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Toxic and Biochemical Effects for Certain Natural Compounds on the Peach Fruit Fly, *Bactrocera zonata* (Diptera, Tephritidae)

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ABSTRACT

The Peach Fruit Fly (PFF), *Bactrocera zonata* (Saunders) is a serious pest of horticulture of crops in Egypt. The present study aimed to evaluate toxicity of the biopolymer Chitosan (Chitosan1) and its derivatives, viz., Chitosan2 (N-(2-nitrobenzyl)), Chitosan3 (N-(2-chloro, 6-fluorobenzyl)), Chitosan4 (N-(4-propylbenzyl)), Chitosan5 (N-(3,4-methelynedioxybenzyl)) as well as the bio-pesticide, Bio-fly under laboratory conditions. Also, the effect of the tested compounds on AChE and ATPase activities of both male and female flies of the PFF was assessed. Results showed that Bio-fly was the most toxic compound against both female and male flies of PFF (LC_{50} = 2408 and 2049 and 1333 and 1145 mg L^{-1} after 24 and 48 h of treatment, respectively). Among Chitosans, Chitosan2 and Chitosan4 were the most potent compounds against female and male flies after 24 and 48 h, respectively. The respective LC_{50} values were 4993 and 4817 and 6115 and 51775 mg L^{-1}. AChE and ATPase activities were significantly reduced whether for female or male flies at 48 h post-treatment. Bio-fly exhibited the highest inhibition of AChE activity, whereas Chitosans gave the highest inhibition of ATPase activity. Chitosan2 and Chitosan4 could be incorporated in the integrated management programs of the PFF.

Key words: Peach fruit fly, *Bactrocera zonata*, insecticidal activity, Bio-fly, Chitosan derivatives, AChE, ATPase

INTRODUCTION

The Peach Fruit Fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a very serious polyphagous pest which causes devastating loss of fruit production in the whole Mediterranean basin (Clarke *et al*., 2005; Duyck *et al*., 2004). PFF is an active insect pest throughout the year in Egypt and it has four to six considerable economically generations per year (El-Gendy and El-Saadany, 2012). Where, it is considered as a quarantine pest.

Current control methods of the PFF rely heavily on the aerial application pesticides specially malathion by bait spray or ground cover spray (Roessler, 1989). It is known that pesticides have harmful environmental effects on beneficial insects. So, the friendly environmental control methods are highly requested. Hence, Chitosan compounds might be used as alternatives of pesticides because of it might possess insecticidal activity and their non-toxic effect to vertebrates and humans (Rabea *et al*., 2003a; Badawy *et al*., 2005). The insecticidal activity of Chitosan was first reported against the lepidopterans, the cotton leafworm *Spodoptera littoralis* (Boisduval)

(Rabea *et al*., 2003b), *Helicoverpa armigera* (Hübn) and *Plutella xylostella* (L) and aphids such as *Aphis gossypii* (Glover), *Aphis nerii*, *Metopolophium dirhodum* (Walker), *Hyalopterus pruni* (Geoffroy), *Rhopalosiphum padi* L., *Sitobium avenae* (Fabricius) and *Myzus persicae* (Sulzer) (Badawy and El-Aswad, 2012; Zhang *et al*., 2003).

Biological control agents are increasingly used to control fruit pests. Bio-fly is a bio-pesticide of the fungus of *Beauveria bassiana* (Hajek and St. Leger, 1994). *B. bassiana* is a vital entomopathogenic fungus currently used as a bio-control agent for a diversity of insect pests such as pupae and adults of *Ceratitis capitata* (Wiedemann) (Dimbi *et al*., 2003; Ekesi *et al*., 2005; Konstantopoulou and Mazomenos, 2005; Quesada-Moraga *et al*., 2006).

The public demand for insecticide-free fresh fruit is encouraging the use of friendly-environmental bio-pesticides for pest control (Hsu and Feng, 2006). Thus, the present study aimed to evaluate the biological activity of Chitosan, its derivatives and bio-fly pesticides against male and female flies of PFF as well as their impact on some biochemical parameters of the flies.

