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Biochemical effect of *Eucalyptus* and Neem-based formulations on *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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The 3rd instar larvae of the peach fruit fly, *Bactrocera zonata* resulted in larval artificial diet treated with 0.5% of crude extracts (CEs) and essential oils (EOs) of *Eucalyptus* and Neem were chemically analyzed to determine their effects on certain enzymatic and non-enzymatic activities. The CEs and EOs significantly and differently reduced acetylcholinesterase in the 3rd instar larvae. Both CE and EO of *Eucalyptus* reduced α-esterases. On the other hand, CE and EO of Neem increased the amount of α-esterases in larvae of *B. zonata*. The amounts of β-esterases were high significantly and differently influenced in treated larvae. *Eucalytus* EO reduced the amounts of β-esterases in treated larvae. The amounts of chitinase in the 3rd instar larvae was low significantly differed according to the treatments. *Eucalyptus* EO increased chitinase compared to untreated individuals. The amount of protease in 3rd instar larvae of *B. zonata* resulted in treated larval artificial diet with CEs or EOs of *Eucalyptus* and Neem low significantly. Meanwhile, the increase in the total carbohydrates content of treated larvae was observed in all treatments compared with control except *Eucalytus* oil. While the lipid and protein contents were significantly decreased in all treatments except only in Neem oil-treated larvae.

Keywords: *Bactrocera zonata, Eucalyptus*, Neem, biochemical effect.

INTRODUCTION

Bactrocera zonata (Saunders) is dangerous, multivoltine, polyphagous and cosmopolitan insect pest which are considered the most destructive pest since it infests fruits directly causing a great damage to them. The control measures for fruit flies adopted mainly on contact conventional insecticides which cause destructive effects on the environment, human health, wild life and biological control agents (Purcell & Schroeder, 1996; Mochi et al., 2006 and Stark & Vargas, 2009). Many researchers tried to study the toxic and developmental adverse effects as well as biochemical actions of several extracts or essential oils of many wild, medicinal and aromatic plants against more than one species of insects (Saleh,1995; Bazzoni et al., 2000; Agar et al., 2005; Pavela, 2009; Kamaraj et al., 2010; Benelli et al., 2013 and Kumrungsee et al., 2014). The present study aims to provide informations about the effects of crude extracts (CEs) and essential oils (EOs) of certain medicinal and aromatic plants (*Eucalyptus* and Neem) on certain enzymatic and non-enzymatic activities in *B. zonata* (Saunders)

MATERIALS AND METHODS

Insect Rearing

A colony of *B. zonata*, was obtained from Agricultural Research Center, Plant Protection Research Institute, laboratory of Horticulture Insects Department, Dokki, Giza under conditions of 25 ± 3ºC and 60 ± 5% R.H. Larvae were reared on an artificial diet as described by Shehata et al., 2006.

Plants Used

Both CEs and EOs of different parts of medicinal and aromatic plants were leaves of Long leaved eucalyptus, *Eucalyptus longifolia* (Myrtaceae), and seeds of Neem, *Azadirachta indica* (Meliaceae). These CEs and EOs were obtained from El-Abgy Factory for Extracting Oils, District of El-Bistatin, Cairo, Egypt.

To study effect of the tested plants on certain enzymatic activities, about 50 individuals of full grown 3rd instar larvae of the peach fruit fly resulted in larval artificial diet treated with 0.5% of CEs or EOs of the tested medicinal and aromatic plants (long leaved eucalyptus, *Eucalyptus longifolia* and Neem, *Azadirachta indica*) were obtained. The respective used concentrations were prepared. These individuals were homogenized in a chilled glass Teflon homogeneizer (ST-Mechanic-Preczyina, Poland). Centrifugation was done by a refrigerated centrifuge (6 MR, USA). Double beam ultraviolet/ visible spectrophotometer (sectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances. The insects were homogenized in distilled water (50 mg/1ml). Homogenates were centrifuged at 8000 rpm for 15 min at 5ºC in a refrigerated centrifuge. The deposites were discarded and the supernatant were kept in a deep freezer till use.

Determination of acetylcholinesterase (AchE)

AchE activity was measured according to Simpson et al. (1964) method, using acetylcholine bromide (AchBr) as substrate.

Determination of non-specific esterases

α-esterases and β-esterases were determined according to Van Asperen (I962) using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively.

