Biochemical effects of certain organophosphorus pesticides against *pectinophora gossypiella* (saund) in fayeom and Dakahlia governorates Egypt.

Mona K. El-Hadek
Centeral Agriculture pesticide laboratory, Agricultural research center

ABSTRACT: The toxicity of thirteen organophosphorus insecticides against *pectinophora gossypiella* (saund) laboratory and two field strains (collected from Fayeom and Dakahlia governorates were investigated. The results indicate that, resistance ratios (RR) varied from one governorate to another. In faoum governorate data can be classified to three categories. The first category showed low resistance, where the resistance ratios were (6.86 and7.91) folds. The second category was moderate resistance the resistance ratios and fluctuated between 11.64and 26.2 fold. As for The third category., resistance ratios were high and ranged from 40.43- to 96.00- fold for insecticides against *p. gossypiella*. while dakahlia governorate, also data can be classified to three different categories. However the levels of resistance to the tested insecticides were higher in Fayoum governorate thanthose in Dakahlia governorate. Determination activity of alkaline phosphatase, acetylcholinestrase and total proteins were also determined in both field strains. Data showed a decrease in alkaline phosphatase activity in both field strains; while acetylcholinesterase activity and total protein content were increased in field strains as compared with laboratory strain.

Keywords: *Pectinophora gossypiella* (saund.) organophosphorus insecticides op, Alkline phosphates total protein Ache, resistance ratio (RR)..

1.Introduction

In Egypt, cotton is one of the most important crops and represents more than half the income of two million small-scale farmers. Moreover cotton is attacked by many insect species. Cotton bollworms are the most destructive insect infesting cotton plants. Pink bollworm , *Pectinophora gossypiella* (saund.) and spiny bollworm , *Earias insulana* (Boisd) but infest many cotton producing areas of the world and cause a severe reduction in cotton yield and quality **(Lohag and Nahyoon, 1995)**.

The pink bollworm, Pectinophora *gossypiella* (Saund.) is the most destructive pest of cotton plants in Egypt. Protection of cotton plant and mass production of harvest cotton fibers depends mainly on the efficient control for this pest Chemical control is still adopted as one of the major techniques for combating these serious pests. The effectiveness of different pesticides against bollworms were studied by several authors **(Khan *et al.*, 2007, Balakrishman *et al.*, 2009 and Magdy *et al.*, 2009)**. Besides, the two pests.Treatment of orgaophosphorus and pyrethroid insecticides were effective in reducing larvae and adult numbers of pink bollworm **(Leonard *et al*)**. 1988). The continuous application of insecticides leads to development of insect resistance in different Egypt governorates **(Abo- Sholo *et al.*, 1998)**.The esterase enzymes exhibit a greater degree of polymorphism than other enzymes because they action a class of molecules many of which come directly fromexternal environment **(Kojima *et al.*, 1970)** The role of phosphatases are defined as enzymes hydrolyzing any phosphorus esters o anhydride bond **(O' Brien, 1967)**. The acid and alkaline phosphatases play an important role in insect development, nutrition and egg maturation. They also, involve in the mechanisms of active transport as well as the activity of secretion in the midgut **(Nath and Butler, 1971)**.The present work aimed to monitoring the resistance of several organophosphorus insecticides against Pink bollworm. *pectinophora gossypiella* (Saund.) collected from two governorates during August to November 2015 season. The determination activity of alkaline phosphatase, acetylcholinestrase and total protein were also studied in the research.

2.Materials and methods

2.1.tested insect:

Two locations field of the pink bollworm *Pectinophora gossypiell*a (Saund) were collected from the cotton fields in several locations (Dkahlia and Fayoum) during August to November 2015 cotton season.

The infested green bolls were collected in the end of cotton growth. Season the disposed larvae were released from the double infested seeds and reared under constant temperature 27 ± 2 ◦C and relative humidity 70 – 75 %.

Five concentrations were used for each insecticide and three reolicates of ten adults each were used for each concentration. Glass chiney cages (6 x 9 cm) were dipped method in each concentrations of insecticide for 20 seconds and left to dry at room temperature. The newly moth (zero- day- old) were exposed to residual insecticides in glass chiney cages and covered with muslin cloth to allow air circulation. After twenty-four hours of exposure the mortality percentage were inspected. Abbott's formula **(Abbott, 1925)** was adapted to correct the mortality data for natural mortalities in the control and concentration- mortality relationship was determined using the computer program LDP line, based on probit analysis **(Busvine, 1957)**. The resistance ratios were expressed as susceptible strain which has been reared in laboratory condition for more than 15 generations without exposed any insecticides.

Resistance ratio= LC_{50} of the field strain / LC_{50} of the susceptible strain.

