Resistant Pest Management Newsletter

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Resistance Management from Around the Globe

Phenoloxidase dependence of the agglutinating activity of Colorado potato beetle (Leptinotersa decemlineata Say.) hemolymph under the action of chitin high-molecular derivatives

ABSTRACT
The formation of chitosan-induced levels of agglutinating activity of Colorado potato beetle hemolymph after the injection of microorganisms depending on phenoloxidase activity has been investigated. It is shown that under the action of the substances belonging to the pathogen as an immune stimulus (in this case the degradation products of chitin macromolecules) in insects can be implemented different ways of opsonization of microorganisms.
Key words: *Leptinotarsa decemlineata*, agglutinin, phenoloxidase, chitosan, chitosan succinate.

INTRODUCTION

Agglutinins play a mediator role in immunity of insects, participating in the processes of recognition and opsonization of foreign objects. It is shown that agglutinins carry out these processes in conjunction with components of phenoloxidase system. Thus, *Blaberus discoidalis* agglutinins enhance recognition and phagocytosis of microorganisms by the activation of phenoloxidase system (Chen et al., 1995). On the other hand, numerous experimental data confirm a close relationship of the prophenoloxidase cascade with the system of recognition of foreign material in arthropods (Nappi et al., 2005). In particular, the components of the phenoloxidase system have been shown to involve the process of recognition, performing the cross-linking of cell surface with the corresponding receptors (Charalambidis et al., 1996). In insects, opsonization may be carried out by hemagglutinins in different ways. Hemagglutinins can agglutinate microorganisms, which are then easily phagocytized and encapsulated. In addition opsonization can be carried out through activation of phenoloxidase system. Inhibition of phenoloxidase by phenylthiourea shows that some agglutinins induce phagocytosis through activation of phenoloxidase system, while others carry out phenoloxidase independently of opsonization (Wilson et al., 1999). There is also a third - intermediate - way of opsonization, which involves phenoloxidase dependent and independent mechanisms for creating the lectin-induced level of phagocytosis. It is possible that the mechanism of phenoloxidase participation in opsonization is based on the above-mentioned cross-linking of tyrosine intermediates with receptors on the surface of hemocytes. According to the results of our recent studies in the hemolymph of *L. decemlineata* these factors of immune recognition are induced with different intensity by high-molecular chitinous derivatives - chitosan and chitosan succinate (Gaifullina et al., 2010). The aim of this study is to evaluate of the significance of phenoloxidase activity in the formation of chitosan-induced levels of agglutinating activity of Colorado potato beetle hemolymph after injection of microorganisms.

METHODS

L. decemlineata larvae of fourth instar were collected from potato field and reared in glasses with volume 0.5 dm^3. Insects in experimental variants one day prior to the analysis were fed with potato foliage moistened with 0.01% water suspension of chitosan (M=200 кDa, deacetylation degree 82%) or 0.01% water solution of chitosan succinate (M=330 кDa, deacetylation degree 95%). Inhibition of phenoloxidase was performed by injecting in hemocoel of 2mkl of phenylthiourea (PTU) at concentration of 0.01% by syringe with a sterile needle. Cellular suspension of *Bacillus subtilis* (strain) in titer $2x10^8$ cells/µl was used as a foreign agent. This suspension was also injected into hemocoel by syringe with a sterile needle. Phosphate saline buffer (PSB) was used as control injection. Measurement of phenoloxidase activity and titer of agglutinins in hemolymph was performed one hour after injection.

Phenoloxidase (PO) activity was determined in trys-HCl buffer (pH 7.5) with respect to substratum L-digydroxiphenilalanin using spectrophotometer

Shimadsu at 475 nm 37ºC by optical density change during 5 min (Raushenbach, 1997). Activity of this enzyme was evaluated in protein concentration, which was measured by the Bradford method (Skopes, 1985). The enzyme activity was expressed in units/min/protein mg.