MATERIALS AND METHODS

Peach Fruit Fly (PFF) culture: The initial culture of the Peach Fruit Fly (PFF), *Bactrocera zonata* (Saunders) had been obtained in May 2011 from infested mango fruits which were collected from a farm at El-Dalangat City, El-Beheira governorate, Egypt.

Mass rearing technique: The infested mango fruits were transferred to Laboratory of Fruit Flies Eradication Program at El-Beheira Governorate. Fruits were maintained in plastic jars furnished by sterilized sand and incubated under laboratory conditions (27±1°C, 60±5% R.H. and 14:10 L: D photoperiod) until complete pupation. Pupae were collected and placed in Petri-dishes inside the rearing cages. The newly emerged flies were provided with sugar mixed with hydrolyzate protein (3:1 w/w) and a source of water. The deposited eggs were collected every 24 h and washed with tap water and placed on an artificial diet (El-Gendy, 2002). After pupation, pupae were placed in Petri-dishes inside the rearing cages to start a new generation following the above mentioned methods (El-Gendy, 2002). The first generation of PFF in the laboratory was used for toxicity and biochemical assays.

Chemicals used: A biopolymer Chitosan (Chitosan1) and its derivatives (Fig. 1), Chitosan2 (N-(2-nitrobenzyl)), Chitosan3 (3N-(2-chloro-6-fluorobenzyl)), Chitosan4 (N-(4-propylbenzyl)) and Chitosan5 (N-(3,4-methelynedioxybenzyl)) were parchased from sigma-Aldrich Co., (St. Louis, USA). Bio-pesticide, Bio-fly, *Beauveria bassiana* fungus was purchased from E1-Nasr Bioinsecticides and fertilizers Company, E1-Sadaat, Egypt and applied at a rate of 100 cm^3 100 L^{-1} water. Buminal (Hydrolyzed Protein 39.78% SL) was purchased from Bridge Trade Co., Germany. Acetylthiocholineiodide (ATChI), adenosinetriphosphate (ATP), Bovine Serum Albumin (BSA), 5,5'-dithio-bis (2-nitrobenzoic) acid (DTNB), trichloroacetic acid (TCA), Folin-Ciocalteu phenol reagent and Tris-HCl were purchased from Sigma-Aldrich Chemical Co., USA.

Toxicity assay: Ten male and female flies of PFF (6-7 days old) were confined in plastic cup covered with a muslin cloth, without food and water for 12 h before treatment. Fiber-broad papers (1.0 inch diameter and 0.1 thicknesses) were immersed in a series of concentrations of Chitosan, its derivatives and Bio-fly for 10 min. Test concentrations were mixed with attractive feeding material 10% (Buminal). Five replicates were used for each concentration while untreated flies

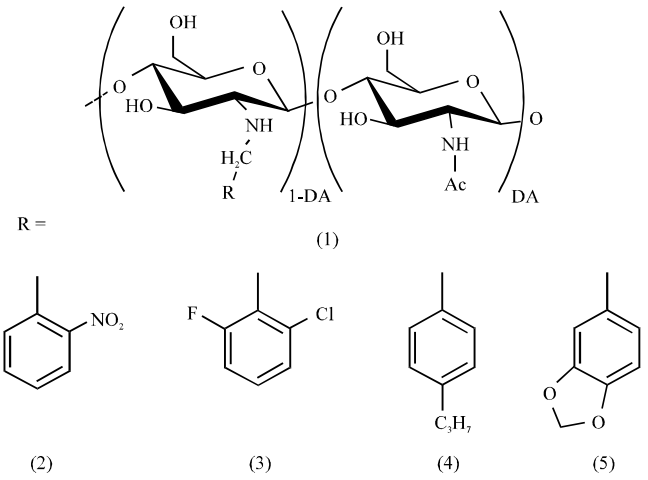

Fig. 1: Chemical structure of Chitosan and its derivatives

(control) with attractive feeding 10%. The Fiber-broad papers were hung inside the cups (20 cm height and 7 cm diameter). Mortality for each sex was observed and recorded after 24 and 48 h post-treatment and dead flies were removed daily.

