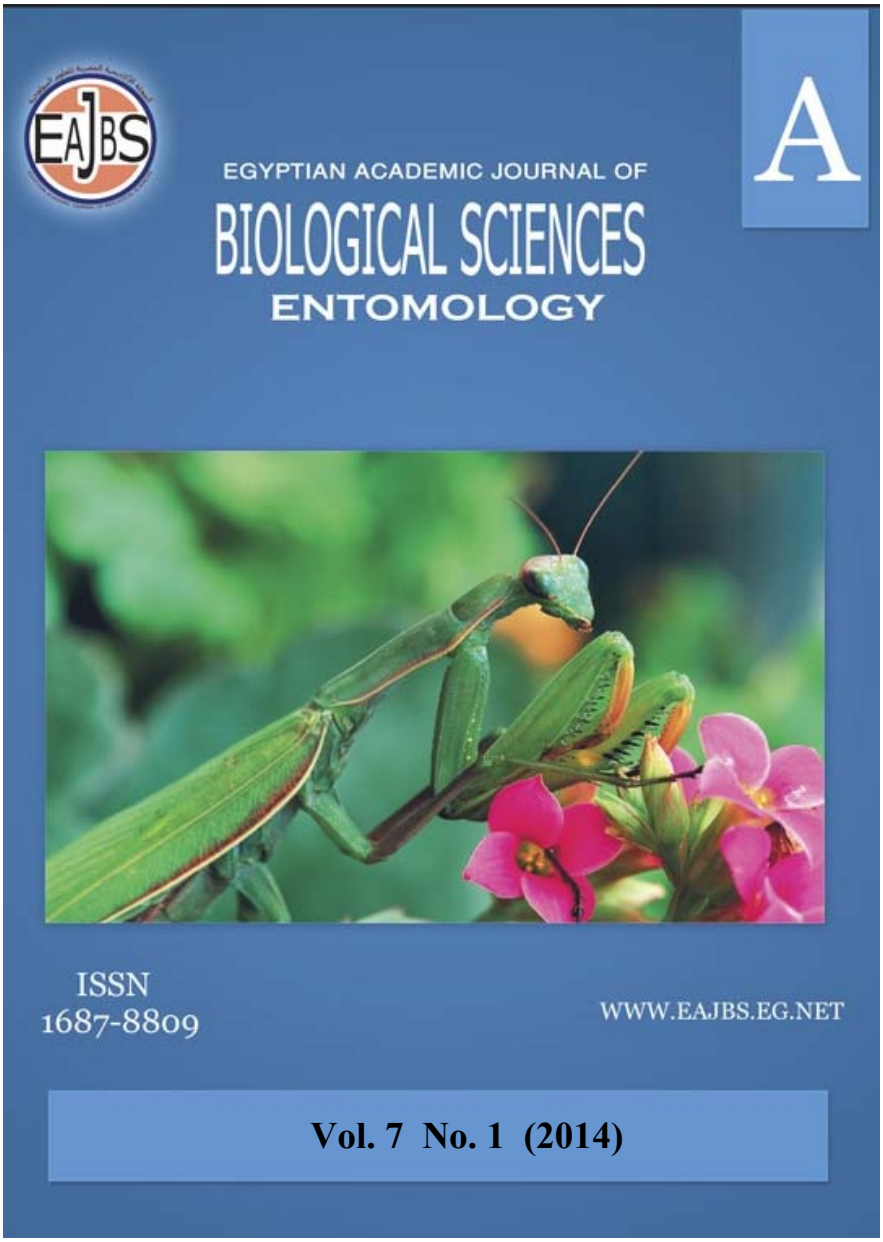

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Monitoring resistance in different strains of pink bollworm, *Pectinophora gossypiella* (Saund.) to profenofos and its relationship of some biochemical aspects

Seleman, Laila E.
Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza

ABSTRACT

Profenofos resistance was found in field strains of the pink bollworm, *Pectinophora gossypiella* (Saunders) collected at late season of 2013 from four Governorates in Egypt. Resistance was high in regions where profenofos were frequently used. Resistance ranged between 31.31 and 59.60% in the tested strains. Profenofos resistance was the highest in the individuals collected from Gharbia Governorate as compared with the other field strains; whereas the lowest resistance percentage was noticed in individuals collected from Kafrel-Sheikh Governorate. Studies carried out through estimation of the detoxification enzymes activity indicated that enhanced acetylcholinesterase, alkaline & acid phosphatases and α & β nonspecific esterases as well as total protein contents were probably important mechanisms for profenofos resistance in field strains. Colorimetric assays showed a significant positive correlation between increased resistance to profenofos in strains of *P. gossypiella* examined and the hydrolytic enzymes activity. The information presented illustrates the importance of proper Integrated Pest Management programs (IPM) to avoid the consequences associated with improper insecticide used.