Determination of chitinase

Preparation of substrate

According to Bade and Stinson (1981), colloidal chitin was prepared as follows: 4.0 gm of purified chitin powder was suspended in 100 ml water at 4ºC and stirred in cold. At 4ºC, 30 ml of concentrated H_2SO_4 was added drop wise to the suspension. Through glass wool, cold viscous chitin solution was filtered into 1800 ml ice-cold 50% ethonal with rapid stirring. The precipitated colloidal chitin was washed with distilled water to pH 5. Before use as substrate, it was buffered with phosphate buffer (pH 6.5, 0.2 M).

Assay of enzyme

According to Ishaaya and Casida (1974), with some modifications, the reaction mixture consisted of 200 ml 0.5% colloidal chitin, 200 ml enzyme solution and 1 ml phosphate buffer (0.2 M, pH 6.5). After 1.5 hour incubation at 37 ºC, enzyme activity was terminated by boiling test tube. Centrifugation for 15 min at 8.000 rpm sedimented undigested chitin. The supernatant was taken for determination of N-acetylglucoseamine that produced as a result of chitin digestion by chitinase.

Determination of N-acetylglucoseamine

According to Waterhouse et al. (1961), N-acetylglucoseamine wsa determined. From the supernatant, the volume of the appropriate aliquot was adjusted to 1 ml with phosphate acetate buffer (0.2 M, pH 6). For each determination, a series of N-acetylglucoseamine standards (10, 20, 40, 60 and 80 µg) and one ml buffer blank in 1 ml buffer were set. Each tube was shaken and heated in a boiling water bath for 10 min after the addition of 0.3 ml saturated sodium borate solution. 8 ml glacial acetic acid was added to each after the tubes were then transferred rapidly to cold water. In 50 ml glacial acetic acid and 2.5 ml concentrated HCl, the 1 ml freshly prepared, modified Ehrlich reagent (1 gm p-dimethylaminobenzoate was added. At room temperature, the tubes were shaken, and allowed to stand for 30 min. At 540 nm, the optical density was read against the buffer blank. The enzyme activity was expressed as µg N-acetylglucoseamineNAGA) × 10^3 / min / gm fresh weight.

Determination of proteolytic activity

According to Tatchell et al. (1972), proteolytic activity was measured with some modifications. During one hour incubation at 30º C, the increase in free amino acids split from substrate protein (albumin) was measured. According to Lee and Takabashi (1966) method, amino acids were colorimetrically assayed by nonhydrin reagent.

Total carbohydrates determination

In acid extract of sample by the phenol-sulfuric acid reaction, total carbohydrates were estimated by Dubois et al., (1956). According to Crompton and Birt (1967) total carbohydrates were extracted and prepared for assay**.**

Total lipids determinations

According to **(**Knight et al., 1972), total lipids were estimated by using phosphovanillin reagent.

Total proteins determinations

According to Bradford (I976), total proteins were determined.

Statistical Analysis

Using one-way analysis of variance (ANOVA), the data were statistically analyzed supported by Duncan's multiple range test (Duncan, 1955) running on CoStat statistical software (1990). At 5% significance level, means were compared using L.S.D.

RESULTS

Effect of tested crude extracts and essential oils on the enzymatic activities:

Data given in Table (1) the activity of acetylcholinesterase (AchE) of *B. zonata* larvae was determined after treatment with 0.5% conc. of oils and extracts of *Eucalyptus* and Neem. The results showed that AchE activity of *B. zonata* larvae was significantly decreased with the *Eucalyptus* extract followed by Neem extract, then *Eucalyptus* oil and Neem oil compared with control with the percentage of change was 65.52, 37.24 and 17.8%, respectively.

The effect of the tested oils and extracts (0.5%) on *B. zonata* α- esterases activity of larvae was recorded (Table 1). As shown from the results a significant ($p<0.05$) decrease in α-esterases activity was induced in the *Eucalyptus* oil- and extract-treated larvae as compared with control (Table 1). A reduction percentage 27.19 and 11.89% in α- esterases activity was induced by *Eucalyptus* oil and extract, respectively. However, the α- esterases activity was significantly increased in larvae treated with both Neem oil and extract compared with untreated larvae being percentage change was -5.09 and -32.91%.

In addition, the activity of ß- esterases of *B. zonata* larvae was determined after treatment with 0.5% -oils and -extracts of Eucalyptus and Neem (table 1). The results showed that ß- esterases activity of *B. zonata* larvae was significantly decreased with the *Eucalyptus* oil compared with control with the percentage of change was 13.65%, respectively. However, the ß- esterases activity was significantly increased in larvae treated with *Eucalyptus* extract, Neem oil and its extract being percentage changes were -10.42, -5.53 and -3.55%, respectively compared with untreated larvae.

