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BIOCHEMICAL EFFECTS OF CERTAIN PLANT OILS ON THE LESSER GRAIN BORER, *RHIZOPERTHA dominica* (F.)

[50]

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ABSTRACT

Treatment of the adult of the lesser grain borer, *Rhizopertha dominica* (F.) with the LC_{50} of six essential oils extracted from the corresponding medicinal plants, peppermint, Marjorana, Lavander, Sweetbasil, Spearment and Artemisia resulted in ACh-estrases, Glutamic Transaminase (Gpt and Got), and Amylase activities in the body of the treated insect. In addition, the tested oils caused depletion in total proteins and total carbohydrates in the body tissues of the tested adults. But these oils increased the total lipids in the treated insects comparing with the check.

Keywords: Plant oils, Lesser grain borer, Biochemical effects

INTRODUCTION

The lesser grain borer, *R. dominica* (F.), is a major cosmopolitan insect pest of stored wheat. Females lay eggs on the exterior of wheat kernel, and the first instar larvae hatch and bore into the kernel upon reaching the adult stage, bore out of the kernel and create a large exit hole. Because the majority of the development occurs inside the kernel, *R. dominica* is difficult to kill with contact insecticides applied directly to stored wheat **(Tripathi *et al* 2003).**

Essential oils are potential sources of alternative compounds to currently used fumigants. Essential oils have low toxicity to warm blooded animals, high volatility and toxicity to stored grain insect pests, **(Shaaya *et al* 1991, Regnault *et al* 1993 and Shaaya *et al* 1997).**

Essential oils are easily distilled from suitable plant material. Their major constituents, monoterpenes, are also of interest to industrial markets because of other potent biological activities in addition to their toxicity to insects, **(Kurita and Koike, 1982, Gamer *et al* 1990; Arakawa *et al* 1992; Crowell *et al* 1992; Kubo *et al* 1994 and Basilico & Basilico 1999).**

Herein, we determined the biochemical effects of LC_{50} of tested essential oils from six medicinal plants on adult of *R. dominica*. Studied biochemical aspects

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were total proteins, total lipids, total carbohydrates, and the activity of amylase, transaminase and AChE in the whole body tissue of the normal as well as treated. insects.

MATERIAL AND METHODS

1. Essential Oils used

Five essential oils from plants belong to family Labiatidae were tested, i.e., Peppermint (*Mentha piperita*); Marjorana (*Marjorana hortensis*), Lavander (*Lavandula officinalis*), Sweetbasil (*Ocimum basilicum*), Spearment (*Mentha virids*). Also another oil from plant belonging to family Compositae was used *(Artemisia herba alba).*

2. Preparation of essential oils

Samples of six medicinal plants were hydro-distilled for 3hrs, using a cleavenger type apparatus. 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 obtained from laboratory strain of *R.dominica* ‹which was reared on wheat grains under room conditions (26 ± 2°C and 60 ± 10%, R.H) away from insecticidal contamination. Experiments were conducted under the same conditions.

3.2. Insecticidal activity of tested oils

Seven grams of wheat grains in Petri dishes were mixed with 0.1, 0.08, 0.05, 0.025, 0.02, 0.015, 0.01 microgram, which were dissolved in 1 ml acetone. Control insects were treated with acetone only. Every treatment was replicated three times. Twenty adult insects were introduced into each dish and numbers of insects (dead and alive) were counted 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 transformed into probits and values obtained were regressed on logarithm of concentrations of the essential oils to obtain the lethal concentration for 50% (LC_{50}) of the tested insects.

3.3. Biochemical determinations

About 100 mg of the control and LC50 treated adults of *R.dominica* in each replicate were crushed in 5 ml of 0.15M NaCl solution at 4°C with the help of a motor driven teflon-glass homogenizer, cooled with ice. The homogenate was centrifuged at 4.900 g for 10 min. at 4°C. The supernatant obtained was used for the estimation of the activities of Acetylcholine- esterase Transaminases, (Got and Gpt), and Amylase. Also, the total proteins, lipids and carbohydrates were determined in the total body tissues homogenates.

3.3.1. Determination of Acetylcholine- esterase

Acetyl choline-estrase (AChE) was measured according to the method described by **Simpson *et al* (1964),** using acetylcholine bromide (AChBr).

3.3.2. Determination of transaminases (Gpt and Got)

Transaminases were determined colorimetrically according to the method of **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 Swirisiki (1976).** The free aldehydic group of glucose formed after starch digestion was determined using 3-5 dinitrosalicylic acid reagent.

Determination of the main components

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

Statistical analysis

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

RESULTS AND DISCUSSION

The arrangement of oils toxicity were *Lavandula officinalis* (22.36ppm), *Mentha virids* (22.71), *Artemisia herba alba* (31.28), *Marjorana hortensis* (39.93), *Ocimum basilicum* (50.59) and *Mentha piperita* (57.85).