Hemagglutination assays. Agglutination activity was determined by method of two-fold serial dilutions of hemolymph in U-bottomed wells in microtitrator plates alongside the use of trypsin-treated human erythrocytes fixed with glutaraldehyde (Stynen et al., 1982). Human blood of group O^- has been given by the laboratory of the Republican clinical hospital. Presence and agglutination degree was considered visually by registration of formed agglutinates on a four scale. The greatest dilution of a hemolymph at which else there was agglutination was used to determine the titer of agglutinins. Agglutination titer was calculated as $-\log_2 X$, where X – the last dilution providing agglutinate formation.

Statistical data analysis was performed using the arithmetic mean and error of the mean. The reliability of distinction of mean values was determined by Student t-test (Lakin, 1990).

RESULTS AND DISCUSSION

Control injection of PSB in hemocoel of Colorado potato beetle larvae did not change the titer of agglutinins lowering phenoloxidase activity in all variants (Tab. 1-3). According to literature data, this reduction of phenoloxidase activity is temporary and is typical stress response to cover damage of insects (Glupov, 2001).

In the normal injection of PTU in larvae hemocoel, as expected, significantly reduced phenoloxidase activity compared with PSB injection. At the same titer of agglutinins in the hemolymph decreased to 10 which indicate a phenoloxidase dependent mechanism of erythrocytes agglutination (Tab. 1).

Table 1: Agglutinating and phenoloxidase activity of *L. decemlineata* Say. larvae hemolymph after *B.subtilis* and PTU injection

Variant	Agglutinin titer	Phenoloxidase activity, units/min/protein mg
Without Injection	12	0.842±0.034
PSB	12	0.562±0.042*
PTU	10*	0.060±0.004*
B.subtilis	8*	0.106±0.003*
B.subtilis+ΦTM	10*	0.048±0.004*

* - value significantly different from control, P≤0.1

Injection of *B. subtilis* suspension caused 8-fold decrease in activity of phenoloxidase and reduction a titer of agglutinins to 8. This may be the result of binding of part of agglutinins with the microorganism and participation in this process phenoloxidase system leading to depletion of active enzyme. On the other hand, the activation of prophenoloxidase in insects can be carried out by the endogenous agglutinins (Yu, Kanost, 2003). Thus, reducing of the free agglutinins amount in the hemolymph may lead to lower the intensity of the proenzyme activation process.

Multiple decreases in activity of phenoloxidase was registered also with the simultaneous injection of a suspension of *B. subtilis* and PTU. At the same time titer of agglutinins fell to 10, that is, as a variant with an injection of only PTU. This observation indicates the absence of binding of part of agglutinins with the microorganism and, consequently, the phenoloxidase independent mechanism of *B. subtilis* agglutination.

Under the action of chitosan succinate analogous changes of agglutinin titer were observed upon injection of *B. subtilis* suspension and PTU, which differ from the control variant a higher level of agglutinating activity of hemolymph (Tab. 2). Phenoloxidase dependent character of *B. subtilis* agglutination was not observed under the action of chitosan, which indicates a somewhat different spectrum of agglutinins induced by these derivatives of chitin (Tab. 3). We have previously shown that chitosan succinate in comparison with the chitosan has a stronger immunostimulatory effect with respect to *L. decemlineata*, inducing a larger number of agglutinins with more broad spectrum of carbohydrate specificity (Gaifullina et al., 2010). Thus, the amount of agglutinins binding to oligomers, consisting of 15 molecules of N-acetyl-D-glucosamine, as well as chitosan succinate increased in insect hemolymph under the action of chitosan. Effect the chitosan succinate added to these agglutinins recognizing molecules capable of binding to N-acetyl-D-glucosamine and N-acetyl-D-galactosamine. Obviously, agglutinins having an affinity for these monomers, implement phenoloxidase dependent opsonization mechanism of the microorganism.

