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RESEARCH ARTICLE

The role of detoxification enzymes in insecticide resistance in the field populations of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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Abstract

Using biochemical and molecular assays, we indentified distinct patterns of detoxification enzymes activity in insecticide resistance in field populations of *Bactrocera zonata* (Saunders) which suggested that certain enzymes are involved in the development of resistance. Field populations of *B. zonata* showed increased activity of detoxifying enzymes compared with susceptible populations. Correlation analysis showed that enzyme activity was positively correlated with resistance to malathion, lambda-cyhalothrin, imidacloprid and spinosyn. However, an inverse correlation has been found between enzyme activity and methomyl resistance. The results showed that the synergists of PBO and Sesamex had good inhibitory activity on detoxifying enzymes, with the highest inhibitory potency on monooxygenases. These results showed that pesticides should be used judiciously and in prescribed ways to integrate pesticides into IPM programs of *B. zonata*.

Keywords: *B. zonata*; Biochemical and molecular assays; insecticides; Fruit fly; resistance.

1. Introduction

Peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the most destructive pests of horticultural crops with more than 50 hosts (Agarwal and Kumar, 1999; Dhillon *et al*., 2005; Anonymous, 2005; Ghanim, 2009; Hosni *et al*., 2013). In Egypt, *B. zonata* has been recorded in many governorates, where the host plants are available (Amro and Abdel-Galil, 2008; El-Gendy and El-Saadany, 2012; Anonymous, 2021). El-Heneidy (2012) mentioned that the *B. zonata* poses a threat to many Egypt's export markets. For controlling fruit flies, the majority of growers depend on cover spray, and chemical control has been the most effective method against fruit flies. Pesticides including organophosphates and carbamates were initially used to control tephritide flies, followed by pyrethroids and new chemical groups are used for controlling fruit flies (Anonymous, 2008).

Several reports have documented insecticide resistance in *B. zonata* in Egypt according to Radwan (2012) which, a field population of *B. zonata* exhibited significant malathion resistance. The field strain of *B. zonata* showed a range of insecticide resistance rates, with diptrex (65.32) being the most resistant at 24 hours, followed by Profenofos (13.20), Imidacloprid (7.12), bifenthrin, (5.97), Lambda-Cyhalothrin (5.73), malathion (5.54), and deltamethrin (2.35) (Haider *et al.*, 2011). Ahmad *et al.* (2010) demonstrate that, *B. zonata* (from Faisalabad and Multan zone) has developed resistance to trichlorfon and its usage as a cover spray and in baits represents a threat. Understanding the mechanisms by which pests develop resistance to insecticides is extremely important.

In our laboratory, compared to the susceptible population, 3 resistant populations of *B. zonata* (EL-Maragah, Tahta, and Shatwra) from Sohag

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governate, Egypt, were shown to have developed resistance to malathion, methomyl, lambda-cyhalothrin, imidacloprid, and spinosad (Toughan *et al*., 2022) They reported that, El-Maragah population showed moderate resistance ratio among these populations, with a range of 26.16 to 62.45-fold against spinosad, malathion, -cyhalothrin, and imidacloprid, and the maximum resistance ratio of 307.94-fold against methomyl. As well, Spinosad, malathion, and λ-cyhalothrin had intermediate resistance ratios against Tahta population, ranging from 27.08 to 84.53-fold, whereas methomyl and imidacloprid had high resistance ratios of 178.95 and 241.17-fold, respectively. Furthermore, similar results showed to Shatwra population which recorded a high resistance ratio of imidacloprid and methomyl 101.68 and 462.09-fold, respectively, whereas spinosad, malathion, and λ -cyhalothrin showed moderate resistance ratios ranging from 19.88 to 64.23-fold (Toughan *et al*., 2022).

There are three primary categories of pesticide resistance mechanisms in insects, with the 1st being target site sensitivity. This category involves allelic variations in target protein expression that change the binding sites for insecticides (Gunning and Moores, 2001; Bloomquist, 2001; Siegfried and Scharf, 2001). The 2nd category of pesticide resistance mechanisms is reduced cuticle penetration. This occurs when the cuticle, which is the outer layer of insects, absorbs insecticide molecules at a much slower rate than susceptible insects (Strycharz *et al.*, 2013; Kasai *et al*., 2014). The 3rd category of pesticide resistance mechanisms is metabolic degradation, which includes detoxifying enzymes such, esterases, microsomal cytochrome P450 dependent monooxygenases and glutathione-s-transferases (Field *et al.*, 2001; Scott, 2001). The metabolism of insecticide can be divided into two phases. The 1st phase involves the biotransformation of the insecticide where the water-insoluble and non-polar substance is converted into a polar compound through enzymatic conversion. The second phase involves the conversion of the products of the first phase into a water-soluble form through a non-synthetic reaction. The enzymes that are responsible for the detoxification of xenobiotics in living organisms belong to large multigene families of esterase's and glutathione-s-transferees', and they modify the normal enzymatic metabolism of the insect to prevent activation of insecticides or increase their detoxification. Therefore, the current aims of study to identify the role of detoxification enzymes in insecticide resistance in the field populations of *B. zonata* and the possible role of piperonyl butoxide and sesamex as enzyme inhibitor.

