Insecticide resistance evolved in field populations of pink boll-worm, *Pectinophora gossypiella* (Saunders) in 2017 Egypt cotton season

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Abstract: Resistance in pink ball worm (PBW) (*Pectinophora gossypiella*) collected from eight governorates against three different insecticides were evaluated. Bioassay results revealed that fluctuated from one governorate to another and from insecticide to another. On the other hand highest resistance was found in Menofia governorate against the insecticide agristar, PBW collected form governorates. Significantly higher AChE activity compared with laboratory and susceptible strain. The highest activity was shown in moths collected from Menofia governorate which increased by 50 %. On the other hand PBW from Beni suif, Gharbia and Kafr elsheikh governorates had significantly lower activity than in the S strain. The activity of glutathione s-transferese was much greater in moths from Menofia by 4.6 times than in the susceptible strain. In similar trend, this activity significantly increased by 3.7, 3.5 and 3.4 fold in Fayoum, Kafr Elsheikh and Dakahlia governorates respectively, as related to control. The total protein recorded were 14.89, 15.45, 13.94, 15.54, 13.89, 15.26, 15.14 and 14.41 g/dL for Fayoum, Dakahlia, Menofia, Sharkia, Beni-Swif Behera, Gharbia and kafrelshek, respectively as compared with laboratory strain (16.75 g/dL). This results suggest that the practical application of the concept integrated insect management should be followed.

Keywords:: Resistance ratio (RR), (lamnbda cyhalothrin, profenophos, chlorpyribos-methyl) *Pectinophora gossypiella* (PBW), LC_{50} and biology , Biochemical Enzymes, GST, AChE and Total Protein

1.Introduction

Pink bollworm *Pectinophora gossypiella* is one of the most damaging insect pests of cotton, causing substantial losses in production and quality of cotton **(Henneberry and Naranjo, 1998)**. The damage is caused by the larvae which feed on buds, flowers, bolls and seeds within growing bolls. Chemical control using insecticides remains the most effective method for controlling such insect pest **(Pimentel, 2009)**. However, due to repeated and recurrent use of these insecticides, Pink bollworm (PBW) developed resistance to such insecticides particularly pyrethroids and organophosphates. Evalution of insecticide resistance is a global concern worldwide and considered as the most serious threat to the development of sustainable integrated insect management **(Labbe *et al*., 2005)**. The development of insecticide resistance in insect field populations has resulted in failure of insect control **(Ishtiaq *et al*., 2012)** and thus searching for novel insecticides will be needed. Monitoring of insecticide resistance would be useful in detecting any alterations in susceptibility levels, especially to widely used insecticides and it would provide a tool for insect resistance management **(Osorio *et al* 2008).** It would also help to envisage the spread of resistance among different areas based on geography, and history of insecticides used. Hence, understanding the basis of insecticide resistance offers useful information on improving insect control and sustain the effectiveness of insecticides available **(Whalon,*et al* 2008)**. Determination of the activity of enzymes that are known to be responsible for conferring the resistance is one of the major factors that help in the early detection of resistance. In Egypt, cotton farmers are recently complaining about poor control of PBW although they are using insecticides belonging to different chemical classes which pose a threat to cotton production. Therefore, in this work the resistance monitoring against three commonly used insecticides namely; lambda-cyhalothrin, profenofos, and chlorpyrifos, were detected. The resistance was evaluated in field populations of PBW collected from eight different governorates representing main cotton growing regions in Egypt. The roles of certain metabolic enzymes commonly confer resistance; acetylcholinesterase (AChE), and glutathione S- transferase (GST) were investigated and the changes in the protein content associated with insecticide resistance were also examined.

2.Materials and Methods

2.1.Chemicals and insecticides used:

All chemicals used for biochemical studies were purchased as technical grade from Gamma Trade company for scientific services and consultation and Technogene Co.(Cairo, Egypt) unless stated otherwise (Table 1).