2.2. Insecticides used:

Organophosphorus insecticides:

Profenofos (Teleton, cord, and kamakron EC72%), Chloroprifos (Dursban, Dorsel, Chlorozan, Helban, Pestban bereban,dora, renoban and pyrifous elnasr) EC48%.

2.3. Biochemical assays

The use 200mg of laboratory and field moths of pink bollworm were homogenized in tris buffer pH 7.8. The cold crud extracts were centrifuged at 10000rpm for 30min at 4°C. The supernatant was taken carefully into new tubes and preserved at -20°C until the biochemical determinations. Determination the total protein content was measured by using Buriet reaction **(Gornal *et al.*, 1949)**. Activity of acetylcholinesterase (Ache) enzyme was assayed according to the method of **Ellman *et al.*, (1961)**. Alkaline phosphatase was measured by using the method of **Belfield and Goldberg (1971)**.

Statistical analysis of the data

Statistical analysis was carried out according to **Fisher (1970)**. LSD (Least squares difference) test was used to compare the significant differences between means of treatment **(Waller and Duncan, 1969)**.

3.Results and Discussion

3.1.Resistance ratios of organophosphorus insecticides against two strain of *pectinophora gossypiella* during August to November 2015 season.

Resistance ratios of organophosphorus insecticides tested against two field strains of *P. gossypiella* collected from two governorates, Fayoum and Dakahlia during 2015 cotton seasons are shown in Table (1). The results indicate that, resistance ratios fluctuated from one governorate to another. In fayoum During cotton season 2015.

Data in Table (1) persented classified to three categories during cotton season 2015, the first category showed low resistance, where the resistance ratios were (6.86 and 7.91) fold for pereban and pestiban respectively. The second category was moderate resistance where the resistance ratio were 11.64, 17.6,18.42, 21.17, 21.33 and 26.2 fold respectively for telethon, kamkron, renoban, dorsal, dora and nasr-fous respectively. Finally,where resistance ratios were high the third category included cord, pesteban, helban, dursban, chlorozan and chlorozed. The RR were (40.43, 48.68, 48.84, 96 and 96 fold. Respectively .

On the other hand; data obtained from Dakahlia governorate showed in table(1) also classified to three category. The first category give moderate of resistance where the RR were (12,73,16.61, 16.27, 19.54, 25.56 and 28.84) fold respectively for kamakron, telethon,pereban, nasr-fous, cord and dora. The second category included helban, dorsal, renoban, chlorozan, chlorozed and dursban, where RR were 46.4, 53.67, 63.11 56.84, 67.46 and 80.42 fold then the third category included pestban which showed high level of resistance (118.07) fold.

Nour El-Hoda A. Zidan (2012) Found the efficiency of five insecticides bolelonging to three different chemical group, pyrethroids, OPs and carbamate on the bollworms which infested cotton plant were evaluated in a field trial during 2010 and 2011 seasons. The number of the larvae of pink and spiny bollworms in the green bolls were recorded before and after treatment and the percent of reduction. The obtained results indicated that, toxic effect of the tested compounds against pink bollworm (PBW) in 2010 cotton season, however they were applied once, twice and triple. While based on the general mean of reduction percentage in infestation of PBW it was ranged between 63.03 to 81.96 %.

Radwan and El- Malla (2015) evaluated in laboratory the toxicity of organophosphorus insecticides against laboratory and field female moths of *P. gossypiella* collected from three lower Egypt governorates female moths profenofos was very high levels of resistance against insects in Sharkia, Menofia and Gharbia insects governorates respectively compared with laboratory. Chlorpyrifos and fenpropathrin had the lower toxic effect (69.8, 46.5 and 40.1 ppm) with high levels of resistance (25.5, 48.4 and 31.6 fold)for Ghabia, menofia and sharkia insects respectively.

Table (1): Resistance ratio to some organophosphours insecticides against bollworm *P. gossypiella* (Saund.) collected from two Governorates fayoum and dkahlia during 2015 seasons.

no	Insecticides OP	Susceptible strain		Field strain					
				Fayoum			Dakahlia		
		LC_{50}	slope	LC_{50}	slope	RR	LC_{50}	slope	RR
1	Telton	9.18	1.97	106.9	1.47	11.64	147	1.97	16.61
2	Nasr-fos	15.25	2.23	399.6	0.925	26.2	298	2.16	19.54
3	Cord	3.01	2.13	121.7	0.616	40.43	76.95	2.14	25.56
4	Kamecron	9.56	1.98	168.6	1.73	17.64	121.7	2.34	12.73
5	Dora	11.25	2.23	240	1.244	21.33	324.5	2.15	28.84
6	Helban	10.34	2.15	5.5	0.906	48.84	480	2.73	46.42
7	Dursban	29.25	2.16	1598	0.75	54.63	2352	2.58	80.41
8	Preban	39.89	2.52	274.7	0.616	6.89	649	1.98	16.27
9	Dorseel	21.24	2.14	449.6	1.85	21.17	114	2.25	53.67
10	Renoban	40.12	2.58	739.1	1.139	18.42	2532	2.13	63.11
11	Pestiban	41.23	2.73	326	0.925	7.91	48.68	2.23	118.07
12	Chlorzan	16.89	2.34	418	1.119	46.25	96.0	2.23	56.84
13	Chlorzed	14. 23	2.25	1375	0.904	96.63	96.0	2.52	67.46