Keywords: resistance - *Pectinophora gossypiella* - biochemical aspects - profenofos

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) is the key pest of cotton in Egypt, 75% of insecticides use on cotton is directed against this pest. The main infestation on cotton occurs between July and September. The economic threshold is 3 percent of infestation in green bolls. It is capable of causing tremendous financial losses of cotton yield. As a result of continued massive use of recommended synthetic insecticides against cotton pests in Egypt, tolerant as well as resistance strains have been developed. The occurrence of resistance and cross-resistance in addition to persistence of insecticides in the environment resulted in expiration of the biotic potential of many insecticides.

Detoxification of insecticide by metabolism is the common mechanism that has evolved to protect insects (Price, 1991). A correlation between hydrolases activity and resistance to organophosphate was reported from *Helicoverpa armigera* (Khidr, 1982) and *P. gossypiella* (Abdel-Baset, 2009). Many more cases of esterases activity mediated OP resistance have been reported for example in tobacco budworm, *H. virescens* (Harold and Offea, 2000) mosquito, *Culex tarselis*, (Whyard *et al*., 1995) and colarado potato beetle, *Leptinotarsa decemlineata* (Say) (Anspaugh *et el*., 1995).

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P. gossypiella has developed resistance to most insecticides groups including organophosphates, carbamates and synthetic pyrethroids (kranthi *et al.*, 2001b). Insecticide resistance is a major impediment to the effective management of field populations of the pink bollworm. Early detection of insecticide resistance provides a basis for the management of resistance pest populations (Khidr *et al*., 2002). A successful resistance management strategy depends on the nature, frequency and evolution of resistance mechanisms in field population of pest insects (Brent, 1986). Rapid biochemical assays coupled with biological assays are potential tools for estimating both the intensity and the frequency of resistance in the field (Brown and Brogodon, 1987).

The aim of this study is to measure the resistance in different field strains in relation to some biochemical aspects.

MATERIALS AND METHODS

Insect and culture:

Five strains of pink bollworm, *Pectinophora gossypiella* (Saunders), were used in this study. A laboratory strain which was used as a baseline in insecticides and biochemical assays. It was obtained from the Bollworms Department, Plant Protection Research Institute, Agricultural Research Center, Dokki Egypt. Newly hatched larvae were reared in glass tubes (2x7 cm) containing 2-3 cm artificial diet until larval pupation. Pupae were separated and kept in tubes until moth's emergence. The larval artificial diet was prepared according to Abdel-Hafez e*t al.* (1982). Four field strains were isolated from natural population in cotton fields of Menoufia, Gharbia, Kafrel-Sheikh and Dakahlia Governorates during the late season of 2013. These fields were exposed regularly to insecticides applications for the pest control according to the routine program set annually by the Administration of pest control, Ministry of Agriculture for controlling cotton bollworms. The discriminating concentration was the LC_{99} for the laboratory strain (Gunning *et al.*, 1984) was used for monitoring resistance percentages in the four field strains.

Insecticide used:

Organophosphate:
Common name: Profenofos
Trade name: Teleton, 72% EC.
Chemical name: O-(4-bromo-2-chlorophenyl)-)-ethyl S-propyl phosphorothioate.