Enzyme assay: Twenty of treated and un-treated flies of each sex of PFF were collected after 48 h post-treatment with Chitosan1, its derivatives and Bio-fly as above mentioned method. The flies were confided in Eppendorf tubes under freezing conditions.

Acetylcholinesterase (AChE) assay: Ten heads of treated and un-treated female and male flies of the PFF were isolated on ice and homogenized in Potassium Phosphate Buffer (PPB), pH 7.0 using a glass/Teflon Homogenizer at 4°C. The homogenates were centrifuged at 5000 rpm for 20 min at 0°C. A mixture of PPB, the supernatant (crude enzyme), DTNB and ATChI (substrate) was incubated for 30 min at room temperature (27±2°C) and followed by the measurement of the Optical Density (OD) spectrophotometricaly at 412 nm using Jenuway 6305 UV/Vis spectrophotometer (Ellman *et al*., 1961).

Adenosinetriphosphatase (ATPase) assay: Ten heads of treated and un-treated female and male flies of the PFF were isolated on ice, homogenized in Tris-HCl buffer (pH 7.4) and centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was re-centrifuged at 17.000 rpm for 30 min at 4°C. The pellet was re-suspended in the same buffer. The suspension was used as a crude enzyme source for ATPase assay. OD was measured at 740 nm using Jenuway 6305 UV/Vis spectrophotometer (Koch, 1969).

Total protein assay: Total protein contents of adult insect heads were determined by the adoption of the method of Lowry *et al.* (1951).

Statistical analysis: The concentration required to kill (LC_{50}) and/or inhibits 50% of enzyme activity (I_{50}) of treated flies were calculated according to Finney (1971) using Ldp line® software. The analysis of variance was performed by subjecting to ANOVA (Duncan's) using CoStat Software (CoStat, 1990) to evaluate whether there are any significant differences ($p \leq 0.05$) between whole treatments and their interactions.

RESULTS

Acute toxicity assay: Toxicity of the biopolymer Chitosan (Chitosan1), its derivatives (Chitosan2 (N-(2-nitrobenzyl)), Chitosan3 (N-(2-chloro-6-fluorobenzyl)), Chitosan4 (N-(4-propylbenzyl)), Chitosan5 (N-(3,4-methelynedioxybenzyl)) and the bio-pesticide (Bio-fly) against both female and male flies of the Peach Fruit Fly (PFF), *B. zonata* after 24 and 48 h post-treatment is presented in Table 1-2. A broad range of susceptibility to tested compounds was obtained not only among female and male flies but also between tested times 24 and 48 h. Results in Table 1 revealed that Bio-fly possessed the highest toxic effect against female flies along the tested times based on LC_{50} values, 2408 and 2049 mg L^{-1} at 24 and 48 h post-treatment, respectively. Chitosans were ranked in toxicity second after Bio-fly. Toxicity of Chitosans are arranged in a descending order as followed: Chitosan2, Chitosan4, Chitosan5, Chitosan3 and Chitosan1. The corresponding LC_{50} values were arranged asendingly as followed; 4993, 5452, 5944, 5996 and 10948 mg L^{-1}, respectively at 24 post-treatment. The LC_{50} values were inversely with elapsed tested times, where Chitosans toxicity was as the following in a descending order; Chitosan2, Chitosan5, Chitosan4, Chitosan3 and Chitosan1; 4817, 4923, 4996, 5055 and 9216 mg L^{-1}, respectively at 48 h post-treatment. Significant differences ($F = 157321.73$, $df = 5$, $p \leq 0.001$) were obtained between treatments with respect to toxicity as well as, high significant interaction ($F = 2427.26$, $df = 5$, $p \leq 0.001$) was obtained between tested compounds and time, 24 and 48 h.

