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Toxicological evaluation of some botanical oils on biochemical aspects in the Indian meal moth *Plodia interpunctella* HB. (Lepidoptera: Pyralidae)

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ABSTRACT

The biochemical changes in the haemolymph of *Plodia interpunctella* larvae treated with sublethal concentrations of two volatile oils and three fixed oils were studied. All oil treatments increased the levels of haemolymph lipids and protein but decreased their carbohydrate contents. However, electrophoretic assays on the haemolymph of the treated larvae revealed the presence of additional protein patterns due to oil treatments. The metabolic features of both oils are considered having juvenilizing action, where some morphogenetic abnormalities in treated larvae as well as developed pupae and adults were observed.

KEYWORD: *Plodia interpunctella*, biochemistry, morphogenetic

INTRODUCTION

In Egypt, losses caused by insects infesting stored grains can amount to the equivalent of 12 million pounds every year (Kamel 1974). The caterpillars eat meal, flour and farinaceous products, dried fruits, nuts, and some pulses and cereals in storage. The caterpillars eat the stored produce, causing direct damage, especially when they selectively eat the germinal part of the seeds; and the cause indirect damage in the contamination of the foodstuffs by frass, bodies and silk webbing (Hill 1987).

Several plant extracts have proved effective as insecticides, insect growth inhibitors and antifeedants against a variety of insect species (Khalaf 1998; Hussein 2002). The toxicological and histopathological effects of plant fixed oils (from *Trigonella foenum-graceum, Rumex dentatus* and *Acacia nilotica*) and volatile oils (from *Piper cubebae* and *Salvia officinalis*) on the Indian meal moth, *Plodia interpunctella*, have been studied previously by Shoukry *et al*. (2002 a & b 2003). There is no information about the effect of these plant oils on biochemistry and morphogenetic effects on this insect species.

Therefore, the present study deals with morphogenetic aberrations and biochemical properties of the effectiveness of two volatile and three fixed oils on 20 day old larvae of *Plodia interpunctella*, including toxicological and growth inhibition and their correlation to biochemical changes.

MATERIALS AND METHODS

The stock culture used in the present study consists of specimens collected from infested corn meal obtained from a mill in Sharqia province. They were reared in laboratory for many generations according to the method of Hassan *et al*. (1962), before being used in experiment. Extraction of fixed oils was performed from the seeds of *Trigonella foenum-graceum, Rumex dentatus* and the leaves of *Acacia nilotica* according to the method of Harborne (1984). Plant volatile oils were extracted by steam distillation from the seeds of *Piper cubebae*, and the leaves and stems of *Salvia officinalis* (Anderson *et al*. 1980). 20-day-old larvae were treated with LC_{50} of different oils in diet (10 ml of acetonic solution of each oil/100 g of corn meal media/100 larvae) for 24 hr, equal to 250, 320, 400, 420 and 760 ppm for *Trigonella foneum graceum, Acacia nilotica, Rumex dentatus, Piper cubebae*

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and *Salvia officinalis*, respectively (Shoukry *et al*. 2002a,b); then larvae completed their development on untreated diet. In the control experiment the larvae were treated only with acetone. Deformed larvae, pupae and adult moth resulting from previously treated larvae were recorded.

Because of small available amount of haemolymph to be taken from larva (about 1-2 µl/larva), a modified special technique was adopted in collecting haemolymph samples. Larvae were washed several times with cold distilled water and dried on a piece of absorbent paper; they were then immobilized by cooling at 4°C for 30 minutes. Each larva was carefully punctured with a fine dissecting needle, avoiding injury to the gut or other organs. About 15 to 20 larvae were then transferred to a microcentrifuge tube (91 ml) with finely perforated bottom fitted into another 2-ml microcentrifuge tube previously cooled on an ice bath and provided with phenylthiourea. The tubes with an ice jacket were centrifuged for 3 minutes at 1200 g. The whole process was repeated several times until a total of 0.5 ml of haemolymph was collected (Hassanein 1995). Excess of phenylthiourea crystals were added to the pooled haemolymph samples. The collected haemolymph was centrifuged for 5 minutes at 3750 g to separate the fat layer and any residue precipitated from the haemolymph, carefully withdrawn through a puncture on the side of the tube using a syringe, and then transferred to another ice-cooled tube and centrifuged once more at the same speed for 5 minutes. The accumulated pooled haemolymph was dispensed into 0.1-ml aliquots and stored at -18°C (Hassanein 1995). Haemolymph from treated larvae were assayed for carbohydrates, lipids, proteins and protein fractions according to the methods of Singh & Sinha (1977), Frings *et al*. (1972), Bradford (1976) and Davis (1964), respectively.