***RR resistance ratio LC_{50} field strain/ LC_{50} susebtable strain**

1- Biochemical studies

Data in Table (2) shoed the activity of alkaline phosphatase in laboratory (L) strain moths and both field strain moths (Fayoum [F] and Daqhliya [D]). The enzyme activity increased in L strain" 0.920µmol/mg protein/min; while it recorded a very high significant decrease in both of field strain, where ctivites were "0.744 and 0.693µmol/mg protein/min" for both F and D strains, respectively.Phosphatses enzymes are reported to be the biosensors on their inhibition by organophosphorus compounds **(Sohail *et al.,* 2004)**. This result is in conformity with **Radwan and El-Malla (2015)**, who reported that alkaline phosphatase activity was decreased in field moths of the Pink bollworm than the laboratory strain.Results also indicated that both field strains had a high significant increase in acetylcholinesterase activity ("50.64% and 87.91%") when compared with L strain moths. AChE is an important enzyme in insect nervous system which hydrolyzes acetylcholine neurotransmitters to yield nerve impulses, it is the primary target of OP and carbamate insecticide. Present data showed that, increase in resistant to insecticides in field strain moths seems to be associated with AChE activity.

AChE insensitivity plays an important role in insecticide resistance mechanism in field strains, these results agree with that obtained by Nour El-Hoda *et al.*, **(2012)**, who found that, AChE activity was increased in field strain than laboratory strain.

Table (2): determination activities of alkaline phosphates, actylcholinesterase and total protein in homogenate of *P. gossypiella* in laboratory and field strains from fayoum and Dakahlia governorates.

Strains / parameters	Alkaline phosphatase µmol/mg protein/min		Acetylcholinesterase		Total protein mg/100mg	
	Mean ±S.E.	Change (100%)	Mean ±S.E.	Change (100%)	Mean ±S.E.	
Field strain Fayoum (F)	*** 0.744 ± 0.0104	-19.13	** 6.854 ± 0.191	+50.64	*** 0.758 ± 0.0101	Change (100%)
Field strain Daqhliya (D)	*** 0.693 ± 0.003	-24.67	** 8.55 ± 0.394	+87.91	* 0.473 ± 0.003	+4.42
Laboratory (L)	0.920 ± 0.0026		4.55 ± 0.543		0.453 ± 0.006	

*Significant
** high significant
*** very high significant

The total protein content increased in both field moths strains when compared with laboratory strain when compared with laboratory strain moths, it reached (0.758mg/100mg b.w.) in F strain and (0.473mg/100mg b.w.) in D strain. These results is in agreement with that found by **Nour El-Hoda *et al.,* (2012)**, who showed that, the concentration of total protein has been increased in field population of the Pink bollworm than of the susceptible strain. Proteins are important compound that binds the foreign compounds and the increase of total

protein of field or treated insects may lead to the increase in enzymes activity related to various insecticides.

References

Abbott, W. S. (1952). A method of [computing the effectiveness of an insecticide. *Econ. Entomol., 18:265-267.*

ABO- Sholo, M. K.; Nassef , M. A. and Watson , W. M. (1998). Changes in susceptibility of the larvae of pink bollworm, *Pectinophora gossypiella* (Saund.) To synthetic pyrethroids. (Egypt.J.Agric . Res. 78 (2):665-673.

Belfield, A. and Goldberg, D. M. (1971). Colorimetric determination of alkaline phosphatase activity. Enzyme.12:561–568.

Balakrishman, N.B. Kumar, V. and Sivasubramanan P. (2009). Bioefficacy ofbifenthrin 10 EC against sucking insects, bollworms and natural enemies in cotton.Madrase *Agricultural journal 96 (1/6): 225-229.*

Busvine, J.R. (1975). A criticalreview of technique for testing insecticides. Commonwealth Inst. *Entomol. London.*

Gornal, A. G.; Bardawill, C. J. and David, M. M. (1949). Determination of serum protein by means of Biuret reaction. J. Biol. Chem. 177: 571-576.

Ellman, G. L.; Courtney, K. D.; Andres, V. Jr. and Featherstone, R. M.(1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7: 88–95.

Fisher, R. A. (1970). Statistical Method for Research Workers, Oliver and Boyed, Edinburgh. pp.140-142.