2. Materials and methods

2.1. Insects

A pupal stage of a reference susceptible population of *B. zonata* that has been reared for several years without exposure to insecticides were received from Pests & Plant Protection Department of the National Research Center (NRC), Dokki, Giza, Egypt. *B. zonata* field populations were gathered from the guava fruits infested and dropped from Shatwra, Tahta, and EL- Maragha, Sohag governorate, Egypt, and were maintained under the same conditions. Susceptible and field populations were reared at (27±3 C° and 65- 70% RH) using artificial dite according to Mahmoud (2004).

2.2. Enzymatic assays

2.2.1. Sample preparation for enzymes purification

From each population, 200 adults of *B. zonata* were homogenised in 10 ml of sodium phosphate buffer (0.1 M, pH 7) and then centrifuged at (10,000 rpm and 4°C for 20 min). the supernatant was transferred to a new Eppendorf tube and kept at -20°C for later use as the enzyme stock and all handling was performed on ice.

2.2.2. Esterases

To estimate the total esterase activity was used the rate of hydrolysis of the model substrate, α-

naphthyl acetate, as described by Kranthi (2005). Each reaction tube included an aliquot of 10 µl of the enzyme stock, which was mixed with 500µl of phosphate buffer (40 mM, pH 6.8) and 5 ml of a substrate solution containing (0.3 mM α-naphthyl acetate) for 20 min at 37 °C. Blank was prepared without the addition of any enzyme. There were three replications. One ml of the staining solution (1 parts of 1% fast blue: 2 parts of 5% sodium dodecyl sulphate) was added after incubation for 30 minutes in the dark. The enzyme activity was calibrated using the α-naphthol standard curve, and the absorbance was measured at 590 nm using a UV/VIS-double beam spectrophotometer (Perkin Elmer λ3B).

2.2.3. Glutathione- s-transferase activity

Glutathione-s-transferase activity towards 1-chloro-2,4-dinitrobenezene (CDNB) was estimated according to Habig *et al.* (1974), with minor modification. The reaction mixture consists of 40 µl 1.0 mM reduced glutathione, 20 µl 1.0 mM 1-chloro-2, 4-dinitrobenzene (CDNB), 200 µl phosphate buffer (100 mM, pH 7.0), and 20 µl of supernatant. Blank was prepared without the addition of any enzyme. After the reaction had taken place for five minutes, the absorbance was recorded for five minutes at 340 nm. Change in the absorbance per minute was converted into nano-moles of CDNB conjugate/min/ mg of protein, by using extinction coefficient (ε) of 9.6 mM cm-1 for CDNB-GSH conjugate.

$$\text{CDNB} - \text{GSH conjugate formed (nM/mg of protein/min)} = \frac{AB/s(change\ in\ 5\ min)\ x0.28x1000}{9.6x0.5\ (path\ length)x\ protein\ in\ mg}$$

2.2.4. Acetylcholinesterase (AChE) activity

The rate of hydrolysis of the model substrate AChI (acetylthiocholine iodide) was used to estimate AChE activity as described by Kranthi (2005), with minor modification. The reaction mixture comprised of 100 µl of homogenate, 30µl of 0.1M AChI, 10µl of 0.01M of DTNB (5,5-dithiobis-2-nitrobenzoic acid) in Na_3PO_4 buffer (0.1M, pH 8.0) containing 1.5% Na_2CO_3 and 2.86 ml Na_3PO_4buffer (0.1M, pH 8.0). The reaction was started by the addition of the substrate AChI and the reagent DTNB. Three replications were used. Blank was prepared without adding enzymes. The acetylcholinesterase activity was estimated by recording the increase in absorbance at 412nm for 10 minutes.

$$\text{AChE activity in µmoles/min/ml of enzyme} = \frac{\Delta E\ x1000\ x\ 3.0}{1.36\ x\ 104\ x\ 0.10}$$

Were ΔE is change in absorbance / minute, 3.0 is the total volume (ml) of reaction mixture,0.1 is the volume (ml) of enzyme, 1000 is the factor to obtain µ moles, 1.36 $x10^4$ is the molar extinction coefficient of the chromophore at 412 nm.

2.3. Cytochrome P450 monooxygenase (CYPM) activity

2.3.1. Carbon-monoxide method

To determine the potential role of cytochrome P450 monooxygenase (CYPM) in *B. zonata* insecticide resistance, the enzyme's activity was evaluated in adult homogenates prepared as previously described for the selected population. The carbon monoxide difference spectra were utilized to evaluate the activity of CYPM after reaction with sodium dithionite, as described by Omura and Sato (1964). The activity of CYPM was estimated in eight replications.