Table 2: Agglutinating and phenoloxidase activity of *L. decemlineata* Say. larvae hemolymph after *B.subtilis* and PTU injection under the chitosan succinate action

Variant	Agglutinin titer	Phenoloxidase activity, units/min/protein mg
Without injection	14	1.041±0.014
PSB	14	0.405±0.011*
PTU	12*	0.036±0.004*
B.subtilis	10*	0.059±0.009*
B.subtilis+ФТМ	12*	0.033±0.002*

* - value significantly different from control, P≤0.1

Table 3: Agglutinating and phenoloxidase activity of *L. decemlineata* Say. larvae hemolymph after *B.subtilis* and PTU injection under the chitosan action

Variant	Agglutinin titer	Phenoloxidase activity, units/min/protein mg
Without injection	13	1.012±0.009
PTU	13	0.745±0.033*
PTU	11*	0.094±0.009*
B.subtilis	11*	0.049±0.002*
B.subtilis+ФТМ	11*	0.134±0.012*

* - value significantly different from control, P≤0.1

Recognition mechanisms, which include carbohydrate components, mediate phagocytosis of foreign particles, causing activation of the phagocyte actin-myosin contractile system. As a result, the phagocytes extend pseudopodia, internalizing foreign object in the phagosome. We have recently shown that hemocyte activation processes also include both phenoloxidase dependent and independent mechanisms (Gaifullina et al., 2011). Thus, significant decrease in the proportion of active granulocytes after PTU injection into the hemocoel demonstrates phenoloxidase dependence of these phagocytes activation process. Thus, phenoloxidase system activating by endogenous agglutinins, mediate opsonization of foreign objects and activation of the granular form of phagocytes.

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Population dynamics of cotton bollworm, *Helicoverpa armigera* (Hübner) on Non-*Bt* and *Bt*-cotton hybrids in Raichur, Karnataka, India

ABSTRACT
Studies on population dynamics of *Helicoverpa armigera* were carried out at University of Agricultural Sciences, Raichur during the year 2008-10. It was observed that during 2008-09 and 2009-10, egg incidence of *H. armigera* on *Bt* and non-*Bt* cotton hybrids spread across nearly three months which coincided with peak vegetative stage to boll maturation stage. However, maximum egg incidence was observed during square formation stage to boll maturation stage. Similar trend was followed by *H. armigera* larval population on non-*Bt* cotton during the two years of observation with a slight shift in the peak larval population across the seasons and hybrids. In *Bt*-cotton no larval population was recorded during 2008-09. However, a considerable larval population was observed on *Bt*-cotton for a short period from boll formation stage to maturation stage during 2009-10.
Key words: *Helicoverpa armigera*, non-*Bt* and *Bt*-cotton, population dynamics

INTRODUCTION

Among the natural fibre yielding crops cultivating in India, cotton "the white gold" stands first which provides ecological niche to 164 insects and mites, of this, only 12 having economic importance. Among them *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), popularly known as American bollworm, is the most dreaded one. The outbreak of this pest in the cotton ecosystem of Andhra Pradesh during 1987-88 resulted in heavy economic loss. Yield loss by *H. armigera* in cotton ecosystem of Tamil Nadu and Karnataka was up to 40% per cent during past two decades resulting in decreased area and production as well as forcing the farmers' to use insecticides worth US$ 330 billion every year (Rai *et al.*, 2009).

At present, India is the second largest producer and consumer of cotton after China with an area of about 9.5 million hectares (mha) representing approximately one quarter of the global cotton area of 35 million hectares and accounting for a little over 21% of the global cotton production in 2008-09. Much of this success owes itself to the introduction of *Bt*- cotton [containing gene(s) from soil bacterium *Bacillus thuringiensis* which is integrated into the genetic makeup of cotton plants designed to produce *Cry* toxin in the entire plant system which is detrimental to bollworms] in 2002 (CICR, 2009). However, extensive cultivation of *Bt*-cotton can impose a continuous and intense selection pressure on bollworms leading to the latter's development of resistance to the toxin (Kumar, 2004). Hence, monitoring the *Helicoverpa armigera* population on *Bt*-cotton is the most important criteria to assess the status of resistance development in this pest.