Discriminating concentration technique:

A discriminating concentration technique was used for rapid monitoring of the insecticidal resistance in field-collected *P. gossypiella* larvae. Roush and Miller (1986) calculated that testing all of an insect sample at a discriminating concentration is more efficient than estimating dose-response regression lines in monitoring for resistance. The diagnostic concentration is a single concentration that can discriminate between susceptible and resistant individuals. Gunning *et al*. (1994) suggested that the discriminating concentration was the LC_{99} values for susceptible insects. The LC_{99} of the susceptible *P. gossypiella* 4^{th} instar larvae were estimated previously from the adult vial technique by probit analysis and used to estimate a diagnostic concentration for profenofos tested, based on considerations discussed by Roush and Miller (1986). To estimate a concentration that would reliably cause approximately 99 % 4^{th} instar larvae mortality of the *P gossypiella* susceptible strain, 2 ml. of the insecticidal solution was pipetted into a glass vial (2.5x7cm), then vial was rolled for approximately 2 min. to ensure that all surfaces received the insecticidal treatment. Vials were air dried (vertically) at room temperature for 24 hours. Ten larvae were placed in ten vial, one larvae for each vial. The treatments were replicated four times. Vials with

treated larvae were placed in incubator at 27+-1 º C, and the mortality was assessed after 24 hours.

Resistance percentages to the tested insecticide in the field colony strains were determined by calculating mortality percentage of the discriminating concentration of profenofos and the correcting the control mortality and percentage of survival in laboratory insect at the discriminating concentration used. To calculate the resistance percentage, the following formula adopted by McCutchen *et al.* (1989) was used.

- Resistance percentage = 100-(MF/MSx100)

Where MF= % mortality at discriminating concentration in field collected larvae.
MS= % mortality (constant) at discriminating concentration in laboratory larvae.

$$\text{- Relative resistance} = \frac{\text{The highest resistance percentage in the field strain}}{\text{Corresponding resistance percentage of each strain}}$$

Biochemical assays:

Preparation of insect homogenates:

Different strains of the 4th instar larvae of the pink bollworm, *Pectinophora gossypiella* collected from four Governorates; namely Kaferl-Sheikh, Dakahlia, Gharbia and Menoufia as well as the laboratory strain were weighed and counted. Each batch was mechanically homogenized in 10 volumes (W/V) of 0.1 M phosphate buffer, pH7 for 2 minutes using a Teflon homogenizer surrounded with a jacket of crushed ice. The homogenates were then centrifuged at 4000 rpm for 30 minutes at 4°C using cooling centrifuge. The resultant supernatant was used to determine the activities of acetylcholinesterase (A.Ch.E.), α and β non-specific esterases, acid and alkaline phosphatases, as well as the total soluble protein content.

Hydrolases activity:

Determination of acetylcholinesterase (AChE) activity:

Acetylcholinesterase activity (0.4 ml of enzyme solution was measured colorimetrically according to the method described by Ellman *et al.* (1961), which is based on the hydrolysis of Acetylcholine (ASCH) as a substrate. Thiocholine reacts with 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB as a color developer) to produce 5-thio-2-nitrobenzoic acid giving a yellow colour, which can be determined, spectrophotometrically at a wave length of 214 nm. AChE activity was measured and expressed as mg substrate hydrolyzed/individual/min.

Determination of alkaline and acid phosphatases activity:

The activities of alkaline and acid phosphatases were determined according to the method described by Powell and Smith (1954). The reaction mixture consisting of 1 ml carbonate buffer (pH10.4) for alkaline phosphatase or 1 ml citric buffer (pH4.9) for acid phosphatase, 0.01 M disodium phenyl phosphate (substrate) and 0.1 ml from tissue supernatant. It was incubated for 30 minutes at 37°C. The reaction was stopped by the addition of 0.8 ml of 0.5 N NaOH then 1.2 ml of 0.5N $NaHCO_3$, 1ml of 4-aminoantipyrine solution and 1 ml of potassium ferricycyaimde were added to produced a red or purple color. The amount of phenol liberated in the media corresponds to the enzyme activity hydrolyzing the substrate, i.e., the more activity of the enzyme on the substrate, the more liberation of phenol. The produced colour was measured colorimetrically at 510 nm. The enzymatic activity is expressed as mg phenol/larva/min.

Determination of non-specific esterases activity:

Alpha esterases (α-esterases) and beta estrases (β-esterases) were determined according to Van Asperen (1962) using alpha α-naphthylacetate or β-naphthyl acetate as substrates; respectively.