2.3.2. TMBZ method

The activity of CYPM was evaluated in *B. zonata* adults from the selected populations according to Vulule *et al*. (1999) with minor modification. Reaction mixture consists of 20 µl of supernatant, 200 µl of 3, 3, 5, 5-tetramethyl benzidine (TMBZ) solution, [TMBZ solution was made by dissolving 0.01g TMBZ in 5 ml ethanol and 15 ml 0.25 M Ch_3COONa buffer (PH 5.0)], 100 µl of 0.625M K_3PO_4 buffer at pH 7.0 and 30µl of 3% H_2O_2. Blank was made without the addition of enzymes. 3 replications were used. The readings were recorded at 620 nm after ten minutes. The amount of monooxygenases was estimated using a cytochrome C standard curve.

2.4. Protein determination

The content of each sample of protein was determined according to Lowery *et al*. (1951) and the standard was used the bovine serum albumin fraction V (Sigma). Protein content enzyme was used to calculate the specific activity of each enzyme.

2.5. Enzyme profile

Non-denaturing PAGE assays were maintained out on poly acrylamide gel to investigate the enzyme profile of glutathione s- transferase, esterase and identifying the enzyme responsible for insecticide resistance. Electrophoresis (Native) was performed on a 20 × 20 cm vertical slab gel unit with a continuous tris-glycine running buffer system (50 mM, pH 8.8), using 12% separating gel and 5% stacking gel. 100 µl of the enzyme stock from each sample containing 20 µg protein was diluted with an equal volume of the 2X sample buffer (traces of bromophenol blue (BPB) dissolved in 1 ml glycerol +1 ml deionized H_2O), before loading. Electrophoresis was performed for approximately 30 minutes at 75 V constant voltages and 25 milliamperes until the samples entered the resolving gel, after which the current was gradually increased to 150 V and 50 mA until the marker dye (BPB) reached the end of the gel and the temperature was assumed at 12 ± 0.1 °C with a circulating water bath.

2.5.1. Esterase

To visualize the esterase bands, the gels were incubated in 48.5 ml of Tris-base (0.05 M, pH 7.1) 1.5 ml of 1% α,β-naphthyl acetate solution (12.5g α-naphthyl acetate+12.5 β-naphthyl acetate in 2.5 ml of deionized H_2O 1:1 acetone) and incubated for 20 min at laboratory temperature with continuous mild shaking, then, the gel was incubated in 50 ml of Tris-base (0.05 M, pH 7.1) + 50 mg of fast blue RR in dark for 30 minutes at room temperature with continuous moderate shaking until dark brown (greenish-black) coloured bands appeared. Add 2 mL formaldehyde to retain the transparency of the background gel matrix. The gels were stained and tested for the presence or absence of esterase isozymes.

2.5.2. Glutathione s- transferase (GSTs)

To visualize the GSTs bands, the gel was incubated in freshly prepared staining solution-I (100 ml of 0.1 M K_3PO_4 buffer, (pH 6.5), containing 4.5 mM reduced glutathione, 1 mM 1-chloro-2,4-dinitrobenzene CDNB, and 1 mM nitro blue tetrazolium NBT) in the dark at room temperature for 20 minutes with continuous mild shaking. The gel was then transferred to the staining solution-II (100 ml of 0.1 M Tris-HCl buffer, pH 9.6, containing 3 mM phenozine methosulfate PMS) and incubated for 5-10 minutes at room temperature with intermittent shaking. Except for the glutathione-s-transferase sections, blue insoluble formazan appears on the gel surface. (Ricci *et al.,* 1984).

2.5.3. Determination the role of the synergists, Piperonyl butoxide and sesamax as enzyme inhibitors

In vitro inhibition activity of PBO and sesamax, was assessed by incubating 500 µl of each enzyme stock for 20 minutes with various dilutions of the two synergists and measuring enzyme activity as previously described. The amount of inhibitor required to inhibit 50% of enzyme activity was used to calculate inhibitor activity.

3. Result and discussion

In our previous study **(**Toughan *et al.,* 2022), compared to the susceptible strain, the three field populations of peach fruit fly (Maragah, Tahta, and Shatwra) exhibited varying degrees of resistance to the tested pesticides. Methomyl showed the highest resistance ratio in adult flies of resistant field strains (Shatwra, Maragah, and Tahta), with ratios of 462.09, 307.94, and 178.95, respectively. Additionally, adult flies of the resistant field populations (Shatwra and Tahta) displayed a high resistance ratio to Imidacloprid (101.68 and 241.16-fold, respectively). In comparison to the susceptible strain, the resistant

field populations Shatwra, Maragah, and Tahta had the lowest resistance ratios to Spinosad (19.88, 26.15, and 27.88-fold, respectively). Maragah strain had a low resistance ratio against Malathion and λ-cyhalothrin (29.37 and 30.28-fold, respectively), whereas adult flies of the resistant field populations (Shatwra and Tahta) had moderate resistance ratio to Malathion and λ-cyhalothrin (51.62, 76.95, and 84.52, 64.23-fold, respectively). Maragah also had a moderate resistance ratio to Imidacloprid (62.44-fold).

3.1. Enzymatic assays

3.1.1. Esterase activity

Data in (Table 1) the esterase specific activity of the four selected populations of *B. zonata.* Tahta population found to have the highest activity of 1.548 µmol/mg protein/min with relative activity which was 4.27-fold higher than of the susceptible population. The relative activity found in El-Maragah and Shatwra populations were 2.74 and 1.85-folds compared to the susceptible population.