MATERIALS AND METHODS

The present study was carried out at a cotton experimental plot at UAS campus Raichur, during the year 2008-10 to assess the population dynamics of *Helicoverpa armigera* on *Bt* and non-*Bt* cotton. During 2008-09, the population dynamics of *H. armigera* were studied on *Bt* and Non-*Bt* versions of RCH 2 hybrids sown on 27 July 2008 with a spacing of 45 X 22.5 cm. Whereas, during 2009-10 the similar study was carried out on MRC7918 (Non-*Bt*) and MRC6918 (*Bt*) hybrids sown on 18 August 2009. All the recommended package of practices were followed to raise a good crop except plant protection measure against bollworms.

Egg and larval population was recorded from different plant parts on 30 to 135 days old crop which coincided with peak vegetative stage to boll maturation stage. From peak vegetative stage to square formation stage, the plant parts such as top three leaves and growing shoots were observed, whereas after square formation stage even the squares, flowers and bolls were observed for recording egg incidence. For larval incidence, leaves, squares, flowers and bolls were examined thoroughly from peak vegetative stage to boll maturation stage.

From each *Bt* and non-*Bt* field 50 plants were selected randomly to record egg and larval incidence at an interval of one week. The data obtained from each standard week (Std.Week) was pooled and mean and standard deviation was calculated. Differences across the *Bt and* non-*Bt* cotton *hybrids* with respect to egg and larval incidence was tested for significance using the independent sample t test.

RESULTS

The data on population dynamics of *H. armigera* on cotton during the year 2008-10 are presented in the Table 1. During 2008-09, the incidence of eggs of *H. armigera* was observed on Non-*Bt* cotton from last week of September - 39th Std. Week (0.22 eggs/ plant) to third week of November - 47th Std. Week (0.10eggs/ plant), with a peak during fourth week of October- 43rd Std. Week (0.76eggs/ plant). Whereas, in *Bt*-cotton hybrid eggs were observed from last week of September - 39th Std. Week (0.60eggs/ plant) to third week of November - 47th Std. Week (0.04 eggs/ plant) with a peak during first week of October-40th Std. Week (0.88 eggs/ plant). Similarly in 2009-10, the occurrence of eggs was observed on Non-*Bt* cotton from third week of September-38th Std. Week (0.52eggs/ plant) to second week of December-50th Std. Week (0.04 eggs/ plant), with two peaks, one during third week of October – 42nd Std. Week (0.76 eggs/ plant) and second during second week of November-46th Std. Week (0.88 eggs/ plant). Whereas in *Bt*-

cotton, egg incidence was observed from third week of September (0.76 eggs/ plant) to second week of December (0.02eggs/ plant) with a peak during third week of October- 42nd Std. Week (1.04 eggs/ plant).

Table 1: Population dynamics of *H. armigera* on non-Bt and Bt cotton hybrids during the year 2008-10

Month	Standard week (Std. Week)	2008-09				2009-10			
		Eggs/Plant* (Mean±SD)		Larvae/plant* (Mean±SD)		Eggs/Plant (Mean±SD)		Larvae/plant* (Mean±SD)	
		RCH 2 Non-Bt	RCH 2Bt	RCH 2 Non Bt	RCH 2Bt	MRC 7918 Non Bt	MRC 6918Bt	MRC 7918 Non Bt	MRC 6918 Bt
September	38	--	--	--	--	0.52±0.86	0.76±0.93	0	0
	39	0.22±0.55	0.60±1.03	0.16±0.47	0	0.46±0.61	0.54±0.73	0.24±0.59	0
October	40	0.48±0.76	0.88±1.06	0.24±0.43	0	0.70±0.70	0.84±0.47	0.18±0.44	0
	41	0.68±0.96	0.76±1.00	0.28±0.57	0	0.62±0.63	0.88±0.79	0.24±0.43	0
	42	0.48±0.79	0.58±0.83	0.32±0.59	0	0.76±0.87	1.04±0.94	0.38±0.66	0
	43	0.76±0.87	0.70±0.89	0.44±0.67	0	0.76±0.86	1.0±0.90	0.44±0.54	0
	44	0.68±0.79	0.68±0.96	0.30±0.54	0	0.52±0.74	0.84±0.96	0.36±0.48	0
November	45	0.34±0.74	0.54±0.88	0.29±0.54	0	0.40±0.67	0.74±0.56	0.50±0.54	0
	46	0.26±0.56	0.34±0.79	0.18±0.44	0	0.88±1.24	0.66±0.48	0.50±0.54	0
	47	0.10±0.30	0.14±0.49	0.08±0.27	0	0.58±0.66	0.46±0.70	0.50±0.61	0
	48	0	0	0.08±0.27	0	0.28±0.60	0.10±0.30	0.44±0.54	0.26±0.47
December	49	0	0	0	0	0.08±0.61	0.04±0.19	0.14±0.35	0.38±0.49
	50	--	--	--	--	0.04±0.19	0.02±0.14	0.08±0.27	0.16±0.37
	51	--	--	--	--	0	0	0.02±0.14	0.02±0.32
	52	--	--	--	--	0	0	0.02±0.14	0
January	1	--	--	--	--	0	0	0.04±0.29	0
Seasonal mean		0.44	0.58 (χ^2=0.99)	0.237	--	0.490	0.60 (χ^2=0.99)	0.272	0.054