The reaction mixture consisted of 5 ml substrate solution (3x1o4Mα-orβ-naphthylacetate, 1 % acetone and 0.1M phosphate buffer, pH7) and 20µl of larval homogenate. The mixture was incubated for exactly 15 min at 27ºC, then 1 ml of diazoblue color reagent (prepared by mixing 2 parts of 1 % diasoblue B and 5 parts of 5 % sodium laurel sulphate) was added. The developed color was read at 600 or 555 nm for α- and β-naphthol produced from hydrolysis of the substrate; respectively.

Determination of total proteins:

Total soluble proteins were determined according to Bradford (1976).

Preparation of protein reagent:

Coomassie Brilliant and Blue G-250 (100 mg) was dissolved in 50 ml 95% ethanol. To this solution 100 ml 85% (W/V) phosphoric acid were added. The resulting solution was diluted to a final volume of one liter.

Protein assay:

Sample solutions 50 µl were pipetted into a test tube and the volume was adjusted to 0.1 ml with phosphate buffer (pH 6.6) the, 5 ml of protein reagent were added to the test tube and the contents were mixed by overtaxing.

The absorbency at 595 nm was measured after 2 min and before 1 hr against blank prepared from 0.1 ml of phosphate buffer (pH 6.6) and 5 ml of protein reagent. The weight of protein was plotted against the corresponding absorbance resulting in standard curve used to determine the protein in unknown samples.

RESULTS AND DISCUSSION

Discriminating concentration of profenofos against the laboratory strain of the pink bollworm, *Pectinophora gossypiella* fourth instar larvae clarified in Table (1) and was carried out for rapid monitoring the insecticidal resistance in the field colony strains.

Table 1: Effectiveness of profenofos against the 4th instar larvae of the laboratory strain of *P. gossypiella.*

Conc. in ppm	Corrected mortality %
10	86.4
5	80
2.5	70
0.1	55
0.05	45
LC50	0.875
LC_{90}	15.525
LC_{99}	163.842
Slope	1.026

The obtained results concerning the response of *P. gossypiella* 4th instar larvae, which collected from different Governorates to discriminating concentration of the organophosphate insecticide, profenofos are represented in Table (2).

Table 2: Response of the pink bollworm. *Pectinophora gossypiella* fourth instar larvae collected from different Governorates to discriminating concentration of profenofos using surface film technique.

Strain	Mortality%	Resistance%*	Relative resistance**
Dakahlia	48	51.62	1.16
Gharbia	40	59.60	1.00
Menoufia	56	57.17	1.37
Kafrel-Sheikh	60	39.39	1.51

In this study, surface film application of the insecticide was used. The obtained results revealed that the discriminating concentration of the organophosphate insecticide; profenofos (LC_{99}) caused 48, 40, 60, and 56 % mortality for the 4th instare larvae of *P. gossypiella* collected from Dakahlia, Gharbia, Kafrel-Sheikh and Menoufia Governorates; respectively.

Moreover, Gharbia field colony strain exhibited the highest resistance value to profenofos toxicity. The corresponding resistance value was 59.60 %; whereas Karel-Sheikh strain recorded the lowest resistance value to profenofos action, it was 39.39 %. On the other hand, Dakahlia and Menoufia Governorates occupied the middle situation among the two strains that mentioned previously.

Concerning the relative resistance values, resistance in the 4th instar larvae of *P. gossypiella* collected from Gharbia to the toxicity of profenofos treatment recorded 1.16, 1.51 and 1.37 fold as the resistance in the 4th instar larvae of Dakahlia, Kafr el-Sheikh and Menoufia strains; respectively.

Determination of biochemical aspects in field colony strains of *p. gossypiella* 4th instar larvae collected from different Governorates compared with the laboratory strain:

Hydrolases activity:

The activities of the determined hydrolyzing enzymes, i. e alkaline and acid phosphatases, non-specific esterases and acetylcholinesterase were determined in the 4th instar larvae of the pink bollworm, *P. gossypiella* collected from cotton fields cultivated in four Governorates, namely Karel-Sheikh, Dakahlia, Gharbia and Menoufia in comparison with the laboratory strain. Determination of the enzymes activity may shed light on the inductive effect of chemicals on *P. gossypiella* larvae.