Table 1. Activity of sterase in the adult of different populations of peach fruit fly, *B. zonata*.

Population	Esterase specific activity (ESA) (µmol/mg protein/min)	*RA
Susceptible	0.362	1
Tahta	1.548	4.27
Shatwra	0.671	1.85
El-Maragah	0.995	2.74

**Relative activity (RA) = Activity of esterase in field population / Activity of esterase in susceptible population.*

Toughan *et al.* (2022), reported that, the three field populations of peach fruit fly (Maragah, Tahta, and Shatwra) exhibited varying degrees of resistance to the tested pesticides compared to the susceptible strain. They stated that, methomyl showed the highest resistance ratio in adult flies of resistant field strains (Shatwra, Maragah, and Tahta), with ratios of 462.09, 307.94, and 178.95, respectively. Additionally, adult flies of the resistant field populations (Shatwra and Tahta) displayed a high resistance ratio to Imidacloprid (101.68 and 241.16-fold, respectively). The resistant field populations of Shatwra, Maragah, and Tahta had the lowest resistance ratios to Spinosad (19.88, 26.15, and 27.88-fold, respectively) compared to the susceptible strain. Whereas, Maragah strain had a low resistance ratio against Malathion and λ-cyhalothrin with an average 29.37 and 30.28-fold. In addition, adult flies of the resistant field populations (Shatwra and Tahta) had moderate resistance ratio to Malathion and λ-cyhalothrin (51.62, 76.95, and 84.52, 64.23-fold, respectively). Also, Maragah had a moderate resistance ratio to imidacloprid (62.44-fold).

Mesbah *et al*. (2016) the field strains of *B. zonata* (Alexandria, El-Fayoum, and Siwa) were shown to have higher esterase activity than the laboratory strain. In addation, Nasouri (2017) reported that, the activity of esterase in the malathion resistant (M-R) strain was higher than that in the susceptible strain. These differences were significant in the eighth generation and in females of the sixth generation. No significant variations were found in esterase activity between the lambada-cyhalothrin resistant (L-R), spinosad resistant (S-R) strains and the susceptible strain.

3.1.2. GSTs activity

The field populations of *B. zonata* showed higher GSTs activity compared to the laboratory population (Table 2). Tahta strain showed the highest GSTs activity (1.01 µmol/mg protein/min) which was 1.7 times that of the laboratory population. The glutathione-s-transferase relative activity in El-Maragah and shatwra strains was 1.5 times than that of susceptible population with specific activity of 0.90 and 0.91 µmol/mg protein/min, respectively.

Table 2. GSTs activity in the adult of different populations of *B. zonata.*

Populations	GSTs specific activity (µmol/mg protein/min)	*RA
Susceptible	0.60	1
Tahta	1.01	1.68
Shatwra	0.91	1.52
El-Maragah	0.90	1.50

**Relative activity (RA) = GSTs activity in Field strain / GSTs activity in susceptible population.*

These results are in agreement with Nasouri (2017) which elevated GSTs activity in malathion, lambdacyhalothrin and spinosad resistant population of *B. zonata*. Also, Bakr *et al*. (2016) observed significant increase in the activity of GSTs in malathion and spinosad-resistant adults of *B. zonata.* Whereas, Yaqoob *et al*. (2013) reported that the activity of insecticide detoxification enzymes was higher in the survivor's flies. They stated that, trichlorofon and malathion treated flies had the same activity of GSTs. In contrary the tested colonies of *B. dorsalis* did not show significant difference with respect to enzyme activity of GSTs. Morevere, Shehab (2011) indicated that elevated GSTs activity conferred resistance to both malathion and methomyl but partially to fenitrothion in *B. zonata*.

3.1.3. Acetylcholinesterase (AChE) activity

Data presented in (Table 3) revealed that the AChE activity was found to be higher in field populations of *B. zonata* compared to the laboratory strain. The population of Tahat had the highest AChE activity (11.96 µmol/mg protein/min), which was 4.47 times higher than the laboratory strain (2.67 µmol/mg protein/min). Whereas, in Shatwra population AChE activity found (4.01 µmol/mg protein/min), which was 1.5 times that of laboratory strain while the relative AChE in El-Maragah population was 1.33-fold with activity of (3.56 µmol/mg protein/min).

Table 3. AChE activity in the adult of different populations of *B. zonata.*

Populations	AChE specific activity (µmol/mg protein/min)	*RA
Susceptible	2.67	1
Tahta	11.96	4.47
Shatwra	4.01	1.50
El-Maragah	3.56	1.33

**Relative activity (RA) = AChE activity in field strain /AChE activity in susceptible strain.*

Our results are in agreement with Bakr *et al*. (2016) which observed that AChE activity was significantly increased in malathion and spinosad resistant adults of *B. zonata*. Whereas, El-Agamy *et al*. (2021) reported that, the field strains of *C. capitata* recorded remarkable higher levels of the activity of AChE than the laboratory strain and also detected positive correlation between resistance levels in the different strains and the enzymatic activity levels in these strains.

