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Biochemical and morphogenic activeities induced by some botanical oils on the date Moth, *Ephestia cautella* [Lepidoptera: Pyralidae]

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Abstract: ***Ephestia cautella is a serious insect pest of dates either in the field or in the form of stored product. Also, this insect pest infests other stored products and causes high loss in all these fruits and cereal grains and their products. Results indicated that, the sensitivity of Ephestia cautella to the oils treatment which effect on biochemical and morphological characters. Plant oils have most effective as insecticides, insect growth inhibitors and antifeedants against a variety of insect species. The biochemical changes in the haemolymph of Ephestia cautella larvae treated with different sub lethal oils concentrations of two fixed oils (Black seed and Cumin) and three volatile oils (Clove, Ginger and Garlic) were studied. All oils treatment increased the levels of haemolymph lipids & protein but decreased the carbohydrate contents. Treatment with above mention oils induced some morphogenic abnormalities in the treated larval stage and subsequent developmental stages were also studied and discussed.***

Key words: Ephestia cautella, fixed oils, volatile oils, biochemical and morphogenic abnormalities.

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I. INTRODUCTION

Insect pests present a major constraint in crop production, especially in developing countries (Fan et al.2011). Using the chemical insecticide in the pests controlling, may have drawbacks, including toxicity to nontargets, development of pest resistance and resurgence and environmental contamination (Sahaf, et al.,2008; Subramanyam, et al.,1995 and Talukder, et al.,1993). Hence there is a worldwide interest in the development of alternative strategies in traditional methods of pest control.

The plant kingdom can be a rich source of a variety of chemicals with the potential for development as successful pest control agents. The use of natural compounds, such as essential oils control agents are receiving increase attention as an alternative because of their largely accessible and non-toxicity to humans and the environment. Moreover, essential oils easily biodegrade in the environment (Huang, et al.,2000; Lee, et al.,2001 and Ngamo et al.,2007)

This study planned to show some biochemical and morphogenic changes associated with the treatment with some fixed (Black seed and Cumin) and volatile (Clove, Ginger and Garlic) plant oils.

II. MATERIALS AND METHODS

Mass rearing of *Ephestia cautella*

A laboratory culture of Ephestia cautella (Walker) moths were obtained from a mass culture maintained from the Stored Products Research Laboratory, Plant Protection Research Institute, Agricultural Research Center, El-Dokki, Giza, Egypt. The rearing of the moths of E. cautella, were done on an artificial diet composed of wheat flower 65%, glycerin 15%, dry yeast 10% and icing sugar 10%. This media used as the standard laboratory diet for rearing of E.cautella, (Burges, 1956).

The emerged adults were collected daily by glass tube and placed in two-sided open glass cages with screen bottom to obtain eggs. Eggs that fall through lower screen bottom were collected daily in large Petri dish and transferred to glass tubes for the other experiments, (Ryne et al., 2004).

Collecting larvae and adults:

During adult collection, the two sexes were easily separated since the male larvae are distinguished by their dark testes, easily seen through the body integument Larvae of each sex were placed in a separate container, so virgin adults were obtained. For experimental purposes, individual adults are required, 1-2 days old, all insects were separated from the food, and the jars were kept again at the controlled conditions in the rearing room. This procedure was repeated several times in order to obtain large numbers of the adults needed to carry out the experiments during this study under laboratory conditions. The food in the jars was renewed whenever needed, (Boles and Marske, 1966).

BIOCHEMICAL STUDIES

Collection of haemolymph

Because of small available amount of haemolymph be taken from third larval instar, a modified special technique was adopted in collecting haemolymph samples. Larvae washed several times with cooled distilled water and dried on a piece of absorbent paper, were 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 micro centrifuge tube (91 ml) With finely perforated bottom fitted in another 2 ml micro centrifuge tube previously cooled on an ice bath and provided with phenylthiourea. The tubes with an ice jacket were centrifuge for 3 minutes at 1200 g. the whole process was repeated several times until a total sum of 0.5 ml of haemolymph was collected **(Hassanein, *et al*.1995).**

Access 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 haemolymph and then carefully with drawn through a puncture on the side of the tube using 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, *et al*.1995).**

Preparation of samples for biochemical assay

Treatment of third larval instar of *Ephestia cautella,* with sub lethal concentration of volatile (Clove, Garlic and Ginger) and fixed (Black seed and Cumin) plant oils **(Khalaf *et al*. 2022)** for 48 hours. The biochemical analysis of haemolymph carbohydrates, lipids and protein were determined.