RESULTS

Deformed larvae, pupae and adult moth resulting from previously treated larvae with the plant oils (volatile and fixed oils) exhibited some morphogenic abnormalities.

As compared with normal larvae (Plate I), larval treatment with *Trigonella*, *Acacia* and *Rumex* fixed oils, and with *Piper* or *Salvia* volatile oils, showed the presence of macerated larvae. Among this stage, larval-pupal intermediates were detected (Plate II_A). These intermediates retained some parts of their last larval skin with their anterior end. They were often completely sclerotized. Others failed to complete their pupal period and died. Sclerotization and the darkening process of the mature larvae occurred before the formation of the puparium.

Treatment of *Plodia* larvae with the tested plant oils resulted in pupae with various morphogenic abnormalities. A large number of aberrant C-shaped pupae were observed with abnormal abdominal arches (Plate II_B), differing from normal pupae.

From plate I (normal adults) and plate II (treated adults), most of the puparia failed to reach adults (aborted puparia). However some emerged adult had various degrees of morphological abnormalities. Some individuals showed a dominance of incomplete adult eclosion (Plate II_C) varying from complete eclosion of adults with only their legs or wings partially attached to the puparium, to (in most cases) only the head and thorax emerged from the puparium. Some adults were completely free but possessed an abnormal appearance such as severely crumpled wings, and deformations of the thorax and abdomen (Plate II_D).

Treatment of 20-day old *Plodia* larvae with sublethal concentrations (LC_{50}) of fixed and volatiles plant oils induced large changes in haemolymph carbohydrates, lipids, proteins and protein fractions. Data in Table 1 show that the level of haemolymph carbohydrate of normal larvae is significantly reduced in those treated with different oil

extracts. The fixed-oil extracts were more effective than volatile-oil extracts. Application of all three fixed oils produced a highly significant decrease ($p<0.001$) in the total carbohydrate content, and similar though smaller reductions in the two volatile oils.

Data on haemolymph lipids (Table 1) show that application of the three fixed oils produced highly significant increases ($p<0.001$) in total lipid content relative to the control. Of the two volatile oils, application of *Piper* oil produced a highly significant increase ($p<0.001$), but treatment with *Salvia* oil produced a highly significant decrease. The haemolymph proteins (Table 1) were increased by treatment with both fixed and volatile oils.

Haemolymph proteins of control and treated *Plodia* larvae were electrophoretically separated using SDS-PAGE using 10% acrylamide as a separating gel. Plate III and plate IV, show the electrophoretic patterns (electropherograms) obtained. Protein standards were used to estimate the molecular weights of the separated protein bands.