The effect of essential oils on total body tissue proteins, lipids and carbohydrates of *R.dominica* are shown in Table (1). Data clearly indicated that, all plant oils adversely affected protein synthesis on the whole body tissue of *R.domininca.* The percentage of decreasing in protein level were 56.98, 54.32, 53.64, 50.44, 41.73 and 33.98% comparing with the check) for *Mentha piperita, Artemisia herba alba, Ocimum basilicum, Marjorana hortensis, Lavadula officinalis,* and *Mintha virids,* respectively.

The above results showed that, LC_{50} of the tested oils caused highly significant decreasing in total protein levels for *R. dominica.* These results clearly indicated that the highest percentage of reduction in protein level were, 56.98 and 54.32%) for *Mentha piperita* and *Artemisia herba alba,* respectively. 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 also been reached by **El-Kordy *et al* (1994)** and **Aly (1999).**

Total lipids

Data in Table (1) show that, the highest percentage of reduction in lipid in the whole body tissue after treatment with the plant oils comparing with check, was 76.24 and 35.12(% for *Lavandula officinalis* and *Artemisia herba alba*, respectively. On the other hand, treatment with the LC_{50} of *Ocimum basilicam, Marjorana hortensis, Mentha peperita* and *Mentha virids* caused a significant increase in the level of total lipid (37.13, 24.78, 11.58 and 3.36%), as compared with the check, respectively.

Table 1. Protein, Carbohydrates and lipids contents of the treated adult of *R. dominica* with the essential oils

Plant oils tested	Homogenate contents					
	Total Protein	% of change	Total Lipid	% of change	Total carbohydrate	% of change
Mentha piprita	6.177 ±0.27	-56.98	29.22 ±1.32	11.58	3.247 ±0.23	-54.65
Mentha virids	9.48 ±0.45	-33.98	27.067 ±1.65	3.36	4.66 ±0.31	-34.92
Marjorana hortensis	7.117 0.32	-50.44	32.67 ±1.9	24.78	1.86 ±0.05	-74.02
Ocimum basilicam	6.657 ±0.33	-53.64	35.91 ±2.13	37.13	2.18 ±0.03	-69.53
Lavandula offieinalis	8.367 ±0.56	-41.73	8.58 ±0.35	67.24	4.517 ±0.12	-36.91
Artemesia herba alba	6.65 ±0.5	-54.32	16.99 ±1.53	35.12	4.313 ±0.13	-39.76
Control	14.36 ±0.34	—	26.187 ±1.6	—	7.16 ±0.21	—

(+) increase (-) decrease

Total carbohydrates

Feeding the adult of *R.dominica* on wheat grain treated with the LC_{50} of the tested oils caused a significant decrease in the level of carbohydrates as shown in Table (1). Data clearly indicated that the order of reduction in carbohydrates level were 74.02% (*Marjorana hortensis*) > 69.53% (Ocimum *basilicum*) > 54.64% (*Mentha pirperita*) > 39.76% (*Artemisia herba alba*) > 36.91% (Lavandula *officinalis*) > 34.92% (*Mentha virids*). The depletion of carbohydrate in whole body tissue may be due to their hyperactivity caused by essential oils treatment. Hence the results of the present study are in agreement with Singh, (1986).

Also results obtained by Saleem *et al* (1998) stated that, the adult of *Tribolium castaneum* that was treated with the LC_{25} of Ripcord (Cypermethrin), showed utilization of carbohydrates, proteins and Lipids in the given order.

As a conclusion, the reduction in carbohydrates, may be explained due to production of extra energy to combat insecticidal stress.

Effect of the essential oils on Enzymatic activities in *R. domimica*

The effect of LC_{50} of the six tested plant oils on Amylase, Transaminase and ACh.estrease are shown in Table (2). The results indicated that, amylase activity was reduced for all tested essential oils in *R. dominica* compared with untreated check. The order of reduction of Amylase activity were 21.59% *Marjorana hortensis* >21.5% *Lavandula officialis* >17.32% *Mentha piperita* >16.93% *Ocimum basilicam* >13.4% *Artemisia herba alba* > and >10.39% *Mentha virids*.

Glutamic Oxaloacetic Transaminase (GOt)

The effect of the LC_{50} of the essential oils on the activity of glutamic oxaloacetic transaminase (Got) of the adult of *R. dominica* is shown in Table (2). Data clearly indicated that, there are a significant reduction of Got activity being 76.1% *Lavandula officinalis* >52.27% *Artemisia herba alba* > 51.47% *Mentha virids* >50.93% *Ocimum basilicum* >48.65% *Mentha piperita* > 30.0% *Marjorana hortensis*.

Glutamic Pyruvic Transaminrase (Gpt)

The activity of glutamic pyryvic transaminase in homogenate of the adult of *R. dominica* was determined after treatment with LC_{50} of the essential oils. The results are tabulated in Table (2). The data revealed that, there was a significant reduction of Gpt activity for all tested plant oils. The order of reduction was 50.25% *Lavandula officibalis*> 47.1 % *Mentha piperita*> 38.07% *Ocimum basilicum* > 37.95 *Mentha virids* > 30.9% *Marjorana hortensis* > 30.45% Artemisia *herba alba* of check.