Alkaline and acid phosphatases activity:

It is generally accepted that the properties of an organophosphorus compound that determined its susceptibility to phosphatase cleavage are the same which enable it to inhibit acetyl cholinesterase, i. e. electrophilic phosphorus atom. The alkaline and acid phosphatases activity levels as indicated in Table (3) were expressed as enzyme unit x 10^3/ gram body weight/min. The obtained results indicated that the alkaline phosphatase activity in the 4th instar larvae of the pink bollworm collected from Kafrel-Sheikh, Dakahlia, Gharbia and Menoufia Governorates as well as the laboratory strain exhibited remarkable much higher enzymatic activity as compared with the acid phosphatase activity.

Table 3: Alkaline and acid phosphatases in the whole homogenates of different field colony strains of *P. gossypiella* 4th instar larvae compared with the laboratory strain.

Strains	Alk. phosphatase *Activity X10^3	Activity** ratio	Acid phosphatase*activity x10^3	Activity ratio**
Kafrel-Sheikh	1327	2.30	601.67	1.10
Dakahlia	1902	3.29	680.33	1.25
Gharbia	3383.67	5.85	710.67	1.30
Menoufia	1448	2.51	691	1.27
Laboratory	578	1.00	544.67	1.00
Average	1727.73	2.99	645.67	1.18

* Enzymatic activity was expressed as enzyme unit x 10^3 / min / g.b.w.

The highest alkaline and acid phosphatases activity was recorded in the larvavae collected from Gharbia Governorate. On the other hand, the lowest enzymatic activity levels associated to both alkaline and acid phosphatases were occurred in the 4th instar larvae collected from Kafrel-Sheikh Governorate. Generally, the alkaline phosphatase activity levels in the 4th Instar larvae collected from cotton fields cultivated in Kafrel-Sheikh, Dakahlia, Gharbia and Menoufia Governorates as well as the laboratory strains were 1327, 1902, 3383.67, 1448 and 578 enzyme unit x 10^3/ g. b. wt./min.; respectively. The corresponding enzymatic activity levels of acid phosphatase activity levels were 601.67, 680.33, 710.67, 691 and 544.67enzyme unit x 10^3/ g.b.wt./ min.; respectively. The mean levels of the alkaline and acid phosphatases activity in the five strains aforementioned were 1727.73 and 645.67 unitx10^3 / g.b.wt./min.; respectively

Through the light on the activity ratio as illustrated in Table (3), the highest enzymatic activity ratios were noticed in the 4th larval instars collected from Gharbia Governorate. The corresponding activity ratios of the alkaline and acid phosphatases relative to the laboratory strain were 5.85 and 1.30; respectively. On the other hand, the lowest enzymatic activity ratios were attained in the 4th larval instars that collected from Kafrel-Sheikh Governorate, where the corresponding enzymatic activity ratios were equal 2.3 and 1.1 for both enzymes, respectively. The enzymatic activity ratio values associated to alkaline phosphatase were ranged between 2.30 and 5.85; whereas the enzymatic activity ratios in case of acid phosphatase were ranged between 1.10 and 1.30.

Non-specific esterases:

Comparison of non-specific esterases activity in the 4th larval instars of the pink bollworm collected from different Governorates as well as those of the laboratory strain are summarized in Table (4). The enzymatic activity levels were expressed as ug naphthol released/ g.b.wt/min. The obtained results indicated that the enzyme activity levels were much higher in the field colony strains as compared with the laboratory strain. On the other hand, α esterases were remarkable much higher in the larvae collected from different Governorates as well as the laboratory strain than the corresponding β-esterases activity. The corresponding mean levels of the enzymatic activity were 1361.51 and 889.13 µg-naphthol/g.b.wt/min.; respectively. Moreover, the larvae collected from Gharbia Governorate showed the highest enzymes activity. The corresponding α and β- esterases activity levels were 1410.33 and 1065.33 µg naphthol releasesd / g. b. wt. /min.; respectively. The lowest non specific esterases activity levels were noticed in the 4th larval instars collected from Kafrel-Sheikh Governorate, where the corresponding enzymes activity levels were 1306.33 and 741.33 µg naphthol released / g. b. wt. /min.; respectively.

Table 4: α and β- non specific esterases activity in the whole homogenates of different field colony strains of *P. gossypiella* 4th instar larvae compared with the laboratory strain.