A total of forty-eight different protein bands were distinguished, with molecular weights ranging from 18-232 KDa. Twenty-two bands were present in control larvae, and 25, 27 and 17 bands from *Trigonella, Acacia* and *Rumex* fixed-oil treatments. 17 and 15 bands were separated from the two volatile-oil treatments of *Piper* and *Salvia.* Among these bands, six (numbers 3, 21, 34, 42, 44 and 48) were always permanent or dominant in both treated and untreated larvae: they had molecular weights of 214, 93, 47, 27, 23 and 18 KDa respectively. However, their relative percentage and intensities fluctuated: they are species-specific proteins. The other protein fractions (42 fractions) are often related to the type of applicable material and therefore they are considered to be treatment-dependent. Band 1 appeared in haemolymph of *Acacia-*, *Rumex-*, *Piper-* and *Salvia*-treated larvae with molecular weights of 227, 232, 229 and 224 KDa respectively. Bands 2 and 15 were detected only in *Trigonella*-treated larvae with molecular weights of 220 and 124 KDa respectively. Bands 4 and 13 appeared only in haemolymph of *Trigonella-*, *Acacia-* and *Piper*-treated larvae with molecular weights of 196/135, 198/135 and 200/136 KDa respectively. Bands 5, 14, 37 and 47 were detected only in control untreated larvae, and had molecular weights of 193, 133, 41 and 20 KDa respectively. Bands 6 and 10 appeared in control, *Trigonella-* and *Acacia*-treated larvae, with molecular weights of 177/153, 186/153 and 187/154 KDa respectively. Band 7 with a molecular weight of 184 KDa appeared only in the haemolymph of *Piper*-treated larvae. Bands 8, 35 and 45 appeared only in haemolymph of *Salvia*-treated larvae, and had molecular weights of 180, 43 and 21 KDa respectively. Band 9 appeared in the haemolymph of control and *Acacia*-treated larvae, and had molecular weights of 172 and 171 KDa respectively. Bands 11, 12, 22 and 26 were detected only in haemolymph of *Acacia*-treated larvae, and had molecular weights of 147, 141, 83 and 70 KDa respectively.

Band 16 appeared in control, *Acacia-* and *Salvia*-treated larvae, with molecular weights of 121, 121 and 123 KDa respectively. Band 17 was detected only in *Rumex-* and *Salvia*-treated larvae, with a molecular weight of 15 KDa. Band 18 appeared in untreated, *Trigonella-*, *Acacia-* and *Piper*-treated larvae, with molecular weights of 110, 110, 110 and 112 KDa respectively. Band 19 with molecular weight of 106 and 104 KDa appeared only in *Trigonella-* and *Rumex*-treated larvae. Band 20 with a molecular weight of 103 KDa appeared in control and *Piper*-treated larvae. Bands 24, 29, 31 and 43 appeared in *Trigonella-* and *Acacia*-treated larvae, with molecular weights of 76, 61, 55 and 24, and 77, 62, 55 and 25 KDa respectively. Bands 25 and 28 with molecular weights 74 and 66 KDa appeared in *Rumex*-treated larvae. Band 27 appeared in control and *Trigonella*-treated larvae, with molecular weights of 67 and 69 KDa respectively. Band 30 appeared in control, *Rumex-* and *Salvia*-treated larvae, with molecular weights of 60, 60 and 61 KDa respectively. Band 31 appeared in *Trigonella-* and *Acacia*-treated larvae with a molecular

weight of 55 KDa. Band 32 appeared in control, *Acacia-*, *Rumex*- and *Piper*-treated larvae, with molecular weights of 54, 53, 54 and 54 KDa respectively. Band 33 appeared in control and *Trigonella*-treated larvae, with a molecular weight of 52 K.Da.

Band 36 appeared in *Trigonella-*, *Acacia-*, *Rumx*- and *Piper*-treated larvae, with molecular weights of 42, 42, 41 and 41 KDa respectively. Band 38 was detected in *Trigonella-*, *Acacia*- and *Salvia*-treated larvae, with molecular weights of 35, 36 and 36 KDa respectively. Band 39 was detected in control, *Rumex*- and *Piper*-treated larvae, with a molecular weight of 35 KDa. Band 40 appeared in *Acacia*- and *Salvia*-treated larvae, with a molecular weight of 33 KDa. Band 41 was present in *Trigonella-*, *Rumex*- and *Piper*-treated larvae, with a molecular weight of 32 KDa. Band 43 appeared in *Trigonella*- and *Acaci*-treated larvae, with molecular weights of 24 and 25 KDa respectively.

In conculsion, the treatment with plant fixed and volatile oils disturbs the protein fractions of treated larvae, the treatment with *Trigonella* fixed oil creates three specific bands (nos. 2, 15 and 23); treatment with *Acacia results in* four specific bands (11, 12, 22 and 26); treatment with *Salvia* also creates three bands (8, 35 and 45); treatment with *Piper* volatile oil creates one specific band (7); while treatment with the other volatile oil (*Rumex*) results in two specific bands (25 and 28).