2.2.Insect populations

The susceptible laboratory strain of the PBW was provided by the (Bollworm Research Division) Plant Protection Research Institute, Agriculture Research Center, Egypt. This strain was reared on an artificial diet in the Central Agricultural Pesticides Laboratory (CAPL) and maintained under laboratory conditions for five years without any insecticide exposure. This

Table (1): Commercial formulations of the insecticides tested

Common name	Chemical class	Trade name	Manufacturer
Lambda-cyhalothrin	pyrethroids	Agristar 5% EC	Kanza Group
Profenophos	organophosphates	Cord 72% EC	El-Helb Pesticides & Chemicals Co
Chlorpyrifos-methyl	organophosphates	Pestiban 48% EC	Agrochem

strain will be used as reference for comparison with the field strain. The eight field populations of pink bollworm were collected from infested bolls from the following governorates: Fayoum, Benisuif, (upper Egypt) and Dakahlia, Menofia, Sharkia, Behaira, Gharbia and Kafr Elsheikh, (Lower Egypt). These populations were returned to the laboratory until collection of moths for resistance studies. Healthy survivors' larvae were released from the infested boll and maintained under laboratory conditions until pupation. All bioassay trials were done on the newly emerged moths of the pink bollworm.

2.3.Insecticide Bioassays:

The control measures are directed against the adult moth due to biological and ecological reasons, assays were performed using (0 day - old) moths of each population. Serial six concentrations of each insecticide formulation in water were prepared such that each was one-half of the previous concentration. Thin layer film method was used to test the insecticides used according to **Plapp *et al.* (1987)**. Cages dipped in water only, served as the controls. Treated insects were held in rearing cups and provided with a 10% sugar solution for food. All bioassays were performed in 5 replicates. The mortality was assessed after 24 h and corrected with Abbott's formula **(Abbott 1925)**. Probit analysis using Ldp-line software was used to calculate LC_{50} values owing to **Finney (1971)**. The resistance ratio (RR) was calculated at the LC_{50} level as follows:

RR = LC_{50} of field population /LC_{50} of laboratory (susceptible) strain.

2.4.Biochemical assays:

One hundred mg of both susceptible and field moths of *P. gossypiella* were homogenized in 4 ml of ice cold Tris buffer pH 7.8. The cold crude extracts were centrifuged at 10000 rpm for 30 min at 4ºC.The supernatant was used as enzyme source. The supernatant was then taken carefully into new tubes and kept at -20ºC until further biochemical studies.

2.5.Acetylcholinesterase (AChE) activity:

AChE activity was measured according to **Ellman et al., (1961)**

2.6.Glutathione S-transferase (GST) activity:

GST activity was assayed as described by the method of **Habig *et al*., (1974)**.

The estimated activities were expressed as mean µmol or mM per minute per mg protein and all experiments were replicated five times.

2.7.Total Protein:

Total protein content was determined colorimetrically according to the method described by **Bradford (1976)**. Protein concentration was expressed as mg protein/100mg body weight. The biochemical determination was performed according to manufacturer's instructions.

2.8.Statistical Analysis:

Data of the enzymes activities were statistically analyzed according to **Fisher R.A.(1970)** Least Significant Difference (LSD) Test to compare the significant differences between means of the calculated values.

3.Results

3.1.Susceptibility of *P. gossypiella* from the eight Governorates

As shown in Table (2) resistance ratios calculated for the three insecticides fluctuated from one governorate to another and from insecticide to another. Population collected from Kafrelshek had the highest resistance ratio for the three tested insecticides. However, the most resistance developed was against pestban as RR was 22.90 fold and the least obtained resistance was against Cord as RR was only 1.02 fold. In addition, resistance was also high against agristar from filed population from Menofia (RR was 17.15 fold). On the other hand, PBW collected from Fayoum governorate was less resistant to all the three tested insecticides as RR values were 2.84, 1.02 and 0.95 fold , respectively for agristar, cord and pestiban.

It can be concluded from Table (2) that the resistance ratios for each field populations are the highest for pestiban, followed by agristar and cord. On the other hand Agristar in Behera Governorate RR was moderate resistance RR was (6.26) fold and in Menofia and Beni swief showed very high level of resistance RR were (17.15 and 15.00) fold, respectively. While in cord insecticide level of resistance was very low in all Governorates RR was fluctuated from (2.39 and

Table (2): values of LC50, slope and resistance ratio to three insecticides for eight Governorates against P. gossypella in 2017 cotton sesons.