Determination of total soluble protein:

total soluble protein (TSP) as described by **Gornall *et al*. (1949),** the principle of this method was based on the presence of an alkaline cupric sulfate, the protein produces violet purple color or blue, the intensity of which is proportional to their concentration. A volume of 0.5 ml of the supernant of the haemolymph add to 5 ml of briuet reagent in a test tube then incubated for 30 minutes at 20-25 c. the total protein content of haemolymph samples was estimated as mg/ml using the following formula.

$$\text{Protein content} = \frac{\text{absorbance - interception}}{\text{slop}} = \text{mg/ml}$$

Determination of total carbohydrate:

Carbohydrates were determined according to **Ishaaya & Swiriski (1976).** A known size (2ml) of the sample was hydrolyzed by 0.8 N sulphonic acid (25ml) for 6 hours under reflux condenser using water bath at 80 C after filtration, the filtrate was neutralized with 0.2 N NaOH, clarified with basic lead acetate and disodium hydrogen phosphate and finally made up to definitive volume with distilled water. 5 ml from the filtrate were taken in test tube and 2ml of 3,5 dinitro salicylic acid were added. Mixed well and heated in a boiling water bath for 10 minutes. After cooling the developing color was determined spectrophotometrically at 550 nm using ''spectronic20''. the total carbohydrate content of haemolymph samples was estimated as mg/ml using the following equation.

$$\text{Carbohydrate content} = \frac{\text{absorbance - interception}}{\text{slop}} = \text{mg/ml}$$

Determination of total lipid:

Serum total lipids concentration was determined according to the method of **Frings *et al.* (1972)** by colorimetric method. A sample of total body homogenate was heated with conc. Sulphoric acid, and the mixture was then reacted with phosphoric acid- vanillin reagent to give red to purple color. The intensity of color was measured by photoelectric colorimeter.

Preparation of lipid reagents:

Vanilline reagent: 0.04 M dissolved 6.1 gm of vanilline in H2O and diluted to 1 litre. This solution is stable for about 2 months in a brown bottle at room temperature.

Phosphovanilline reagent: add 350ml of the vanilline reagent and 50 ml of water to flask. Add with constant stirring, 600 ml of conc. Phosphoric acid (85%). This solution is also stable for about 2 months in brown bottle at room temperature. Phosphoric acid, concentrated reagent grade.

Concentrated sulphoric acid, reagent grade

Preparation of standard lipid solution:

In 100 ml flask 0.5 ml of good grade of olive oil was added. The flask was weighted to obtain the exact weight of the oil. The concentration was adjusted to exactly 500mg/100ml with absolute ethanol. This solution is kept for about one month.

Procedure:

In separate tubes add 0.1 ml of H2O (blank), 0.1 ml of sample and 0.1 ml of the standard solution. To each tube add 0.2 ml of concentrated sulphoric acid. Mix well preferably on vortex mixer. Place all tubes in boiling water bath for 10 minutes. Remove and cool in water to room temperature. To each tube add 10 ml of phosphovanilline reagent and mix well. Incubate at 37 C, in a water bath for 15 minutes. Cool and read standard and against black at 540 nm.

Calculation:

Concentration of total lipid in mg/ml=

$$\frac{\text{Absorbance of the sample}}{\text{absorbance of the standard}} \times \text{concentration of standard} \times 1 / \text{Volume of sample used}$$

Morphogenic studies

Third instar larvae were treated with various sub lethal concentrations of above mention plant oils for 48 hours then complete its life cycle on untreated diet. The morphogenic abnormalities of treated larvae and subsequent developmental stages were recorded.