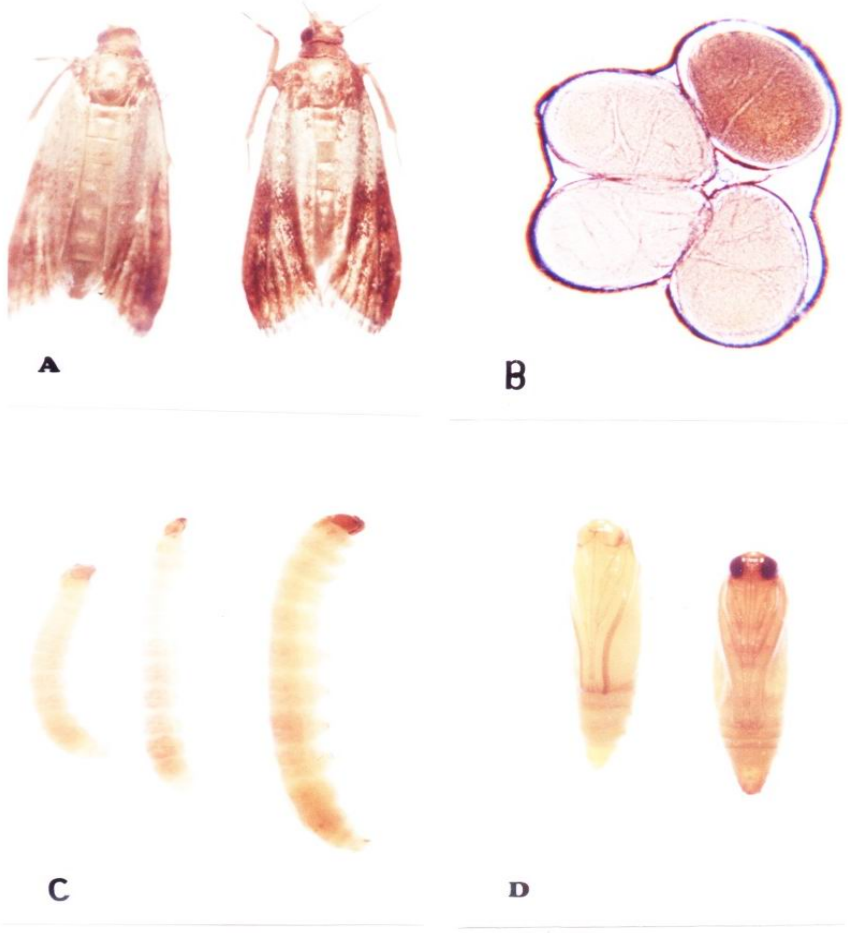

Plate (1): **Normal developmental stages of *Plodia interpunctella*.**
A- Adult B- Eggs C- Larvae D- Pupae

Plate (II): Morphogenic abnormalities of some plant fixed and volatile oils on *Plodia interpunctella*.
A- Larval pupal intermediate B- C-shaped pupae
C- Incomplete eclosed adult D-Adult with crumped wings

Table 1: Effect of plant oils (fixed and volatile) on haemolymph total carbohydrate, lipid and protein content of *Plodia interpunctella* larvae.

Treatment	Carbohydrate content (mg/ml)		Lipid content (mg/ml)		Protein content (mg/ml)	
	mean ± SE (range)	change %	mean ± SE (range)	change %	mean ± SE (range)	change %
Trigonella foenum graceum	0.2 ± 0.57* (0.10-0.30)	-87.50	0.06 ± 0.001* (0.05-0.06)	+31.39	0.25 ± 0.005* (0.24-0.26)	+20.85
Acacia nilotica	0.33 ± 0.003* (0.33-0.34)	-79.30	0.049 ± 0.001* (0.04-0.05)	+2.70	0.406 ± 0.03* (0.34-0.43)	+92.4
Rumex dentatus	0.44 ± 0.005* (0.43-0.45)	-72.50	0.059 ± 0.001* (0.05-0.06)	+24.11	0.29 ± 0.01* (0.27-0.32)	+40.7
Piper cubeba	0.46 ± 0.005* (0.45-0.47)	-71.25	0.055 ± 0.001* (0.05-0.06)	+16.21	0.32 ± 0.008* (0.31-0.33)	+54.02
Salvia officinalis	0.66 ± 0.005* (0.65-0.67)	-58.70	0.04 ± 0.006* (0.03-0.04)	-20.3	0.30 ± 0.06* (0.19-0.41)	+44.07
Control	1.6 ± 0.057 (1.5-1.7)	-	0.048 ± 0.001 (0.04-0.05)	-	0.21 ± 0.02 (0.18-0.25)	-

(-) or (+) refers to the percentage increase or decrease from the control.
* highly significant (P<0.001).