Governorates/ insecticides	Agristar					cord					Pesteban				
	LC_{50} (SC)	slope	LC_{50} (FS)	slope	RR	LC_{50} (SC)	slope	LC_{50} (FS)	slope	RR	LC_{50} (SC)	slope	LC_{50} (FS)	slope	RR
Sharkia	16.25	1.5	38.62	1.43	2.37	2.93	1.8	6.84	0.72	2.3	30.30	2.0	266.09	0.89	8.7
Dakahlia	16.25	1.5	70.95	1.80	4.36	2.93	1.8	5.64	0.94	2.36	30.30	2.0	237.16	1.44	7.82
Menofia	16.25	1.5	278.69	0.86	17.15	2.93	1.8	9.18	0.78	3.18	30.30	2.0	278.69	1.85	9.19
Gharbia	16.25	1.5	89.28	1.56	5.49	2.93	1.8	5.72	0.92	2.39	30.30	2.0	116.98	0.80	3.86
Behira	16.25	1.5	101.85	1.49	6.26	2.93	1.8	5.43	1.05	2.29	30.30	2.0	86.38	0.76	2.85
Kafr-Elshek	16.25	1.5	46.43	1.5	2.85	2.93	1.8	5.72	0.92	2.39	30.30	2.0	696.4	0.92	22.9
Beni-Swif	16.25	1.5	244.47	1.44	15.04	2.93	1.8	161	1.58	2.02	30.30	2.0	61.22	1.58	2.02
Fayoum	16.25	1.5	46.25	1.44	2.84	2.93	1.8	2.45	1.05	1.02	30.30	2.0	28.80	0.97	0.950

1.02) fold. Finally in pestiban insecticide RR was fluctuated in one governorate to anther one resistance ratio was low level in Fayoum, Beniswif, Behera and Gharbia RR was (0.95, 2.02, 2.85 and 3.86) fold respectively. While in Dakahlia, Sharkia and Menofia resistance ratio was moderate level RR was 7.82, 8.7 and 9.19 fold respectively, on the other hand in Kafrelshek Governorate resistance ratio was very high RR was 22.9 fold.

The highest RR to insecticides was found in the Kafrelshek governorate to insecticide pesteban, followed by Agrestar in menofia. This can be explained by the different amounts of insecticides used in these regions. The results also showed that the resistance to pyrethroids is more serious than that to organophosphates except certain Governorate. This seems to be related to the more frequent use of pyrethroids in recent years. Results also agree with those obtained by **Mohamed *et.al* (2017)**. However, results are not in accordance with those obtained by **Radwan and El Malla (2015)** who found that the pyrethroid lambda cyhalothrin was the most toxic to field moths of *P. gossypiella* populations from different governorates. Resistance to insecticides in field populations may also appear in regions with low insecticides use due to the high migration rate of insects and the genetic mixing of populations, which can facilitate the rapid spread of resistance among isolated populations (**Mohamady 2017**). Similar results were also obtained by **Omayma *et.al*. (2014)** who found that in field strains of cotton leaf worm collected from Gharbya, Kafr El-Sheikh and Behera governorates, RC varied from one year to another and from Governorates to another.

All data agree with **Reham *et. al*(2018)** as the toxicity of four pyrthroid insecticides to field strains of *Pectinophora gossypiella* collected from four Goverorates (Fayoum, Menofia, Dakahlia and Sharkia) was investigated. Those results indicated that resistance coefficient (RC) varied from one governorate to another. Cafrothren showed low resistance in Dakahlia, Menofia and Sharkia, but in Fayuom medium resistance was obtained. Cothren El-nasr showed low resistance in Dakahlia, Fayuom and Sharkia but in Menofia medium resistance was also recorded. In fayuom, Menofia and Dakahlia, resistance to sumialfa was medium. On the other hand, Cyperco showed high resistance in Dakahlia, Menofia and Sharkia but in Fayuom the resistance was lower.

In general, these insecticides are still effective against *P. gosypiella* in most governorates under the study.

3.2.Enzymes activities in the field population of *P. gossypiella*:

3.2.1.Acetylcholinesterase (AChE) activity:

Data set up in table (3) indicated that PBW collected form Fayoum, Dakahlia Menofia, Sharkia and Behaira governorates had significantly higher AChE activity compared with control (laboratory). The highest activity was shown in moths collected from Menofia governorate which increased by 50 %. On the other hand PBW from Beni suif, Gharbia and Kafr elsheikh governorates had significantly lower activity than in the S strain. The greatest reduction in AChE activity was in moths from Gharbia which reached 67% compared to laboratory strain.