T-value 0.78^{ns}

*-Mean of 50 observations SD- standard deviation

DISCUSSION

In both years, the period of egg incidence in *Bt* and Non-*Bt* cotton hybrids spread across nearly three months which coincided with peak vegetative stage to boll maturation stage. However, there was a slight shift in the peak egg incidence across the seasons and hybrids. In both the years the maximum egg incidence was observed during square formation stage to boll maturation stage. These studies are in accordance with report of Kengegowda (2003) and Manju (2006) who observed more eggs on *Bt* and Non- *Bt* cotton hybrids between late vegetative stage to square formation stage.

Though the mean egg incidence on *Bt*-cotton hybrids during both years was more than that on non-*Bt* cotton hybrids, it was insignificant (χ^2 =0.99). The present results are in close agreement with the report of Kengegowda (2003), Patil *et al*. (2004) and Manju (2006) who also reported almost similar levels of egg population on both *Bt* and non-*Bt* cotton plants.

In 2008-09, larval population was noticed on non-*Bt* cotton from last week of September -39th Std. Week (0.16 larvae/ plant) to last week of November -48th Std. Week (0.08 larvae/ plant) with a peak of 0.44 larvae/ plant during fourth week of October-43rd Std. Week. There was no larval population on *Bt*-cotton during 2008-09.

During 2009-10, the larval population was observed on non-*Bt* cotton from last week of September - 39th Std. Week (0.24 larvae/ plant) to first week of January- 1st Std. Week (0.04 larvae/ plant) with a peak during 1st to 3rd week of November- 45th to 47th Std. Week (0.50 larvae/ plant). In *Bt*- cotton the larval population was observed during from last week of November- 48th Std. Week (0.26 larvae/ plant) to third week of December- 51st Std. Week (0.02 larvae/ plant) with a peak during first week of December -49th Std. Week (0.38 larvae/ plant).

In 2009-10, the larval population on non-*Bt* cotton was spread across nearly four months, whereas, in 2008-09 the larval population was noticed for three months because of late observation taken after 60 days of sowing which coincided with square formation to boll maturation stage.

In *Bt*-cotton, no larval population was recorded during 2008-09. However in the next season (2009-10), considerable larval population (0.02 to 0.38 larvae/ plant) was found on MRC-6918 (BG-I) during 48th to 51st Std. Week. The present results are in accordance with Udikeri *et al*. (2002) who also reported the incidence of *Helicoverpa* larvae (0.45 per plant) in MECH-184 (BG-I). Patil *et al*. (2004) reported the larval population in MECH-184 *Bt* (0.22 larvae/per plant) whereas, it was 0.28 larvae/ plant in NCS-145 hybrid cotton. Prasad *et al*. (2009) also reported incidence of *H. armigera* on *Bt* (MECH-162 *Bt* (8.8%)), (RCH-2Bt (7.53%)) and non Bt cotton hybrids under unprotected condition. Similarly, Bambawale *et al*. (2004) reported lowest larval population in *Bt*-cotton (0.03 per plant) compared to 0.05 and 0.09 larvae per plant in conventional cotton IPM and non IPM plots, respectively.