On the other hand, as represented in table (1), the proteases activity was significantly decreased in larvae treated with Neem oil, Neem extract, *Eucalyptus* extract and *Eucalyptus* oil compared with control, where the percentages of change were 28.35, 18.81, 6.70 and 3.87%, respectively.

Also, data given in table (1) indicated the effect of the tested oils and extracts (0.5%) on *B. zonata* chitinase activity of larvae. The results showed that chitinase activity of *B. zonata* larvae was significantly decreased with Neem extract, *Eucalyptus* extract and Neem oil where the percentages of change were 14.21, 6.68 and 1.87%, respectively. Meanwhile, the chitinases activity was significantly increased in larvae treated with *Eucalyptus* oil compared with untreated larvae.

Effect of tested crude extracts and essential oils on the non-enzymatic activities:

Data in table (2) showed that the increase in the total carbohydrates content of treated larvae which observed in all oils treatment different significantly compared with control except *Eucalytus* extract and Neem extract. While the total carbohydrates content of *Eucalytus* oil treated larvae (16.22 ± 1.11 mg/g.b.wt.) was significantly lower than that of the control (22.81 ± 1.5 mg/g.b.wt.).

The mean of body lipid contents of the treated larvae by both *Eucalyptus* oil, extract and Neem extract (3.43 ± 0.53, 4.11 ± 0.44 and 4.64 ± 0.84 mg/g.b.wt., respectively) was significantly lower than that of the control (13.33 ± 0.62 mg/g.b.wt.). Meanwhile, Neem oil increase mean body lipid contents of the treated larvae compared with untreated ones (table 2).

Moreover, the changes in total protein content were conducted as a result of extracts and oils treatment in general, the total protein content in the homogenates larvae were significantly reduced as compared to control except for Neem oil treatment.

Table (1): Effect of *Eucalyptus* and Neem essential oils and crude extracts on certain enzymatic activities of *B. zonata* larvae.

Treatmens		Biochemical parameters					Sensetivity %				
		Acetylcholinesterase	α-esterases	β-esterases	chitinase	proteases	Acetylcholinesterase	α-esterases	β-esterases	chitinase	proteases
control		151.70±4.42^{a}	1590.0±20.96^{c}	403±7.45^{d}	353.3±16.80^{b}	38.80±2.85^{a}	--	--	--	--	---
Eucalyptus	oil	95.70±1.86^{c}	1157.7±23.28^{e}	380.0±7.02^{e}	486.7±8.90^{a}	37.30±0.70^{b}	36.91	27.19	5.71	-37.76	3.87
	extract	52.30±2.97^{e}	1401.0±11.34^{d}	445.0±8.37^{a}	329.7±7.91^{d}	36.20±1.98^{c}	65.52	11.89	-10.42	6.68	6.70
Neem	oil	124.70±2.44^{b}	1671.0±14.83^{b}	425.3±8.58^{b}	346.7±12.30^{c}	27.80±0.99^{e}	17.80	-5.09	-5.53	1.87	28.35
	extract	95.20±0.93^{d}	2113.3±68.84^{a}	417.3±7.86^{c}	303.1±5.93^{e}	31.50±1.22^{d}	37.24	-32.91	-3.55	14.21	18.81
LSD 5%		0.325	12.095	6.462	3.161	0.552	--	--	--	--	--

Sensetivity % = control- treatment / control * 100

Table (2): Effect of *Eucalyptus* and Neem essential oils and crude extracts on certain non-enzymatic activities of *B. zonata* larvae.

Treatments		Biochemical parameters			Sensetivity %		
		carbohydrates	lipids	proteins	carbohydrates	lipids	proteins
control		22.81±0.87^{c}	13.33±0.36^{b}	94.40±1.45^{b}	--	--	--
Eucalyptus	oil	16.22±0.65^{d}	3.43±0.31^{d}	74.07±0.62^{d}	28.89	74.49	21.54
	extract	25.27±0.71^{b}	4.11±0.26^{cd}	94.40±0.81^{b}	-10.78	69.24	74.07±0.62
Neem	oil	27.97±0.71^{a}	14.81±0.62^{a}	98.00±1.80^{a}	-22.62	-11.10	-3.81
	extract	24.71±0.95^{b}	4.64±0.49^{c}	81.20±0.76^{c}	-8.33	65.19	13.98
LSD 5%		1.43	0.892	1.25	--	--	--

Sensetivity % = control- treatment / control * 100

The mean of body protein content of the treated larvae was 74.07 ±1.06, 81.20±1.3 and 94.4±1.4 mg/g.b.wt. with *Eucalyptus* oil, Neem extract and Eucalyptus extract, respectively, as compared to 94.4± 2.5 mg/ g.b.wt. in control. The change percentages in the total protein content with the above mentioned extracts were 21.54, 13.98 and 0.00%, respectively (table 2).