3.2. Cytochrome P450 monooxygenasze (CYPM) activity

3.2.1. Carbon-monoxide method

The total CYPM in the selected *B. zonata* populations was estimated using carbon monoxide method. Tahta and El-Maragah populations showed high elevated CYPM activity of (16.31 and 12.12 pmol/mg protein/min) respectively, which was 10.73 and 7.97 times that of the susceptible population (1.25 pmol/mg protein/min). Shatwra population had slightly higher CYPM activity of (1.90 pmol/mg protein/min) which was 1.25 times that of the susceptible population (Table 4).

Table 4. Determination of CYPM activity using carbon-monoxide in the adult of differentpopulations of *B. zonata.*

Populations	CYPM activity(pmol/mg protein/min)	*RA
Susceptible	1.52	1
Tahta	16.31	10.73
Shatwra	1.90	1.25
El-Maragah	12.12	7.97

**Relative activity (RA) = CYPM activity in field population / CYPM activity in susceptible population.*

3.2.2. TMBZ method

Data in Table (5) revealed that, resistance populations of *B. zonata* have enhanced *monooxygenase* activity compared to the susceptible population. Tahta population have 20.43-fold of monooxygenase activity higher than that of the susceptible population. Shatwra and El-Maragah have 11.39 and 4.83 higher monooxygenase activity than that of the susceptible population.

Table 5. CYPM activity using TMBZ method in the adult of different populations of *B. zonata.*

Populations	CYPM activity (µmol/mg protein/min)	*RA
Susceptible	1.003	1
Tahta	20.494	20.43
Shatwra	11.431	11.39
El-Maragah	4.845	4.83

**Relative activity = CYPM activity in field population / CYPM activity in susceptible population.*

These results are in agreement with EL-Agamy *et al*. (2021) which reported that the field strains of *C. Capitata* recorded remarkable higher levels of the activity of mixed function oxidase than the baseline laboratory strain and also detected positive correlation between resistance levels in the different strains and the enzymatic activity levels in these strains. Whereases, Toughan *et al*. (2017) indicated that the field populations of *Psammotermes hypostoma* had enhanced activity of cytochrome P450, which was may be important mechanisms for pyrethroid resistance in the field population. CYP6A51 was found to be overexpressed (13-18-fold) in the resistant strain of *C. capitata*. In the resistant strain W-1K of *C. capitata*, metabolic resistance mediated by P450 appears to be the main resistance mechanism Arouri *et al. (*2015). Another insect, Abd El-Latif *et al*. (2014) reported that *H. armigera* field strains from India (Nagpur and Delhi) have 2.40 and 1.79 times more CYPM activity, respectively, than a susceptible strain. They also identified a strong correlation between CYPM activity and pyrethroid resistance.

Morever, Yaqoob *et al*. (2013) reported that the activity of insecticide detoxification enzymes was higher in the survivor's flies. They also showed that, trichlorofon and malathion treated flies had the same activity of monooxygenases. Furthermore, Hsu and Feng (2006) reported that malathion resistant strains of *B. dorsalis* have higher mixed function oxidase activity than susceptible strains.

3.3. ***Enzymes profile***

3.3.1. Esterase

Esterase profile study of *B. zonata* reported the existence of 7 esterase isozymes which were identified and specified from E1 to E7, the slowest migrate esterase (high molecular weight) E1 to the fastest (lowest molecular weight), E7 (Fig. 1). Important difference in esterase isozyme between the resistance (field) populations and susceptible population of *B. zonata* were observed. Susceptible population had unique esterase isozymes (E5 and E7) with low molecular weight that were not observed in the resistant populations. Susceptible and Shatwra populations found to have E2 isozyme that was not observed in Tahta and El-Maragha populations. On the other hand, susceptible and Shatwra populations lack E3 isozyme which was present in Tahta and El-Maragha populations. Intensity of E6 isozyme was stronger in the susceptible population than to the resistance populations. Toughan *et al.* (2017) reported that in the termite *P. hypostoma*, 5 esterase isozymes were detected and specified as E1 to E5 based on their migration rates in electrophoresis gel, with E1 being the slowest (having the highest molecular weight) and E5 being the fastest (having the lowest molecular weight). Among these isozymes, four esterase bands were present in both susceptible and resistant populations, whereas the susceptible population lacked the E4 esterase isozyme, which was exclusive to resistant populations.