Strains	α – esterases *Activity	Activity **ratio	β-esterases *Activity	Activity ratio
Kafrel-Sheikh	1306.33	1.01	741.33	1.08
Dakahlia	1408.33	1.09	1042.67	1.51
Gharbia	1410.33	1.10	1065.33	1.53
Menoufia	1395.33	1.08	908.00	1.32
laboratory	1287.33	1.00	688.33	1.00
Average	1361.53	1.06	889.13	1.29

* Enzymatic activity was expressed as µg naphthol released / mim / g.b.w.

Concerning the activity ratio values, α non-specific esterases were much higher than those of β-non-specific esterases in larvae of both field colony and laboratory strains. Moreover, the highest enzymatic activity ratios were noticed in the larvae collected from Gharbia Governorate, where the corresponding values of α and β non-specific esterases recorded 1.10 and 1.53; respectively. On the other hand, Kafrel-Sheikh field colony strain

showed the lowest activity ratios for α and β non-specific esterases which measured 1.01 and 1.08; respectively.

Acetyl cholinesterase activity (A.Ch.E.):

Organophosphrus and carbamate insecticides owe their toxicity to the inhibition of the enzyme, acetyl cholinesterase activity, which has a vital role in the maintenance of the nerve activity by removing acetylcholine released in the passage of an impulse synapses and possible also along axons. The biochemical assays of A.Ch.E. activity in the field colony collected from four Governorates as well as the laboratory strain of the *p. gossypiella* 4th larval instars are represented in Table (5). The obtained data showed that the enzyme activity levels were remarkable much higher in larvae of the field colony strains than the correspondent levels in the laboratory strain. The highest enzymatic activity was noticed in larvae collected from Gharbia Governorate; whereas the lowest one was occurred in Kafrel-Sheikh Governorate. Based on the enzymatic activity levels, the larvae of the field colony strains could be descendingly arranged in order as follow: Gharbia, Dakahlia, Menoufia and Kafrel-Sheikh strains. The corresponding enzymes activity levels were 41.97, 39.67, 35.23 and 35.17ug acetylcholine bromide released / min / g. b. wt. and the corresponding activity ratios were1.25, 1.18, 1.05 and 1.04; respectively. The enzymatic activity in the laboratory strain was 33.67 ug acetylcholine bromide released/ min/ g. b. wt.

Table 5: Acetylcholinesterase activity in field colony strains of *P. gossypiella* 4th instar larvae compared with the laboratory strain.

Strains	Activity *	Activity ratio**
Kafrel-Sheikh	35.17	1.04
Dakahlia	39.67	1.18
Gharbia	41.97	1.25
Menoufia	35.23	1.05
Laboratory	33.67	1.00
Average	37.14	1.38

* Acetylcholinesterase activity was expressed as µg AchBr / min / gram body weight.

Total protein contents:

Results illustrated in Table (6) indicated that the total protein contents in the *p. gossypiella* 4th larval instars collected from the different Governorates that aforementioned were remarkable much higher than in the laboratory strain. Gharbia strain showed the highest amount of the total soluble protein contents as compared with the other field colony strains which being 73.30 mg/ gram body weight. On the other hand, Kafrel-Sheikh strain had the lowest amount of the total protein contents as compared with the other field colony strains, which recorded 59.10 mg/ gram body weight. The amount of the total protein contents in the 4th larval instars collected from Dakahlia and Menoufia Governorates occupied middle situation among those collected from Gharbia and Kafrel-Sheikh, the corresponding amounts were 70.60 and 65.33 mg protein contents/ gram body weight; respectively. The average of the total soluble protein contents in the different field colony as well as the laboratory strains were 65.25 mg. protein contents/ gram body weight.

Table 6: Total protein in the whole homogenates of different filed colony strains of *P. gossypiella* 4th larval instars compared with the laboratory strain.

Strains	Mg / g.b.w	Total protein ratio **
Kafre-Sheikh	59.10	1.02
Dakahlia	70.60	1.22
Gharbia	73.30	1.27
Menoufia	65.33	1.13
Laboratory	57.93	1.00
Average	65.25	1.13

Total protein ratio = $\frac{\text{amount of total protein in filed strain}}{\text{total protein in laboratory strain.}}$

The values of the protein ratio in the larvae collected from the different Governorates that aforementioned ranged between 1.02 and 1.27. The highest value of the total protein ratio was noticed in Gharbia strain, which measured1.27. The total protein average in the different strains was 1.13.