With regard to male flies, the Bio-fly exhibited the highest toxic activity after 24 or 48 h post-treatment, LC_{50} = 1333 and 1145 mg L^{-1}, respectively (Table 2), followed by Chitosan4, 2, 5, 3 and 1 with LC_{50} = 6115, 6204, 7575, 7708 and 12030 mg L^{-1}, respectively at 24 h

Table 1: Acute toxicity of Bio-fly and Chitosans against female flies of PPF, *B. zonata*, 24 and 48 h post-treatment

	LC_{50} (mg L^{-1})±S.E after treatment (h)		
Compounds	24	48	Overall Mean±S.E
Bio-fly	2408.00±5.18^{f}	2049.00±3.40^{f}	2228.50±14.05^{f}
Chitosan1	10948.00±3.59^{a}	9216.00±3.85^{a}	10082.00±30.80^{a}
Chitosan2	4993.00±03.12^{b}	4817.00±4.15^{e}	5024.00±5.94^{e}
Chitosan3	5996.00±4.13^{c}	5055.00±3.43^{b}	5525.50±22.70^{b}
Chitosan4	5452.00±4.73^{d}	4996.00±3.75^{c}	5224.00±15.82^{d}
Chitosan5	5944.00±3.86^{e}	4923.00±03.14^{d}	5433.50±23.65^{c}
Overall mean±S.E	5956.83±51.11^{a}	5215.66±46.37^{b}	5586.25±48.87

LC_{50}: Lethal concentration causing 50% mortality after 24 and 48 h, S.E: Standard error, $LSD_{0.05}$ at 24 h: 32.45, $LSD_{0.05}$ at 48 h: 23.94, $LSD_{0.05}$ at time: 10.74, $LSD_{0.05}$ for overall mean of compounds: 18.61

Table 2: Acute toxicity of Bio-fly and Chitosans against male flies of PPF, *B. zonata*, 24 and 48 h post-treatment

	LC_{50} (mg L^{-1})±S.E after treatment (h)		
Compounds	24	48	Overall Mean±S.E
Bio-fly	1333.0±4.77^{f}	1145.00±3.82^{f}	1239.00±10.22^{d}
Chitosan1	12030.0±4.11^{a}	9975.00±5.11^{a}	11002.50±11.21^{a}
Chitosan2	6204.0±3.61^{d}	5515.00±4.41^{d}	5859.50±19.43^{c}
Chitosan3	7708.0±4.27^{b}	6210.00±3.66^{c}	6958.83±14.85^{b}
Chitosan4	6115.0±3.85^{e}	5175.00±3.13^{e}	5645.00±10.72^{c}
Chitosan5	7575.0±4.68^{c}	6876.00±4.46^{b}	7225.50±19.581^{b}
Overall mean±S.E	6827.5±56.92^{a}	5815.94±51.83^{b}	6321.72±54.57

LC_{50}: Lethal concentration causing 50% mortality after 24 and 48 h, S.E: Standard error, $LSD_{0.05}$ at 24 h: 32.50, $LSD_{0.05}$ at 48 h: 30.44, $LSD_{0.05}$ at time: 12.18, LSD 0.05 for overall mean of compounds: 21.09

Table 3: *In vivo* inhibition of (AChE) activity of female and male flies of PPF, *B. zonata*, treated with Bio-fly and Chitosans at 48 h post-treatment

	LC_{50} (mg L^{-1})±S.E after treatment (h)		
Compounds	Female	Male	Overall Mean±S.E
Bio-fly	4155.00±3.64^{f}	2161.00±2.99^{f}	3158.00±33.05^{e}
Chitosan1	11735.00±3.94^{a}	8985.00±4.84^{e}	10360.00±38.81^{b}
Chitosan2	10424.00±4.89^{b}	10827.00±4.23^{a}	10625.50±14.88^{a}
Chitosan3	8974.00±4.72^{e}	10335.00±9.47^{b}	9654.50±27.34^{c}
Chitosan4	9343.00±4.31^{d}	9765.00±6.89^{d}	9554.00±15.28^{d}
Chitosan5	9475.00±5.82^{c}	9918.00±4.78^{c}	9696.50±15.62^{c}
Overall mean±S.E	9017.66±49.25^{a}	8665.17±55.21^{b}	8841.41±52.16

I_{50}: Concentration producing 50% inhibition of enzyme activity after 48 h, S.E: Standard error, $LSD_{0.05}$ for females: 39.62, $LSD_{0.05}$ for males: 80.64, $LSD_{0.05}$ for female and male flies: 42.55, $LSD_{0.05}$ for sex: 24.57

post-treatment. The previous trend was roughly achieved at 48 h post-treatment, where Chitosan4 was in ranked second, followed by Chitosan2, Chitosan3, Chitosan5 and Chitosan1 with LC_{50} = 5175, 5515, 6210, 6876 and 9975 mg L^{-1}, respectively.