Wang *et al*. (2015) found two overexpressed α-esterase genes, BdCarE4 and BdCarE6, which are found in the adult of *B. dorsalis* midgut and fat body, operate to provide malathion resistance. Particularly, these two genes were designated to be largely close to the esterase E3, which is normally involved in the detoxification of organophosphate insecticides. Whereas Teese *et al.* (2013) reported that, seven esterase isozymes were identified from 14 that migrated to two regions of the gel previously associated with organophosphate and pyrethroid resistance in different strains of *H. armigera*. Also, Abd El-Latif and Subrahmanyam (2010) reported that, native PAGE assay was examined the esterase isozymes of both the pyrethroid-resistant and susceptible strains of *H. armigera*, which indicated considerable differences between the two. Only five of the ten esterase isozymes found were shared by the resistant and susceptible strains. Three bands, E2, E6, and E10, were missing in the susceptible strain but present in the resistant strains. The Delhi strain, which was less resistant to deltamethrin (1842-fold), had two additional isozymes (E2 and E6), but Nagpur strain, which was the most resistant (2850-fold), had three additional isozymes (E2, E6, and E10). Importantly, the susceptible strain featured a unique esterase isozyme (E1) with a molecular weight of 57.3 kDa, which was lacking in Nagpur and Delhi strains. Taflkn *et al*. (2007) stated that the Native PAGE analysis presented the esterase band patterns and frequencies for all populations of *Drosophila melanogaster*. The analysis detected a total of 21 esterase bands when substrates α- and β-naphthyl acetate were used, which were categorized into 18 α-esterases, 2 β-esterases, and 1 α/β esterase. Young *et al*. (2005) reported that pyrethriod resistance in field populations of Australian *H. armigera* is primarily sequence of the over production of esterases isoenzymes which metabolite and sequester pyrethroids.

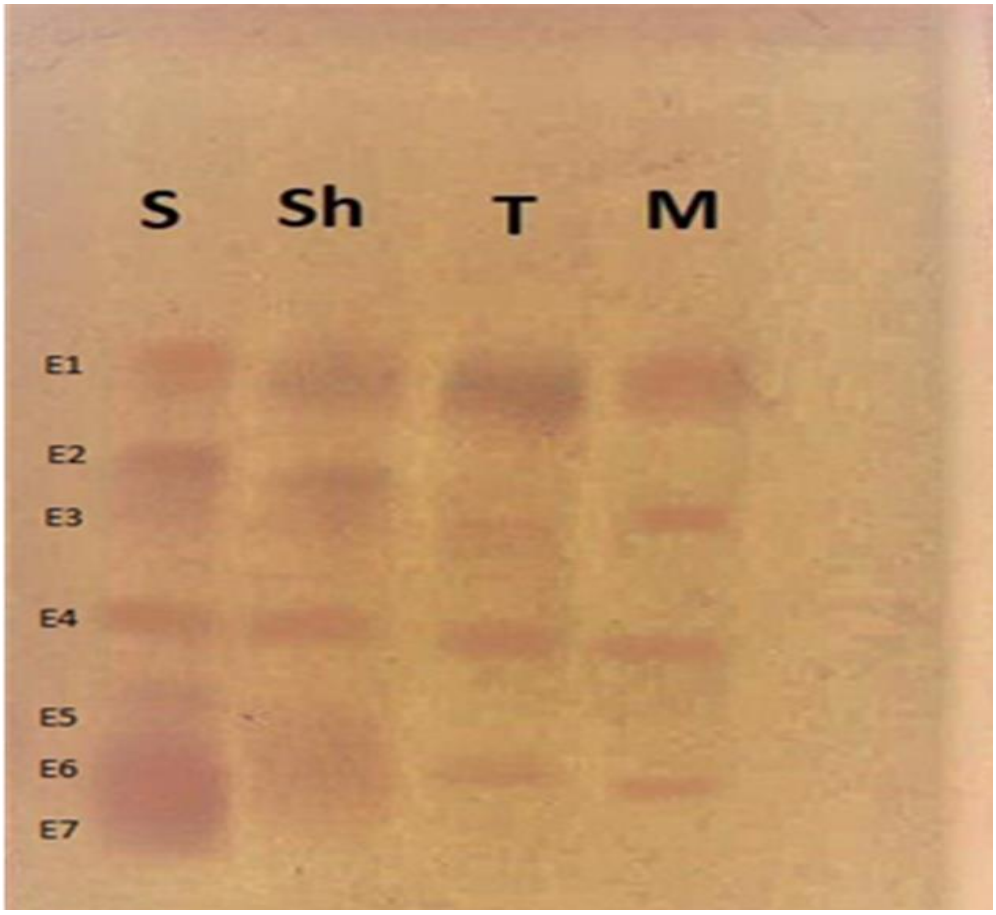

Figure1. Native page profile of esterase isozymes for the adult stage of different populations of *B. zonata*, (S:Susceptible, Sh: Shatwra, T: Tahta and M : Maragah)

3.3.2. Glutathione-s-transferase (GSTs)

Eight isozymes of GSTs were also identified in the tested populations of *B. zonata* (Fig. 2). Susceptible population has extra GSTs isozyme (G8) that was not detected in the resistant populations. On the other hand, El-Maragha population has unique GSTs isozyme (G7) which was not detected in other populations. G1, G2, G3, G4, G5 and G6 isozymes were common in all populations, while the intensity of G2, G3, G4 in El-Maragha population was stronger than that of other populations followed by Tahta population. Grant and Matsumura (1989) isolated two glutathione-s-transferases (GST-1 and GST-2) from both insecticide-susceptible and resistant strains of *Aedes aegypti* mosquitoes. Both isozymes were homodimers and did not share any immunological similarity. GST-1 had a subunit molecular weight of 26800 with a pi value of 5.0, while GST-2 had a subunit molecular weight of 28000 and a pi value less than 5.0. Yawetz and Koren (1984) reported that the GST enzyme from *C. captitata* was isolated and purified to a state of apparent homogeneity. It was observed that the GST consisted of two subunits, with molecular weights of 22000 and 21000, and a pi value of 5.7.