The objective of a resistance-program is to detect resistance before a control failure occurs. The monitoring program should measure frequencies as well as monitoring changes in the frequency of resistance with time (Schouest and Miller, 1988). Laboratory bioassays that closely simulate the field situation help to establish a correlation between the laboratory bioassay and the field by establishing LDP relationship (Ball, 1981&Roush and Miller, 1986). In this respect, Ismailia field strain exhibited the highest level.

The obtained results concerning the response of *P. gossypiella* 4th larval instars, which collected from different Governorates to discriminating concentration of profenofos showed remarkable much high resistance percentages to the toxicity of profenofos which ranged between 39.39 and 59.60 In this respect it is obvious that Gharbia field colony strain larvae exhibited the highest resistance value to the profenofos toxicity as compared with the other field colony strains, i.e. Kafrel-Sheikh, Dakahlia and Menoufia strains. It is interest to note that the difference in the resistance levels may be related to the detoxification of the tested compound by the enzymatic activity. In this field of investigation Hassan and Mostafa (2000) determined the toxicity of three organophosphorus insecticides namely, Malathion, Fenthion and Fentr othion against the 3rd instar larvae of the Mediterranean fruit fly, *Ceratitis capitata.* They revealed that Fenthion was the most toxic compound against the 3rd instar larvae of both field colony and laboratory strain, whereas Fentrothion was the least efficient toxicant. They demonstrated that the resistant ratio values ranged from 2.06 to 2.31 indicating tolerance in the the field colony strain than resistance. They studied the effect of S,S,S-tributyl phosphorothioate(DEF) an esterases inhibitor, N-propyl paraoxon a phosphatases inhibitor on the toxicity of the three tested insecticides against the 3rd instar larvae. They indicated that the synergistic rates were higher in the field colony as compared with the laboratory strain. Khidr *et al*. (2002) developed technique for monitoring resistance in the pink bollworm moths; *P. gossypiella* adult stage of a laboratory strain was tested with six insecticides. An approximate LC_{99} was selected to discriminate for monitoring resistance in the field colony strains. They stated that the resistance percentages in these field strains ranged from 2.32 and 49.50%. The present results are in close agreement with those published by Abou El-Seoud *et al*. (2005). They revealed that resistance measured by the discriminated concentration LC_{99} was high in regions where Chlorpyrifos was frequently used. They found that resistance in *P. gossypiella* adult to the insecticide used ranged for 51.5 and 80.6% in the tested strains collected from four governorates.

Although biochemical monitoring tools such as enzymes activity assays by no means a universal alternative to conventional bioassay techniques, they can complement bioassays and provide information about the dynamics and evolution of resistance among field populations. For example, esterases catalyze hydrolysis of several different types of esters, and many insecticides such as organophosphates, carbamates and pyrethroids contain ester groups. Therefore, these classes of insecticides could be detoxified via hydrolysis of the ester bond. Malathion is one of these insecticides that can be hydrolyzed by esterases activity.

Accordingly, in this study, it was found that Ismailia field colony strain has the highest level of resistance in comparable with other tested field colony strains, showed the highest levels of the enzymatic activity included alkaline& acid phosphatases, α-and β-esterase, and acetylcholinesterase as well as the highest amount of protein contents as compared with the

other field colony strains that recorded less level of resistance. These results strengthen the hypothesis that, the mechanism associated with insecticides resistance had been found in many isects having an increase of such enzymes activity, probably as a result of gene amplification (Cruz *et al.*,1997). Also, Raymond *et al*. (1989) and Piorie *et al*. (1992) reported that the high levels of esterases activity involved in organophosphorus insecticides are caused by an enzyme overproduction. This overproduction is the result of gene amplification and/or gene regulation (Rooker *et al*. 1996). Esterases activity in most resistance strains represented 3-4% of the total protein (Yamamoto *et al.*, 1983).

The results obtained in this study agree with Young *et al*. (2005) who reported that pyrethroid resistance in field populations of Australian *Helicoverpa armigera* is primarily a consequence of the overproduction of esterases isoenzymes which metabolites and sequester pyrethroids. Zhou *et al*. (2003) found a consistent correlation between elevated esterases activity and Methyl Parathion resistance among Nebraska wastern corn rootworm, *Diabrotica virgifera* populations. Hung and Ottea (2004) reported that esterases activity associated with resistance to organophosphorus and pyrethroid insecticides in *Heliothis virescens* larvae were detected.