III.RESULTS AND DISCUSSION

Morphogenic studies

Larval malformations:

Malformed larvae due to the exposure to tested plant oils were showed. The malformations were observed during the larval stage manifested Such as, Larvae with dark cuticle around the anterior portion of abdomen, pigmented Larvae and larvae failed to get rid of the reminder old cuticle plate (1). Similar deformed cases were reported by (**Kamal,1998; Shoukry *et al*.,2003 and Shoukry, 2009)**

pupal malformation:

Treatment of larvae with above mention oils, resulted pupae with various morphological abnormalities, that appeared as elongated pupae and larval pupae intermediate plate (2). Similar results were obtained by **Quraishi and Thorsteinson (1965)** on *musca domestica*, they recorded that, the treated larvae failed to contract to the pupal form but did acquire the melanization of the pupal skin, this phenomenon was due to muscle paralysis. **Kamal (1998),** who found that, the neem extract had many deformative effects against different developmental stage of *C. pipiens* and *M. domestica*.

Adult malformation:

Some of the pupae- failed to reach adults. However, some emerged adult has various degrees of morphological abnormalities. Some individuals showed a dominance of incomplete adult eclosion varying from complete eclosion of adults with only their legs or wings were partially

attached to the puparium, also abnormal appearance such as; severally crumpled wing and deformation in the thorax and abdomen were also recorded Plate (3).

Similar morphogenic activities of the tested plant extracts were recorded by many authors on the different insect's species; the results are agreement with data reported by **Ivbijaro (1986),** who tested neem ethanolic extract against *T. castaneum*. He found that, few pupae that survived metamorphosed into malformed adults. Also, **Rembold *et al*. (1980); Saxena *et al.* (1981)** who found different degrees of the adult eclosion after treatment of some lepidoptera species with azadirachtin and **Shoukry *et al*. (2003)** reported that treatment of larval stage of the *plodia interpunctella* with some plant oils induced some morphogenic abnormalities in the adult stages.

Biochemical studies

Haemolymph protein

Protein is the major cell components which play the most important role in all biological process including reproduction (**El-Halafawy *et al.,*2001**), development, growth and performance of its vital activities **(Rashawn, 2013).**

The obtained data are tabulated in table (1), showed that the haemolymph of the control larvae (third instar) contained 0.25±0.024 mg/ml of total proteins. Application of two fixed oils and three volatile oils produced the same effect in the haemolymph total protein content of the treated larvae. Treatment with sub lethal concentration of both fixed and volatile oils produced significant increase in the haemolymph total protein (P>0.05). The black seed oil and clove oil were the most potent oils which induced higher increased in the protein content.

the increase in the total hemolymph protein may be a kind of detoxification mechanism. In this respect, **Wilkinson (1976)** stated that protein helps to synthesize microsomal detoxifying enzyme which assists to detoxify the toxicants that entered into the animal body. **Radwan and Shaurub (1995)** found that cypermethrin increased the total protein of the treated larvae of the blow fly, *chrysomya albiceps*.

Contrariwise some reports indicated that plant extracts reduced protein level **(Sammour *et al.,*2011 and Shoukry *et al*.,2013)**. The protein content showed mixed result between elevation and reduction in *sitophilus* insects after clove, anise oils, diatomaceous earth, malathion and spinetoram treatment by (**Askar *et al*. 2016).** Similar mixed result was obtained by **Draz *et al*. (2016),** protein content was increased in *S. oryza* after malathion treatment and decreased after jasmine, thyme, camphor, chamomile, mint, sesame and basil oil treatment.