Statistical analysis, showed high significant differences (F = 157321.73, df = 5, $p \leq 0.001$) between treatments in their toxicity, however no significant difference was obtained between Chitosan2 and chitosan4. Highly significant interaction (F = 2116.91, df = 5, $p \leq 0.001$) was obtained between tests compounds and time.

Data clearly revealed that female insects were more susceptible to the Chitosan compounds compared to male flies either at 24 or 48 h post-treatment.

***In vivo* inhibition of AChE:** The inhibitory effects of Chitosan1, its derivatives and Bio-fly to AchE activity of both male and female flies of PFF, *B. zonata* were evaluated at 48 h post-treatment and presented in Table 3. The data revealed a reduction in AChE activity for all treatments. Bio-fly had the highest inhibitory effect to AChE activity in female and male flies based on I_{50} (Concentration required to inhibits 50% of enzyme activity); I_{50} = 4155 and 2161 mg L^{-1}, respectively. Chitosan3 was ranked second (I_{50} = 8974 mg L^{-1}) followed by (in a descending order), Chitosan4 (I_{50} = 9343 mg L^{-1}), Chitosan5 (I_{50}: 9475 mg L^{-1}), Chitosan2 (I_{50}: 10424 mg L^{-1}) and Chitosan1 (I_{50}: 11735 mg L^{-1}) against female flies. With regard to male flies, Chitosan1 was ranked second in the inhibition of AchE activity (I_{50}: 8985 mg L^{-1}), followed by Chitosan4 (I_{50}: 9765 mg L^{-1}), Chitosan5 (I_{50}: 9918 mg L^{-1}), Chitosan3 (I_{50}: 10335 mg L^{-1}) and Chitosan2 (I_{50}: 10827 mg L^{-1}). On the other hand, a significant difference was obtained between male and female flies in inhibition of AChE activity. Where, female were more susceptible than male flies in inhibition of AChE activity by Chitosan2, Chitosan3, Chitosan4 and Chitosan5 compounds. Also, a high significant interaction (F = 3100.7565, df = 5, $p \leq 0.001$) was obtained between sex and tested compounds against PFF.

The overall mean values showed that Bio-fly had the lowest concentration that inhibited 50% of AChE activity (I_{50}) for both female and male flies, followed by Chitosans4. I_{50} of Bio-fly to AChE activity was lower with 0.33, 0.327, 0.325, 0.30 and 0.297-fold than Chitosan4, Chitosan3, Chitosan5, Chitosan1 and Chitosan2, respectively. Also, highly significant differences (F = 37344.697, df = 5, $p \leq 0.001$) were obtained between the tested compounds in inhibition of AChE activity.

Table 4: *In vivo* inhibition of ATPase activity of female and male flies of PPF, *B. zonata*, treated with Bio-fly and Chitosans at 48 h post-treatment

	LC_{50} (mg L^{-1})±S.E after treatment (h)		
Compounds	Female	Male	Overall Mean±S.E
Bio-fly	12313.00±3.70[a]	12000.00±3.58[a]	12156.50±3.34[a]
Chitosan1	8605.00±7.03[b]	6384.00±4.15[b]	7494.50±11.04[b]
Chitosan2	7893.00±9.51[d]	6094.00±9.70[b]	6993.50±31.44[c]
Chitosan3	7845.00±6.37[d]	6091.00±3.81[b]	6968.00±31.00[c]
Chitosan4	7272.00±4.60[e]	6247.00±5.42[b]	6759.50±23.70[d]
Chitosan5	8292.00±5.421[c]	5611.00±3.32[c]	6951.50±12.10[c]
Overall mean±S.E	8703.33±41.41[a]	7123.33±48.98[b]	7913.33±47.01