These data assisted the growers to phase the fact that profenofos rapidly losing its effectiveness and alternative control methods are needed.

The most common mechanism of resistance in the insect species to the tested insecticides is related to several types of metabolic enzymes, Khidr (1982), Siegfried and Zera (1994) and Wilson and Ashok (1998),

Organophosphates, carbamates and pyrethroids can be detoxified by appropriate hydrolytic enzymes such as esterases. Another type of organophophates is based on an increase in detoxification capacity resulting from increased phosphatases activity. The results indicated that non-specific esterases and phosphatases play an important role in the detoxification of the insecticides used for controlling the pink bollworm 4th larval instars

In this field of investigation associated to the resistance mechanism in the insect pests to the different insecticidal treatments; Khidr (1982) studied the effect of S, S, S-tributyl phosphorrthioate (DEF), an esterases inhibitor and n-propyl paraoxon; a phosphatases inhibitor on the toxicity of Malathion, Methyl Parathion and Dimethoate agaist the 4th instar larvae of helicoverpa armigera. He revealed that the synergestic ratio was remarkedly higher for the field colony strain as compared with that of the laboratory strain. He noticed that DEF synergized Malathion greater than both Methyl Parathion and Dimethoate. As known n-propyl paraoxon, the synergistic ratio for the three tested insecticides was in the order; Methyl Parathion, Dimethoate and Malathion. Also, Hassan and Mostafa (2000) studied the effect of DEF an esterases inhibitor, N-propyl paraoxon a phosphatases inhibitor on the toxicity of three organophosphorus insecticides; namely Lebaycid, Malathion and Fenitrothion against the 3rd instar larvae of the Mediterranean fruit fly, *Ceratitis capitata.* They indicated that the synergistic rates were higher in the field colony as compared with the laboratory strain. The enzymatic inhibitors synergized Malathion greater than the other two insecticides.

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ARABIC SUMMARY

تقصى ظاهرة المقاومة فى سلالات مختلفة لدودة اللوز القرنفلية لمبيد البروفينوفوس وعلاقتها ببعض النظم البيوكيمائية

ليلى سليمان
معهد بحوث وقاية النباتات – مركز البحوث الزراعية – وزارة الزراعة- الدقى - الجيزة

أظهرت السلالات الحقلية لدودة اللوز القرنفلية التى جمعت من حقول محافظات كفر الشيخ ، المنوفية ، الغربية والدقهلية فى نهاية موسم قطن 2013 درجات متباينة لفعل مبيد البروفينوفوس . اظهرت المناطق التى عوملت بمبيد البروفينوفوس بطريقة متعاقبة أعلى مستوى مقاومة فى دودة اللوز القرنفلية . تراوحت مستويات مقاومة العمر اليرقى الرابع لدودة اللوز القرنفيلة لمبيد البروفينوفوس بين 31.31 ، 59.60 % فى السلالات المختبرة . أظهرت يرقات دودة اللوز القرنفلية التى جمعت من محافظة الغربية اعلى مستوى مقاومة لفعل مبيد البروفينوفوس مقارنة بالسلالات الثلاثة الاخرى ؛ بينما لوحظ اقل نسبة مئوية لظاهرة المقاومة فى اليرقات التى جمعت من محافظة كفر الشيخ.كما اظهرت الدراسةات التى اجريت على مستوى نشاط إنزيمات إزالة السمية زيادة فى إنزيمات كلا من اسيتيل كولين استيراز ، الفوسفاتيز القاعدى والحامضى ، وألفا وبيتا استيرزات غير المتخصصة وكذلك محتويات البروتين الكلى . قد تكون ميكانيكيات هامة لمقاومة السلالات الحقلية لمبيد البروفينوفوس . أظهرت الاختبارات اللونية علاقة طردية بين زيادة مستوى المقاومة فى السلالات المختلفة لدودة اللوز القرنفلية المختبرة ومستويات نشاط إنزيمات التحلل المائى لذلك ومن المعلومات المتواجدة تبين اهمية تطبيق برنامج الادارة المتكاملة للافات لتفادى تتابع استخدام المبيدات بتوسع .