Khan, R. R.; Ahmed, S; Saleem, M. W. andNadeem M. (2007). Field evaluation of different insecticides against spotted bollworms *Erias spp*.at district sahiwal.*Pakistan.Entomologist., 29(2):129 – 133.*

Kojima, K.; Gillespie, J. and Tobbari, X. N. (1970). A profile of Drosophila species enzyes assayed by electrophoresis.I. Number of alleles, heterozygosites and linkage disequilibrium in glucose metabolizing systems andsome other enzymes, Biochem. Genet. 4: 627-637.

Leonard, B. R.; Graves, J. B.; Sparks, T. C. and Pavioff, A. M. (1988). Variation in resistance of field population of tobacco budworm and bollworm (Lepidoptera: Noctuidae) to selected insecticides J. Econ. Entomol., 81:1521-1528.

Lohag, M. G. M. and Nahyoom, Y. M. (1995).Comparative efficacy of Hostathion, sumicidin and sevin xl R against cotton bollworms Sarhad. *J.Agric. Res.;11(13): 963-368.*

Magdy, A. Z.; Ibrahim, M. M.; shekeban, M. M.and Ebed, H. M. (2009). Effect of threeinsecticides and two insecticides alternatives onpink bollworm *P. gossypii* (saurd) and quality of cotton yield .Alex. *Univ. J. Agric. Res.,54(1):155-163.*

Nath, J. and Butler, L. (1971). Acid phosphates during development of black beetle Attagenus megtoma (Fab.). J. Biochem., 49:311-315.

Nour El-Hoda, A. Z.; El-Naggar, J. B.; Aref, S. A. and E. El-Dewy, M. (2012). Field evaluation of different pesticides against cotton bollworms and sucking insects and their side effects. Journal of American Science, 8(2): 128-136.

O' Brien, R.D. (1967) insecticides action and metabolism (Academic press, New york and London)

Radwan, E. M. M. and El-Malla, M. A. (2015). Toxicity of Insecticides against the Laboratory and Field Moths *Pectionophora Gossypiella* (Saunders). Egyptian Scientific Journal of Pesticides, 1(1): 1-9.

Sohail, A.; Mushtaq, A. S. and Rao, K. S. (2004). Effect of cypermethrin (10EC) and bifenthrin (10EC) on levels of acid and alkaline phosphatases in a strain of Tribolium castaneum (herbst) (coleoptera: tenebrionidae). Pak. Entomol., 26(1): 47-52.

Waller, W. M. and Duncan, D. B. (1969). A Bayes rule for the symmetric multiple comparison problem. Am. State Assoc. J., 64: 1484-1499.

الملخص العربي

التاثير البوكيميائي لمجموعة من المبيدات الفسفورية ضد فراشات حشرة ديدان اللوز القرنفلية في محافظتي الفيوم والدقهلية في مصر

منى قطب محمد الحادق

المعمل المركزى للمبيدات – مركز البحوث الزراعية – الدقى -الجيزة

استهدف البحث دراسة سمية ثلاثة عشر من المركبات الفسفورية ضد فراشة ديدان اللور القرنفلية للسلالات المعملية و الحقلية التى تم جمعها من محافظتي الدقهلية والفيوم . وقد اظهرت النتائج مؤشرات مختلفة لمعامل المقاومة باختلاف المحافظة . يمكن تصنيف النتائج الى ثلاث مجاميع بمحافظة الفيوم . المجموعة الاولي اظهرت نسبة مقاومة منخفضة وكان معامل المقاومة ٦,٨٦ و ٧,٩١ فولد .اما المجموعة الثانية اظهرت مقاومة متوسطة وكانت قيمة معامل المقاومة تراوحت بين ١١,٦٤ و ٢٦,٢ فولد. اما بالنسبة للمجموعة الثالثة معامل المقاومة كان عالي فتراوحت قيمته بين ٤٠,٤٣ الى ٩٦,٠٠ فولد للمبيدات ضد فراشة ديدن اللوز القرنفلية. اما بالمقارنة لمحافظة الدقهلية ايضا تم تقسيمها الى ثلاث مجاميع مختلفة. وعليه فان مستوى المقاومة في المبيدات المختبرة كان عاليا في محافظة الفيوم بالمقارنة بمحافظة الدقهلية . واجرى تقدير نشاط انزيم الفوسفاتيز القلوي والاستيل كولين استريز والبروتين الكلي لكلا من السلالتين الحقليتين وقد اظهرت النتائج انخفاض مستوى نشاط انزيم الفوسفاتيز القلوي في كلا السلالتين الحقليتين في حين انخفض مستوى نشاط انزيم الاستيل كولين استريز والبروتين الكلي في كلا المحافظتين بالنسبة للسلالة المعملية.