3.3.3. Determination the role of PBO and sesamax synergists as enzyme inhibitors

Result of inhibitor activity of the tested synergests indicated that, both PBO and sesamex have inhibitory activity against esterase of all populations. The esterase inhibitory potency of PBO and sesamex was more against the esterase of field populations compared to the susceptible population (Table 6). Compared to sesamex, PBO was more potent as esterase inhibitor in all

populations tested. The esterase of Shatwra was the most sensitive to PBO and sesamex followed by El-Maragah and Tahta populations. PBO and sesamex showed the highest inhibitory activity against monooxygenase compared to other tested enzymes in all populations of *B. zonata.* PBO showed high monooxygenase inhibitory activity compared to sesamex in all populations of *B. zonata.* PBO was more potent against the monooxygenase of Shatwra population (IC_{50} = 58.16 µg/ml) compared to the other populations while sesamex was more potent against monooxygenase of susceptible population (IC_{50} = 76.75 µg/ml) compared to other populations.

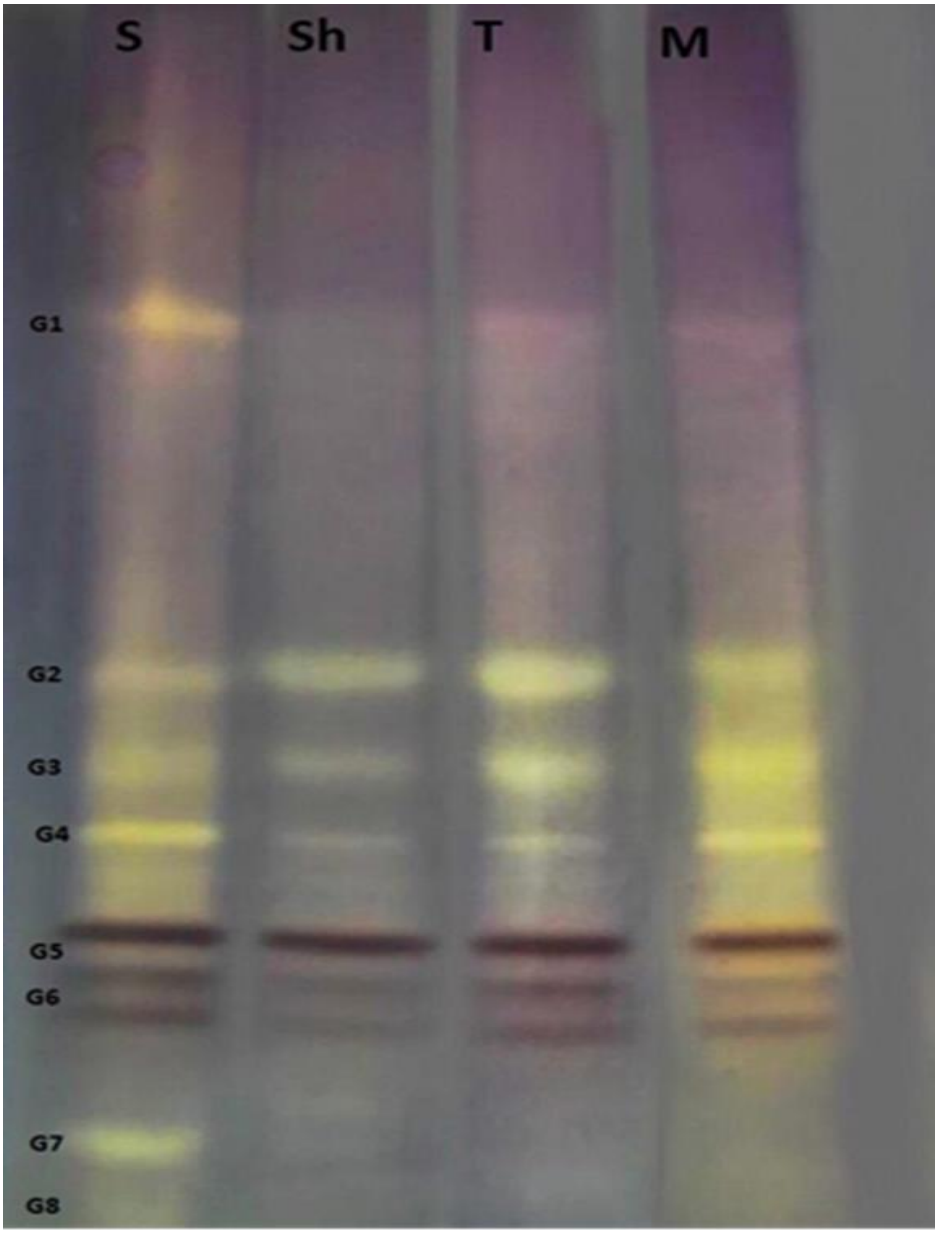

Figure 2. Native page profile of glutathione-s-transferase isozymes for the adult stage of different populations of *B. zonata*, (S:Susceptible, Sh: Shatwra, T: Tahta and M : Maragah).