Haemolymph carbohydrates

In most insect, carbohydrates serve reserves are present as glycogen and trehalose which can be ready converted into glucose for the support of all life processes. Metamorphic changes in insect are usually accompanied by substantial depletion of their

carbohydrate reserves. During this period, glycogen and treholase supply glucose which provides an energy source and a substrate for the synthesis of nymphal and adult tissues, especially the cuticle **(Rashwan, 2013).**

Data in the table (1) show that, the level of haemolymph carbohydrate of normal larvae was 1.7±0.057, while the treatment with different oil extracts caused a significant decrease in the haemolymph carbohydrate contents. The volatile, clove oil and fixed, black seed oil were more effective in decreased the level of haemolymph carbohydrates.

The present investigations are comparable with the findings of **Khalaf (1998)** who reported that, treatment of the second larval instar of *muscina stabulans* with two plant oils of *Cymbopogon citratus* and *posmarinus efficinalis* was induced a significant reduction in the carbohydrate content of the whole pupal period and **ABO EL-Ghar *et al*., (1995)** showed that the petroleum ether extract of *Ammi majus* and *Apium graveolens* fed to six instar larvae of *Agrolis ipsilon* greatly reduced the haemolymph carbohydrates. **El-sherif (1995)** concluded that treatment of the 1st and the 6th instar nymph of *S. gregaria* with JHA pyriproxyfen increased the haemolymph carbohydrate level.

Carbohydrates are of vital importance since they can be utilized by the insect's body for production of energy or conversation of lipids or proteins **(Fahmy,2017).** That explains the total carbohydrate content was decreased for the larvae fed on treating artificial diet. Under stress conditions, more sugars might be metabolized to meet out the energy expenses. This could be the reason for the carbohydrate level depletion in treated insects **(Fahmy,2008).**

Generally, Carbohydrates metabolism is controlled primarily by amylase, invertase and trehalase enzymes **(Wigglesworth,1972).** Alpha-amylase is an enzyme that break down starch to oligosaccharides then hydrolyzed to glucose by glucosidases **(Terra& Ferriera 2005 and Kaur *et al*.,2014).** Alpha-amylase, in physiological status, improve the insect digestive performance, resulting in survival in different conditions and raise their biological fitness (**Kaur *et al.*,2014).**

Invertase enzyme hydrolyzes sucrose into monosaccharides; glucose and fructose so it could be utilized by insects **(Heil *et al.,* 2005).** Trehalose breakdown of trehalose into 2 glucose molecules. So, the defect in the enzyme activities could impede the supply of glucose, which is needed for chitin build up **(Wyatt,1967 and Candy & Kibly,1962).**

Haemolymph lipids

The obtained data was tabulated in table (1). the haemolymph of untreated larvae was 0.050±0.001 mg/ml. Treatment with sub lethal concentration of both fixed and volatile oils produced significant increase in the haemolymph total lipid (P>0.05). the black seed oil and clove oil were the most potent oils which induced higher increased in the lipid content.

The rise in total lipids agrees with the finding of **Mostafa (1993),** who showed 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. And **Ibrahim (2006)** found highly significant increase in total lipid content of *S. littoralis* pupae treated with plant extracts.

Contrariwise some reports indicated that plant extracts reduced lipid level **Abo El-Maaty (2003)** found reduction in total lipids content of third instar larvae of M. domestica treated with *L. pruinosum* and *C. procera*. **Sabry (2004)** showed that the total lipids content significantly decreased with volatile oils treatment. And mixed result between elevation and reduction of the total lipid content on the *Synthesiomyia nudiseta* larvae treated with some fixed and volatile oil was reported by **Shoukry (2009).**

Generally, we concluded that, the Increase the level of protein content may be related to lack of juvenile hormone as reported by **Hill and Izatt (1974)** stated that accumulation of lipids in the fat tissues of one-day old female of desent locust resulted in accumulate lipids in the fat tissues, this might be due to high deposition of lipid in the tissue 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 plants oils extracts may cause degeneration of corpora alata and which may be allow elevation in the mean lipid content. As well as The depletion of carbohydrates content in the treated insect larvae with both volatile and fixed oil may be related to the inhibitions of carbohydrate hydrolyzing enzymes by the treatment with plant oils **(Yacoub,2013; Rashwan,2013; Srour,2014 and Gad 2019)** this might be affecting the growth and molting process and then could explain the morphogenetic abnormalities which induced by these treated oils.