Acetyl Cholinesrease activity

The effects of essential oils on Ach.E activity in *R. dominica* are shown in Table (2). Data clearly indicated that all tested oils decreased Ach. E activity. The order of reduction was 23.9% *Artemisia herba alba* > 22.72% *Marjorana hortensis* > 19.2% *Mentha piperita* >18.41% *Ocimum basilicum* > 15.09% *Mentha virids*> 4.29% *Lavandula officinalis*, comparing with the check.

As a conclusion, these results clearly indicated that, the tested essential oils caused a reduction in, Gpt, Got, Amylase and Ach-E activities. The main compounds of these oils are Y-terpine and sabinent + B-pinene **(Amer *et al* 2001).** A relationship between monoterpine toxicity, inhibition of Ach.E activity and insecticidal effects has been reported, by **Gragza 1985; Grundy & Still 1985; Ryan & Byrne 1988; El-Kordy *et al* 1994 and Lee 2002).** Also, **Aly, (1999)** found that the plant extracts caused a great inhibition in the activity of enzyme that in control.

Acetylcholine estrase may be the target site of essential oil toxicity to *R. dominica.* These results agree with **Mostafa, (1993),** who found that *(Margosan – O)* caused reduction in Ach.E activity of *S. littoralis.* found that the (*Margosan – O*) caused a significant reduction in Ach.E activity of *S. littoralis*.

The essential oils caused a significant decrease in Amylase digestive enzyme in the adult of *R. dominica.* The reduction in Amylase activity as a results of the treatment with the plant extracts was reported also by **Abo-El-Ghar *et al* (1986)** who

Table 2. The activity of Amylase, Acetyl choline. Estrase and trans-aminase of the adult of *R.dominica* treated with the LC_{50} of the essential oils

Enzymes / Plant oil	Amylase		Got		Gpt		Acetyl Choliestrase	
	Activity	% of change	Activity	% of change	Activity	% of change	Activity	% of change
Mentha piperita	1244.34 ± 21.2	-17.32	4.54 ±0.27	-48.65	4.663 ±0.358	-47.1	1230.5 ±46.104	-19.2
Mentha virids	1348.54 ± 63.91	-10.39	4.54 ±0.27	-51.47	5.46 ±0.765	-37.95	1293.9 ±65.53	-15.09
Marjorana hortensis	1180.09 ± 44.86	-21.59	6.13 ±0.38	30	6.077 ±0.42	-30.94	1175.3 ±51.72	-22.7
Ocimum basilicam	1250.17 ± 67.34	-16.93	4.297 ±0.129	-50.93	5.45 ±0.17	-38.07	1243.5 ±67.73	-18.41
Lavandula offieinalis	1181.43 ± 61.66	-21.5	2.093 ±0.184	-76.1	4.38 ±0.21	-50.23	1458.7	-4.29
Artemesia herba alba	1303.05 ± 50.07	-13.41	4.18 ±0.429	-52.27	6.12 ±0.161	-30.45	1159.8	-23.9
Control	1507.93 ±16.21	—	8.757	—	8.8 ±0.141	—	1524.6 ±35.61	

(+) increase (-) decrease

found that, the ethanol extracts of *Melia azedrachiten* and *Vincea rosea* decreased Amylase activity in the haemolymph of 6th instar *Agrotis ipsilon* (Hufugel) larvae.

Data also clearly indicated that all tested oils caused a reduction in Got and Gpt activity in the adult of *R.dominica*. The effect of plant extracts were also reviewed by **Mostafa, (1993)** who found that, plant extract caused a significant reduction in the enzyme activity of *S. littoralis*. Also **Azmi *et al* (1998)** observed that, the neem extract caused decreasing in Gpt activity 57.477%. **Tabassum *et al* (1998)** found that, the neem formulation caused decreasing in Gpt and Got activities in adults of *Alphitbius diaprrus* (52.48 and 12.15%), respectively.

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مجلة حوليات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م٥٠ ، ع(٢)، ٧٢٩-٧٣٧ ، ٢٠٠٥

التأثيرات البيوكيمائية لبعض الزيوت النباتية على حشرة ثاقبة الحبوب الصغرى : ريزوبيرثا دومينيكا (اف)

[٥٠]

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لقد أحدثت معاملة حشرة ثاقبة الحبوب الصغرى *R. dominica* بالتركيز النصفي المميت (Lc_{50}) لستة زيوت عطرية مستخرجه من نباتات طبية هى النعناع البلدي ، البردقوش ، اللافندر ، الريحان ، النعناع الفلفلي ، الشيح انخفاض ملحوظ في نشاط أنزيمات الأستيل كولين أستريز والأميليز والترانس أمينيز داخل جسم هذه الحشرة . كذلك أحدثت المعاملة بالزيوت المختبرة إنخفاض ملحوظ في المحتوى الكلى للبروتينات والكربوهيدرات في أنسجة الحشرات المعاملة عن الحشرات المقارنة وغير المعاملة. على العكس سببت المعاملة ببعض الزيوت النباتية زيادة في مستوى الليبدات عن الحشرات المقارنة .

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