3.2.2.Glutathione S-transferase (GST) activity:

P. gossypiella collected from all the eight governorates had significantly much higher activity of GST related to the laboratory. The activity of GST was much greater in moths from Menofia by 4.6 times than in the susceptible strain. In similar trend, this activity significantly increased by 3.7, 3.5 and 3.4 fold in Fayoum, Kafr Elsheikh and Dakahlia governorates respectively, as related to control. While, slightly significant increase in GST activity by 1.7

Table (3): Biochemical study in laboratory strain and four field strains of P.gossypiella in 2017 cotton season

Insect population	Acetylcholinesterase (mM/min/mg protein)		GST (µmol/min/mg protein)		Total Protein Concentration (mg/100mg body weight)	
	Specific Activity Mean ± S.E.	Change (%)	Specific Activity Mean ± S.E.	Change (%)	Mean ± S.E.	Change (%)
Fayoum	2.25 ± 0.0197	(+) 6.23	0.835 ± 0.073**	(+) 267.84	14.89 ± 0.179**	(-) 11.10
Dakahlia	2.68 ± 0.0729*	(+) 2.65	0.778 ± 0.075**	(+) 242.73	15.45 ± 0.146**	(-) 7.76
Menofia	3.18 ± 0.296*	(+) 50.14	1.043 ± 0.093**	(+) 359.47	13.94 ± 0.173***	(-) 16.78
Sharkia	2.26 ± 0.148	(+) 6.70	0.394 ± 0.070	(+) 73.57	15.54 ± 0.142***	(-) 13.19
Beni-swif	1.14 ± 0.390	(-) 46.18	0.592 ± 0.118	(+) 160.79	13.89 ± 0.202***	(-) 17.07
Behera	2.83 ± 0.180*	(+) 33.62	0.445 ± 0.065	(+) 96.04	15.26 ± 0.145**	(-) 8.90
Gharbia	0.698 ± 0.054**	(-) 67.04	0.635 ± 0.086*	(+) 179.74	15.14 ± 0.277**	(-) 9.61
Kafr-elshek	1.25 ± 0.257*	(-) 40.98	0.788 ± 0.083**	(+) 247.14	14.41 ± 0.241***	(-) 13.97
Laboratory	2.118 ± 0.173		0.227 ± 0.078		16.75 ± 0.121	

fold was observed in adults collected from Sharkia region.

Results on biochemical parameters suggested that acetylcholinesterase and glutathione transferase could be involved in resistance to all three tested insecticides in Menofia governorates. As the highest activity of the two enzyme systems were measured in the moths collected from that governorate. These findings are in consistent with those of **Abd Elhady and Abd El-Aal (2011).**

3.3.The total protein content:

The level of total protein in both laboratory and field moth strains were determined as shown in Table (3). Results showed that the total protein decreased in field strain compared with the laboratory one.

Values recorded were 14.89, 15. 45, 13.94, 15.54, 13.89, 15.26, 15.14 and 14.41 g/dL for Fayoum, Dakahlia, Menofia, Sharkia, Beni-Swif Behera, Gharbia and kafrelshek , respectively as compared with in laboratory strain (16.75 g/dL). Data also illustrated that, Dakahlia ,Behera and Gharbia governorates had the decreased protein content (7.76, 8.90 and 9.61 %), respectively. Similar results were also obtained by **Kandil *et al*., (2013)**, who found that treatment of *P. gossypiella* adults with insecticides, decreased total protein content. The relationships between protein synthesis and transaminase levels were affected by the hormonal control of protein synthesis and neurosecretory hormones which are involved in the regulation of transaminase levels. **(Megahed *et al.*, 2013)**.

Mohamed *et.al* (2017) Toxic influence of Chlorpyrifos, Lambda-Cyhalothrin,Methomyl and Spinosad against lab and field moths of the pinkbollworm, Pectinophora gossypiella (Saund.) were tested under Laboratory conditions. Methomyl had the superior toxic effectfollowed by Lambda-Cyhalothrin, Chlorpyrifos and Spinosad (LC50 =1.16, 2.45, 4.01 and 20.75 ppm respectively) to Lab insects. The field insects from Menoufia Governorate were more tolerant to toxic effectof the tested insecticides especially chlorpyrifos than Bani-Suif Governorate insects. A high significant increase of enzyme activity was determined in Glutathione-S-Transferase (GST) than MFO Cytochrome P450 (PCMAN-demethylase) of Field moths than lab ones. While the depletion in enzyme activity was presented in Acetylcholinesterase enzyme of field insects. These changes in enzymes activity of the field insects may be correlated with their tolerance ability to toxic effect of the tested insecticides