I_{50}: Concentration producing 50% inhibition of enzyme activity after 48 h, S.E: Standard error, $LSD_{0.05}$ for females: 83.06, $LSD_{0.05}$ for males: 367.45, $LSD_{0.05}$ for female and male flies: 52.86, $LSD_{0.05}$ for sex: 30.52

***In vivo* inhibition of ATPase:** The *in vivo* assay of the ATPase activity of both male and female flies of PFF was evaluated after 48 h post-treatment. Data presented in Table 4 revealed that all tested compounds reduced ATPase activity for both female and male flies. Chitosan4 had the highest inhibitory effect of ATPase activity compared to the tested compounds against female flies on the basis of I_{50} value (I_{50} = 7272 mg L^{-1}), followed by Chitosan3 (I_{50}: 7845 mg L^{-1}), Chitosan2 (I_{50}: 7893 mg L^{-1}), Chitosan5 (I_{50}: 8292 mg L^{-1}) and Chitosan1 (I_{50}: 8605 mg L^{-1}), respectively. In case of male flies, the highest inhibitory effect to ATPase activity was obtained by Chitosan5 (I_{50} = 5611 mg L^{-1}), followed by Chitosan3 (I_{50}: 6091 mg L^{-1}), Chitosan2 (I_{50}: 6094 mg L^{-1}), Chitosan4 (I_{50}: 6247 mg L^{-1}) and Chitosan1 (I_{50}: 6384 mg L^{-1}). Bio-fly showed the lowest inhibitory effect of ATPase activity for both female and male flies. With regard to sex, males were more susceptible than female flies to the inhibition of ATPase activity. Furthermore, a higher significant interaction ($F = 686.836$, $df = 5$, $p \leq 0.000$) was obtained between sex and tested compounds against PFF.

High significant differences ($F = 11416.219$, $df = 5$, $p \leq 0.001$) were found between the tested compounds in inhibition to ATPase activity. Nevertheless, there were no significant differences between Chitosan2, 3 and 5 as inhibitory agents of ATPase activity. In general, Chitosans had the highest inhibition to ATPase activity compared to Bio-fly for both female and male flies; I_{50} values of Chitosans against ATPase activity was lower than that of Bio-fly with 0.56, 0.57, 0.57, 0.58 and 0.62-fold for Chitosan4, 5, 3, 2 and 1, respectively.

DISCUSSION

The present study is the first one to investigate the insecticidal activity of the biopolymer Chitosan, its derivatives and Bio-fly for using as alternatives to traditional pesticides against both female and male flies of the PFF, *B. zonata*. Results revealed a broad range of toxicity among the tested compounds. Comparison between different substitutes of Chitosans; Chitosan2 (N-(2-nitrobenzyl)) was the most toxic compound either at 24 or 48 h of treatment against female flies of PFF and followed by Chitosan4. Chitosan4 (N-(4-propylbenzyl)) was the most toxic compound among tested Chitosan either at 24 or 48 h of treatment against male flies of PFF, followed by Chitosan2. An inferior toxicity was obtained with the parent Chitosan (Chitosan1) against both female and male flies of PFF. Several studies were in agreement with the obtained data. The insecticidal activity of Chitosan on *Hyalopterus pruni*, *Rhopalosiphum padi* (L.),

Metopolophium dirhodum and *Aphis gossypii* were 93, 99, 70 and 80% mortality, respectively (Zhang *et al*., 2003). For cotton leaf worm, *Spodoptera littoralis*, the N, O-benzoyl Chitosan was the most active compound among eighteen derivatives with 84.61% mortality Badawy *et al*. (2004), (N-(2-chloro-6-fluorobenzyl)) Chitosan caused 100% mortality with an estimated LC_{50} of 0.32 g kg^{-1} (Rabea *et al*., 2005). N-propylchitosan, N-undecanylchitosan and (N-(3-phenylpropyl)) chitosan derivatives strongly inhibited the larval weight of the cotton leaf worm, *Spodoptera littoralis* (Rabea *et al*., 2006). As well as, the N-(methyl-4H-chromen-4-one) Chitosan exhibited a significant growth inhibition and as an anti-feedant against the larvae of *S. littoralis* (Badawy, 2008). Imidacloprid (Confidor) and *Steinernema carpocapsae* Weiser with chitosan (Biorend R® Palmeras) significantly reduced the mean number of immature stages of red palm weevil, *Rhynchophorus ferrugineus* from 83.8-99.7% (Dembilio *et al*., 2010).