Table 6. *In vitro* inhibition of enzymes activity by the insecticide synergists, PBO and sesamex in different populations of *B. zonata.*

		IC_{50} (µg/ml)			
Enzymes	Synergists	Susceptible	Tahta	Shatwra	El-Maragah
Esterase	Sesamex	133.4	122.62	75.99	81.18
	PBO	118.39	95.34	57.89	56.22
Glutathione-s-transferase	Sesamex	80.99	91.13	88.98	84.70
	PBO	66.98	74.18	70.30	64.89
Acetylcholinesterase	Sesamex	132.862	117.38	124.79	123.87
	PBO	93.21	92.23	89.71	101.37
Monooxygenase	Sesamex	76.75	89.55	79.30	79.56
	PBO	60.70	66.18	58.16	64.49

Obtained data revealed that PBO and sesamex could inhibit glutathione-s-transferase activity in all studied populations of *B. zonata.* In addation, PBO was more potent against glutathione-s-transferase of all populations compared to sesamex. Forthermore, the highest inhibitory activity for PBO (IC_{50} = 66.98 µg/ml) and sesamex (IC_{50}= 80.99 µg/ml) were obtained against the glutathione-s-transferase of the susceptible population. PBO and sesamex showed the least inhibitory activity against acetylcholinesterase compared to the other tested enzymes. While, the obtained IC_{50} values for PBO and sesamex against acetylcholinesterase was less to that obtained for acetylcholinesterase in Tahta population only (92.23 and 117.38). Sesamex showed less inhibitory activity to acetylcholinesterase compared to PBO in all the tested populations of *B. zonata*. The highest inhibitory activity (IC_{50} = 89.71 µg/ml) was obtained against the acetylcholinesterase of Shatwra population while the highest inhibitory activity (IC_{50} = 117.38 µg/ml) for sesamex was obtained against acetylcholinesterase of Tahta population. Abd El-Latif and Subrahmanyam (2010) reported that, PBO reduced the esterase activity in the insecticide-resistant Nagpur and Delhi strains of *H. armigera*, with IC_{50} values of 7.5 and 8.8 mM, respectively. They also indicated that, esterase inhibition was detected 4 and 8 hours after PBO treatment, rather than immediately after the synergist treatment.

The correlation regression between insecticide resistance and enzyme activity of different populations of adult stage of *B. zonata* was displayed in (Table 7). Correlation analysis showed positive correlation between enzymes activity and resistance against malathion, λ-cyhalothrin, imidaclopride and spinosad insecticides. However, negative correlation was recorded between enzymes activity and resistance against methomyl. Malathion resistance showed highest correlation (r = 0.91) with acetylcholinesterase followed by glutathione-s-transferase (r = 0.89), esterase (r = 0.65) and monooxygenase (r = 0.32). λ-cyhalothrin resistance was synchronized with elevated enzyme activity with highest correlation (r = 0.81) with acetylcholinesterase followed by glutathione-s- transferase (r = 0.79), esterase (r = 0.50) and monooxygenase (r = 0.14). The highest correlation value between imidacloprid and enzyme activity was observed for acetylcholinesterase (r = 0.99) followed by glutathione-s-transferase (r = 0.98), esterase (r = 0.83) and monooxygenase (r = 0.56). On the other hand, spinosad resistance was highly correlated with elevated monooxygenase activity (r = 0.99) followed by esterase (r = 0.85), glutathione-s-transferase (r =0.59) and acetylcholinesterase (r=0.56). Abd El-Latif and Subrahmanyam

(2010) found a significant positive correlation between esterase and monooxygenase activity and resistance of pyrethroid in *H. armigera*. Toughan *et al.* (2017) reported a significant positive correlation between esterase activity and pyrethroid resistance in *P. hypostoma*.

Table 7. Correlation analysis between enzyme activity and insecticide resistance in different populations of *B. zonata*.

	Correlation coefficient (r)			
Enzymes / Insecticides	Esterase	Glutathione-s-transferase	Acetylcholinesterase	Monooxygenase using mono-carbon
Malathion	0.65	0.89	0.91	0.32
Methomyl	-0.98	-0.83	-0.81	-0.98
λ-cyhalothrin	0.50	0.79	0.81	0.14
Imidacloprid	0.83	0.98	0.99	0.56
Spinosad	0.85	0.59	0.56	0.99

4. Conclusions

Finally, it can be concluded that the field populations of *B. zonata* have elevated detoxification enzymes compared to the susceptible population. The field populations also showed the highest increase in the detoxification enzymes compared to susceptible population. Insecticide synergist such as PBO and sesamex showed good inhibition activity against the detoxification enzymes with the highest inhibition potency against monooxgenase. Using synergists may be a good solution to overcome the insecticide resistance in *B.zonata*.

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