A. Larva **B. Pupa** **C. Adult**

Normal developmental stages of *Ephestia cautella.*

pigmented larva

larvae failed to get rid of the old cuticle

Plate 1. Morphogenic abnormalities on Larval stage of *Ephestia cautella*.

Larval pupal intermediate

Elongated pupa

Plate 2. Morphogenic abnormalities on Pupal stage of *Ephestia cautella*.

incomplete adult eclosion crumpled wing crumbled thorax and abdomen

Plate 3. Morphogenic abnormalities on adult stage of *Ephestia cautella*

Plant oils	Conc	Total proteins(mg/ml)	Total carbohydrates (mg/ml)	Total lipid (mg/ml)

		Mean ± SE	Change (%) to control	Mean ± SE	Change (%) to control	Mean ± SE	Change (%) to control
Black seed	10	0.40±0.02*	+60	0.39±0.557*	-77.1	0.068±0.033*	+36
	5	0.41±0.02*	+64	0.42±0.557*	-75.2	0.065±0.033*	+30
	2.5	0.39±0.013*	+56	0.47±0.0576*	-72.3	0.062±0.034*	+24
	1.25	0.38±0.01*	+52	0.51±0.0542*	-70	0.060±0.036*	+20
	0.75	0.37±0.02*	+48	0.52±0.057*	-69.4	0.060±0.038*	+20
Cumin	10	0.32±0.06*	+28	0.60±0.056*	-64.7	0.055±0.065*	+10
	5	0.31±0.06*	+24	0.65±0.056*	-61.7	0.054±0.066*	+8
	2.5	0.31±0.05*	+24	0.72±0.053*	-57.6	0.054±0.068*	+8
	1.25	0.29±0.06*	+16	0.79±0.051*	-42.4	0.052±0.066*	+4
	0.75	0.26±0.06*	+4	0.80±0.053*	-52.9	0.052±0.059*	+4
Garlic	10	0.29±0.014*	+16	0.65±0.057*	-61.7	0.057±0.038*	+14
	5	0.28±0.014*	+12	0.70±0.057*	-58.8	0.056±0.038*	+12
	2.5	0.26±0.014*	+4	0.76±0.053*	-55.3	0.055±0.034*	+10
	1.25	0.25±0.013*	+0	0.76±0.045*	-55.3	0.055±0.038*	+10
	0.75	0.23±0.014*	+8	0.79±0.048*	-53.5	0.054±0.032*	+8
Ginger	10	0.33±0.08*	+32	0.65±0.057*	-61.7	0.055±0.019*	+10
	5	0.32±0.08*	+28	0.70±0.051*	-58.8	0.055±0.017*	+10
	2.5	0.32±0.017*	+28	0.72±0.0057*	-57.6	0.055±0.016*	+10
	1.25	0.31±0.06*	+24	0.72±0.0048*	-57.6	0.054±0.016*	+8
	0.75	0.30±0.08*	+20	0.79±0.0057*	-53.5	0.052±0.016*	+4
Clove	10	0.41±0.034*	+64	0.31±0.03*	-81.7	0.063±0.018*	+26
	5	0.42±0.032*	+68	0.32±0.03*	-81.2	0.063±0.016*	+26
	2.5	0.40±0.034*	+60	0.37±0.037*	-78.2	0.062±0.0016*	+24
	1.25	0.39±0.036*	+56	0.40±0.039*	-76.5	0.062±0.0057*	+24
	0.75	0.32±0.034*	+28	0.45±0.033*	-73.5	0.060±0.0016*	+20
Control		0.25±0.024		1.7±0.0577		0.050±0.0014	

Table 1. Effect of plant oils (fixed and volatile) on haemolymph total protein, carbohydrate and Lipid content of *Ephestia cautella*

- Asterisk refers to significant differences from control (P>0.05)
- Non-asterisk refers to non-significant differences from control

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