Results showed that Bio-fly possessed the highest toxic action compared to the tested compounds against both male and female flies of the PFF. Results of our study results were confirmed by Quesada-Moraga *et al*. (2006) who tested effects of myco-insecticides. They found that *Beauveria bassiana* and *Metarhizium anisopliae* recorded mortality ranging from 30-100% of the adult of *Ceratitis capitata*. So, *B. bassiana* and *M. anisopliae* have a mortality effects on both the third instar larvae and pupae of *C. capitata* (Oliveira *et al*., 2010).

The present data revealed that the female flies of the PFF were more tolerable to the Bio-fly and Chitosan1 (Parent) than the adult males. The obtained results are agreed with those obtained by (Stark *et al*., 2004) that adult males of *C. Capitata* were significantly more susceptible to spinosad than female. Furthermore, the female flies of the PFF, *B. zonata* were less sensitive to mehomyl, actra, spinosad and malathion insecticides than males (El-Aw *et al*., 2008a). However, the female flies of the PFF were more susceptible to the Chitosan derivatives than the adult males. No studies are available on female sensitivity than males to insecticides.

Data revealed that the insecticidal activity was time-dependent; the toxic activity of the tested compounds was higher at 48 than 24 h post-treatment. Similar results were obtained by ethanol extract, which increased the mortality of the PFF about 25, 48.66 and 61.67% at 10, 15 and 20th days, respectively at 1000 ppm (Jilani *et al*., 2006). Also, a negative relationship was obtained between time elapsed post-treatment and the LC_{50} of the tested compounds against the PFF (El-Aw *et al*., 2008a).

The obtained data revealed an inhibition of AChE activity after treatments with the tested compounds against PFF. These results may indicate that AChE is a biological site for these compounds. These findings were confirmed by the results of Placencia *et al*. (2005), who reported that chitosan diethyl phosphate performed similarly to organophosphate pesticides and inhibited in ChE of rainbow trout (*Oncorhyncus mykiss*). Also, a decrease in the AChE activity of treated adults of PFF was obtained with Methomyl, Actra and Malathion (El-Aw *et al*., 2008a). Furthermore, AChE activity of treated PFF *B. zonata* with Malathion, Diazinon, Methoxyfenozoide and Lufenuron was decreased compared to untreated adults (Mosleh *et al*., 2011). The confirmed results extended to uncompleted stages where, the specific activity of AChE of 2nd old-day pupae and adults of PFF was decreased after treatment with Beticol, Elsan, Mani, Match, Radiant and Lufox compared to control (Halawa *et al*., 2013). In contrary, an increase in AChE activity was obtained after treating adults of PFF by Spinosad insecticide (El-Aw *et al*., 2008b). As well as, the activity of AChE was increased by Biosad insecticide in the 2nd old-day pupae and, Mani and Radiant in the 6th old day pupae of PFF (Halawa *et al*., 2013).

A significant difference was obtained between male and female flies in inhibition of AChE activity. Where, females were more tolerable than male flies in inhibition of AChE activity by both Chitosan (parent) compound and Bio-fly. In contrary, the treated males by Methomyl, Actra and Malathion were more sensitive than female flies of PFF in the inhibition of AChE activity (El-Aw *et al*., 2008b).

In general, Chitosan2 (N-(2-nitrobenzyl)) was the most toxic of Chitosan compounds to female flies either at 24 or/and 48 h. In contrary, Chitosan4 (N-(4-propylbenzyl)) was the most toxic compound to male flies either 24 or/and 48 h and this may serve as a template for developing a new class of bioactive agents against the peach fruit fly, *B. zonata*. On the other hand, Chitosans were more inhibition to ATPase activity than Bio-fly. Further, the overall mean values showed that I_{50} of AChE was higher than those of ATPase activity by Chitosan treatments. Our results suppose that ATPase is a target to the Chitosan and its mechanism of toxicity needs further investigations.

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