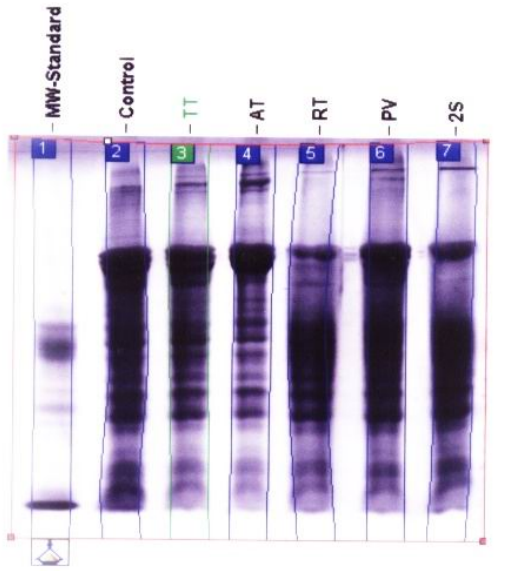

Plate (III): Electrophotograph of haemolymph protein of *P.interpunctella* larvae (SDS-poly-acrylamid gel electrophoresis)
TT- *Trigonella foenum grceum* **AT-*Acacia nilotica***
RT- *Rumex dentatus* **PV- *Piper cubebae***
2S- *Salvia officinalis*

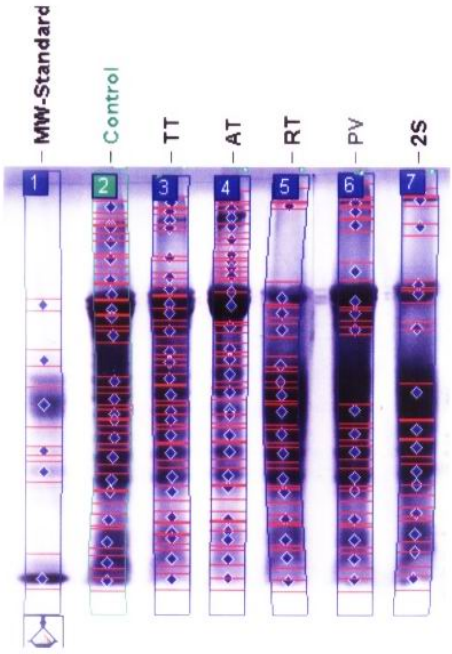

Plate (IV): Computerized diagram of plate (III)

DISCUSSION

Treatments of 20-day-old larvae with various plant oils resulted in morphological aberrations, including the appearance of larval-pupal intermediates and malformed pupae, the later characterized by a C-shape. Similar results were obtained by Naqvi (1986), who found that treatment of *Aedes aegypti* larvae with natural fractions of neem leaves produced larval-pupal intermediates. El-Shazly *et al*. (1996) reported similar findings on *Muscina stabulans* larvae treated with ethanol extract of *Nerium oleander* leaves. Abahussain (1999) studied the morphological effects induced by *Calotropis procera* in *C. pipiens* and *A. multicolor*, and again this produced larval-pupal intermediates and pupal-adult intermediates. A high frequency of incomplete eclosion in adults within the individuals were observed. Complete eclosion with crumped wings or legs in adults were also found. Ivbijaro (1986) tested neem ethanolic extract against *T. castaneum*, and also found that the few pupae that survived metamorphosed into malformed adults. Moursy (1997) on *Sarcophaga haemorroidalis* after treatment with *Cymbopogon citratus* extract, and Hussein (2002) on *Culex pipiens* larvae after treatment with *Thevetia peruvine* active compound, produced similar results.

