على بعض المكونات البيوكيميائية لثلاثة أنواع من الآفات التي تصيب الحبوب المخزونة

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أجريت هذه الدراسة بهدف تقييم فعالية زيت النعناع الطبيعي على بعض الصفات البيوكيميائية لثلاثة أنواع من حشرات الحبوب المخزونة وهي سوسة الارز ، سوسة القمح وثاقية الحبوب الصغرى. والصفات التي تم دراستها هي نسب موت الحشرات الكاملة والمحتوى الكلى من السيروتين ، الدهون والكربوهيدرات ، بجانب الإنزيمات الناقلة لمجموعة الأمين وهي جلوتاميك أوكسيلو اسيتيك وجلوتاميك بيريفيك ترانس أمينيز (GOT أو GPT) بالإضافة إلى إنزيم الأسيتيل كولين. وقد أظهرت النتائج المتحصل عليها أن نسب الموت تزداد بزيادة التركيز وفترة التعريض معتمدة على نوع الحشرة المختبرة.

وقد وجد أن سمية الزيت كانت واضحة (أكثر سمية على حشرة سوسة القمح يليها سوسة الارز ثم ثاقية الحبوب الصغرى حيث كانت الجرعة النصفية المميتة LC_{50} هي ٤.٧ ، ٦.٢ و ١٠.٥ مل / كيلو جرام حبوب لسوسة القمح وسوسة الارز وثاقية الحبوب الصغرى على التوالي.

وأوضحت النتائج المتحصل عليها حدوث انخفاض معنوي في محتوى الحشرات من السيروتين مقارنة بالحشرات الغير معاملة ، بينما أدت المعاملة إلى زيادة معنوية في محتوى حشرتي سوسة الارز وسوسة القمح من الدهون بينما حدث نقص معنوي في محتوى الدهون في حشرة ثاقية الحبوب الصغرى مقارنة بالحشرات الغير معاملة.

بينما أدت المعاملة بالزيت إلى انخفاض معنوي في محتوى حشرتي سوسة القمح وثاقية الحبوب الصغرى من الكربوهيدرات بينما حدثت زيادة معنوية في محتوى الكربوهيدرات في حشرة سوسة الارز مقارنة بالحشرات الغير معاملة.

ومن النتائج المتحصل عليها أيضا حدوث انخفاض معنوي في محتوى الأنواع الحشرية الثلاثة من الإنزيمات الناقلة لمجموعة الأمين (GOT, GPT) وكذلك إنزيم الأسيتيل كولين (AChE) مقارنة بالحشرات الغير معاملة.

وهذه الدراسة أظهرت أن زيت النعناع الطبيعي له تأثير سام على الحشرات المختبرة وتأثيرات غير مباشرة على بعض المكونات البيوكيميائية (البروتينات ، الدهون ، الكربوهيدرات ، GOT ، GPT وكذلك إنزيم الأسيتيل كولين)

لذا يجب إجراء المزيد من الدراسات على هذا الزيت لمعرفة تأثيره على حشرات أخرى ذلك بخلافه مع بعض مواد مكافحة الأخرى.