Conclusion

The pink bollworm is one of the two key insect pests of cotton in Egypt. The reliance on insecticides to control such PBW resulted in development of resistance. Early detection of resistance is crucial factor help in diagnosing of incidence of resistance. Therefore, biochemical assay combined with biological evaluation can be a useful tool for assessment the level of resistance in the field population of an insect pest **(Rauch and Nauen 2003 and Farghaly, 2010)**. Our results draw the attention that there is an urgent need to find solutions to the resistant PBW problem occurred in the cotton-growing governorates under the study in Egypt. This result provides an incentive to continue studying on the resistant populations. If no resistance management strategies are developed soon for the insecticides tested in our study, the resistance phenomenon might become a widespread reality and will threat the sustainability of insecticides used in cotton. On the other hand, reliance on insecticides for PBW control in cotton is expected to continue because there is no effective and reliable alternative options are available for cotton farmers and producers. Consequently, rational strategies to manage this insecticide-resistant insect pest within cotton production system should be adopted through insecticide use diversification. The concept of integrated insect management should be followed by practicing other control measures.

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تطور مقاومة المبيدات الحشرية في المجموعات الحقلية من الدودة الحلزونية الورديّة، البكتينوفورا gossypiella(سوندرز) في موسم مصر للقطن 2017

ريهام السيد على و منى قطب محمد الحاذق

مركز البحوث الزراعية ، المعمل المركزى للمبيدات ، قسم بحوث مقاومة الآفات للمبيدات ، الدقى ، الجيزة

الملخص العربي

تطور صفة المقاومة للمبيدات الحشرية للسلالات الحقلية لدودة اللوز القرنفلية بيكتينوفرا جوسيبلا لموسم القطن لسنة 2017 في مصر . تم حساب مستوى المقاومة لثلاثة مبيدات وهم الاجريستار و الكورد و البستبان وجد ان مستوى المقاومة تذبذب مابين محافظة والأخري وبين مبيد والاخر لدودة اللوز القرنفلية التي تم تجميعها من ثمانية محافظات وهي منوفية دقهلية شرقية كفر الشيخ غربية بحيرة بني سويف والفيوم . اظهرت النتائج ان هذه المبيدات مازالت فعالة على افة دودة اللوز القرنفلية فيما عدا بعض المحافظات. كما تم حساب النشاط الانزيمي لهذه السلالات الحقلية لانزيمات استيل كولين استريز (AChE)ونشاط ((GST جلوتاثيون اس ترانسفيرس والبروتين الكلي.لجميع السلالات الحقلية ووجد فروق معنوية عالية للاستيل كولين استريز مقارنة بالسلالة المعملية (كنترول). حيث وجد في فراشات سلالة المنوفية نشاط عالي للانزيم بنسبة 50 % في حين ان محافظة بني سويف والغربية كان نشاط الانزيم منخفض مقارنة بالكنترول كما حدث انخفاض كبير في نشاط الاستيل كولين استريو في فراشات الغربية 67 % مقارنة بالسلاله المعملية (كنترول).بالنسبة لنشاط اننزيم الجلوتاثيون اس ترانسفيرس لكل الحشرات التي تم تجميعها من الثماني محافظات كان عالي مقارنة بالكنترول كان الاعلى في محافظة المنوفية 4.6 فولد بالنسبة للسلالة المعملية وكان الفرق معنوي وزاد الى 3.7و 3.5و 3.4 في محافظات الفيوم و كفر الشيخ والدقهلية على التوالي مقارنة بالكنترول في حين زادت الفروق المعنوية بعض الشئ في نشاط الانزيم في محافظة الشرقية و كانت 1.7 فولد .واخيرا عند تقدير نشاط البروتين الكلي كان 14.89و 15.45و 13.94و 15.54و 13.89و 15.26و 15.14و 14.4 فولد لمحافظات الفيوم الدقهلية المنوفية الشرقية بني سويف بحيرة غربية كفر الشيخ على التوالي مقارنة بالسلالة المعملية التي كانت 16.75.