Results showed that treatment with plant oils affects the biochemical activities of *Plodia* larvae, leading to disturbances in carbohydrate, lipid and protein levels in the haemolymph, and also protein fractions. The content of haemolymph carbohydrates significantly decreased in treated larvae, with the fixed-oil extracts being the most effective. Khalaf (1998) also reported that treatment of second-instar larvae of *Muscina stabulans* with two plant oils of *Cymbopogon citratus* and *Rosmarinus officinalis* induced a significant reduction in the carbohydrate content during the whole pupal period. Abo El-Ghar *et al*. (1995) showed that petroleum-ether extract of *Ammi majus* and *Apium graveolens* fed to 6^{th}-instar larvae of *Agrotis ipsilon* greatly reduced haemolymph carbohydrates, and the same results were obtained when acetone and ethanol extracts of *Melia azedarach* were used. Shoukry & Hussein (1998) obtained the same findings with

the larvae of *Galleria mellonella* treated with volatile oils of *Lantana camara* and *Vitex aganus custus* plants. Haemolymph lipid content was significantly increased in all treatments except treatment with *Salvia*, which significantly decreased the haemolymph lipid content. Hill & Izatt (1974) stated that all one-day-old female desert locusts accumulate lipids in the fat tissues, perhaps due to high deposition of lipid together with low lipid utilization; they added that lipid accumulation is more likely to be related directly to a lack of juvenile hormone. The tested plant-oil extracts may cause degeneration of corpora allata, which may allow elevation of the mean lipid content. The rise in total lipids agrees also with the finding of Mostafa (1993), who found that the total lipid content was significantly increased in *Trogoderma granarium* as a result of treatment with plant extracts. Abou El-Ela *et al*. (1995) found the same result on *Musca domestica* after treatment with water extracts of some plants. However, Shoukry & Hussein (1998) showed that treatment of third-instar larvae of *Galleria mellonella* with sublethal concentrations of *Lantana camara* and *Vitex agnus castus* reduced the total lipids in the last larval instar.

Protein content significantly increased in all treatment, especially in the cases of fixed oil treatments. Generally, changes in protein content probably reflect the balance between synthesis, storage, transport and degradation of structural and functional nutrients during ontogeny as well as response to particular physiological conditions. The increase in protein content with different oil treatments may be attributed to the increased activity of protein biosynthesis by its tool (amino acids). This line of evidence signified that both fixed and volatile oils treatments in this experiment generated an increase in the incorporation of amino acids in protein, and enhanced the utilization of some amino acids as an energy source. Many workers obtained similar findings on other species of insects (Shakoori & Saleem 1991), attributing the greater protein synthesis with insecticidal treatment to synthesis of the proteinases needed for insecticide detoxification. This may be due to the conversion of carbohydrates and lipids to proteins: Kinnear *et al*. (1971) suggested that increased protein levels was due to increased synthesis of new proteins by the fat body, haemolymph and other tissues of the larvae. The increase in the total haemolymph protein may be a kind of detoxification mechanism. In this respect, Wilkinson (1976) stated that protein helps to synthesize microsomal detoxifying enzyme which assists in detoxification. In contrast, Schloter (1985) found that treatment of the last larval instar of *Epilachna varivestis* with high doses of azadirachtin caused metabolic defects. He also added that storage of proteins in the fat bodies, which is necessary for pupation, did not occur. Abou El-Ela *et al*. (1995) reported a significant decreased in the total protein content in *Musca domestica* larvae treated with plant extracts. Khalaf (1998) showed that the volatile oils of *C. citratus* and *R. officinalis* induced biochemical disturbance in the pupae of *M. stabulans* which decreased protein content, as did Shoukry & Hussein (1998) on the greater wax moth *Galleria mellonella*. The appearance of new protein bands upon treatment may be due to liberation of free radicals which affect nitrogenous compounds directly; this in turn leads to breakdown of the peptide linkage, causing fragmentation of protein molecules. The formation of extra-molecular sizes of one or more molecules is expected (Megahed 1996, Gehad & Shaurub 1997). Another possible source of haemolymph free protein is the haemocytes which play a part in metabolic process such as growth and moulting (Wigglesworth 1959).