DISCUSSION

The reduction obtained in both AchE and both α-, β esterases were with accordance with El (Kady et al., 2008) who found a reduction in Anopheles multicolor and Culex pipiens AChE activity caused by vertimec and spinotram bioinsecticides. In addition, after 24 h of exposure, the exposed mosquito had a decreased α and β-esterases. (Sharma et al., 2011) concluded that Azadiracta indica and Artemicia annua extracts produce significant alterations in the biochemical profiles of larvae of culicine and anopheline. Megahed et al., (2013) found that 4th instar larvae of Spodoptera littoralis treated with abamectin, emamectin benzoate and spinosad had a decrease in AChE activity, total lipid and total protein contents. Gamil et al., (2011) found that M. domestica treated with Curcuma longa (Turmeric) had a significant decrease in protein and total carbohydrate contents.

The amounts of chitinase in both the 3rd instar larvae were high significantly differed according to the treatments. Crude extracts of *Eucalyptus* decreased chitinase in the full grown larvae, whereas essential oils of the same plant increased it compared to untreated individuals. While the decrement in chitinase in larvae caused by crude extract and essential oils of Neem was 14.21 and 1.87%, respectively.

The tested crude extracts and essential oils reduced the amounts of proteases in both treated larvae. *Xanthogaleruca luteola* Mull. and *Eurygaster integriceps* Puton have shown similar reduction in protease and its substrate levels of hemolymph and midgut tissue due to the effect of *Artemesia annua* L. extracts (Zibaee and Bandani 2010). Comparable results have been observed in *S. litura* upon treatment with azadirachtin extracts.

B. zonata larval treatment with *Eucalyptus* extract significantly decreased total carbohydrates content which was in harmony with Sharma et al., 2011. Also, the results agreed with Shoukry et al., (2003) who studied the biochemical changes in the *Plodia interpunctella* haemolymph of larvae treated with sublethal concentrations of three fixed oils and two volatile oils and found that levels of haemolymph carbohydrates contents were decreased in all oil treatments. It also agreed with the finding of (Gareth et al., 2006) who observed that carbohydrates content was decreased in oil-treated insects.

CEs and EOs of the tested plants highly reduced the amounts of total lipids in larvae except neem oil. The reason for the decrease in the total lipid content may be owing to its conversion to proteins to produce supplementary energy or to substitute the reduction in protein content.

Own results agree with these results obtained by many investigations (Abou El-Ela et al., 1998; Abd El–Aziz, 2000; Omar et al., 2005b and Rawi et al., 2011). While Mostafa (1993) found that *T. granarium* treated with plant extracts had increased total lipid content. Also Abou El-Ela et al., (1995) found the similar result on Musca domestica treated with water extracts of some plants, and Shoukry et al., (2003) who recorded the biochemical changes in *Plodia interpunctella* haemolymph larvae treated with sublethal concentrations of three fixed oils and two volatile oils and found that all oil treatments decreased their carbohydrate contents but increased the levels of haemolymph lipids.

In addition, both CEs and EOs reduced the amounts of total protein in treated larvae. The reduction of the protein contents is a result of breakdown of these proteins into amino acids which are used in the compensatory mechanism to supply energy for the insect to recover from insecticidal stress (Ali et al., 2014).

CONCLUSION

It may be concluded the botanicals used had direct adverse effects on the development of *B. zonata*. Among the tested plants the essential oils were more effective than crude extracts. These botanicals are available throughout the country and the farmers may use these plants easily for the management of other related pest species.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Dr. Naeem M. Eesa and Dr. Hanaa A. El-Sherif suggested main topic of the manuscript and chose members of this team work, Mai K. Daif worked experiments of the manuscript, Dr. A.M.Z. Mosallam provided necessary laboratory facilitates and Dr. Dina H. Abd El-Monem designed the experiments, made statistical analysis, wrote the manuscript, followed up work progress and was the corresponding author of this work.

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