In conclusion, the protein bands of treated samples were completely different from those of the control, so there may be differences in biological and biochemical activities (Mostafa *et al*. 1995; El-Bermawy & Abdel Fattah 2000). The application of plant oils greatly changed biochemical activities in *Plodia* larvae; this disturbance may have several causes, such as as antifeedant activities (Kalavathi *et al*. 1991) or enzyme activities (Chun *et al.* 1994). Mostafa *et al*. (1995) reported that the volatile oils of some plants increases

the activity of MDH enzyme and decreased the activity of ME enzyme in *Pectinophora gossypiella* and *Earias insulana* larvae. Abo El-Ghar *et al*. (1995) noticed a significant decrease in carbohydrates digestive enzymes and a considerable increase in the activity of trehalose after treatment of *Agrotis ipsilon* larvae by *Melia azedrachta*.

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الملخص العربى

تقييم سمية بعض الزيوت النباتية على بعض الأوجه البيوكيميائية فى فراشة الجريش الهندية *بيلوديا انترينكتللا* (بيراليدى - حرشفية الأجنحة) المعاملة ببعض الزيوت النباتية

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أدت معاملة اليرقات بالمستخلصات النباتية وبخاصة الزيوت الثابتة إلى ظهور أطوار وسطية بين اليرقات والعذارى مع ظهور بعض التغيرات المورفولوجية على العذراء تمثلت فى ظهور عذراء على شكل حرف C . أما التغيرات المورفولوجية التى حدثت للطور الكامل فقد لوحظ أن معاملة اليرقات بهذه المستخلصات تؤدى إلى خروج حشرات ذات مستويات مختلفة من التشوه تمثل فى الآتى : حشرات تخرج الرأس والصدر من العذراء - حشرات تخرج كاملة من العذراء ولكن تعانى من فقد أحد أرجلها أو التفاف أجنحتها.

كما أكدت الدراسة أن المعاملة بالزيوت النباتية تؤثر على فسيولوجية حشرة فراشة الجريش مما يؤدى إلى اختلال فى نسب كل من الكربوهيدرات والدهون والبروتين فى دم الحشرة وأيضا التركيب البروتينى حيث وجد أن نسبة الكربوهيدرات تقل فى الحشرات المعاملة وأعلى نسبة للانخفاض تلاحظ فى المعاملة بالزيوت الثابتة تم تليها الزيوت الطيارة بينما تزيد نسبة الدهون فيما عدا المعاملة بزيت المرمرية حيث تقل نسبة الدهون وتزيد أيضاً نسبة البروتينات فى كل المعاملات. وقد أثبت تحليل الدم بطريقة الهجرة الكهربية إحتواءه على 48 نوع مختلف من الأجزاء البروتينية والتى يختلف فيها الوزن الجزئيى من 18-232 كيلو دالتون وقد وجد أن ستة منها ثابتة فى كل المعاملات وهم 3, 21, 34, 42, 44 ثم 48. وقد أثبتت الدراسة أن فى كل معاملة يظهر نوع معين من الأجزاء البروتينية، فمثلاً المعاملة بزيت الحلبة يظهر فيها ثلاثة أجزاء بروتينية وهى (2, 15 ثم 23) والمعاملة بزيت السنط يظهر فيها أربعة أجزاء بروتينية وهى (11, 12, 22 ثم 26) والمعاملة بزيت الحميص يظهر فيها ثلاثة أجزاء بروتينية هى (8, 35 ثم 45) بينما المعاملة بالزيوت الطيارة يظهر بها عدد أقل من الأجزاء البروتينية الخاصة ففى المعاملة بزيت الكبابة الصينى يظهر جزء بروتينى واحد وهو 7 بينما المعاملة بزيت المرمرية تظهر بها نوعين من البروتينيات هى 25, 28. ومن هنا يتضح أن لهذة الزيوت النباتية الثابتة والطيارة تأثيراً فعالاً ضد حشرات الحبوب المخزونة وبالتالى يمكن استخدامها وتطبيقها على نطاق واسع كبديل آمن للمبيدات والمركبات الأخرى ذات التأثير الجانبى الضارعلى كل من الإنسان والبيئة.