Based on the earlier reports and the present studies, it is quite evident that the incidence of *H. armigera* on *Bt*-cotton hybrids has been recorded at regular interval since the inception of *Bt* technology in India. Thus, there is a need for continuous monitoring of *H. armigera* population in cotton ecosystem throughout the country.

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Research in Resistance Management

Assaying insecticidal efficacy of OP's against pink bollworm male moth field strains using the attracticide efficacy monitoring technique

ABSTRACT
Experiments were carried out in two successive cotton seasons of 2009 and 2010 in both early and late season. These experiments were done in two locations: in cotton fields of both Kafr El-Dawar District, El-Behera Governorate, and Faculty of Agriculture Farm, Alexandria Governorate, Egypt. Along the course of this investigation, the toxicity parameters indicated that profenofos was the most toxic for Kafr El-Dawar population in the 6 hr assessment with LC_{50} values of 909.1, 970.16, 1167.03 and 1338.63 ppm for early 2009 cotton season, late 2009 cotton season, early 2010 cotton season and late 2010 cotton season, respectively. On the other hand, chlorpyrifos proved to be the most toxic for Alexandria population in the 6 hrs assessment with LC_{50} values of 260, 337, 393.51 and 494.1 for the same periods, respectively. The data from the 12 hr assessments have been taken closely in the same manner to that of 6 hrs assessment. The efficacy ratios study proved that the intensive, the continuous and the repeated use of the same insecticides along the course of controlling pink bollworm caused decrease in their efficacy and lead to control failure. For example, chlorpyrifos efficacy ratios after 12 hrs of treatment in early 2009, late 2009, early 2010 and late 2010 cotton seasons at Kafr El-Dawar were 15.55%, 12.84%, 9.66% and 10.82%, respectively.
Key words: Pink bollworm, *Pectinophora gossypiella*, Efficacy, Insecticides, Resistance.

INTRODUCTION

Cotton, the world's most important fiber, is grown on more than 33.9 million hectares in about 100 countries. Four countries alone (China, the USA, India, and Pakistan) account for approximately two thirds of world output. If we added Uzbekistan and Egypt, six countries would account for three fourths of world cotton production, (Anonymous, 2004). Cotton is a plant that seems to be designated specially to attract a wide range of insect pests. It is green with succulent leaves, open flowers, nectaries on every leaf and flower, and a vast amount of fruit. Each characteristic attracts a variety of insects such as the pink bollworm, spiny bollworm, the tobacco budworm, cotton leaf worm, cotton aphid, boll weevil, cotton fleahopper, spider mites, grass-hoppers, white fly, thrips and many other insects.

The pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) is a worldwide pest of cotton and in some regions of the world is the key cotton pest. Like the boll weevil, the PBW is a well-adapted herbivore of cotton, feeding throughout the growing season on the cotton fruit (square and bolls) and burrowing habits. It has caused loss in yield and costs of insect control, substantial indirect losses occur as result of the destruction of beneficial insects and the development of insecticides resistance in cotton. It has been extremely difficult to control using pesticides but considerable success has been achieved using alternative control tactics.

Formulated insecticides are used in large scale through the world as a major means to manage and control pests. Although insecticides provide numerous benefits in terms of increased production and quality of the product, their efficacy isn't always effective because of the development of pesticide resistance. Efficacy of several formulated pesticides was studied by several investigators using the attracticide resistance monitoring technique (Haynes *et a*l 1986, Albeltagy et. al. 1996, Al-Beltagy *et al* 2001, Khider et. al. 2002, Shekeban *et al* 2003, Albeltagy et. al. 2010, and Albeltagy 2012).

Therefore it is important to study efficacy of insecticides against bollworms in Egypt to establish a program for controlling and reducing the resistance level of these pest to insecticides. Such a program could be efficiently used to reduce the number of insecticide sprays; cost of insecticides, and increase the production of cotton per unit.

In our present study, insecticidal efficacy of selected formulated insecticides, three organophosphorus, were examined against pink bollworm male moth strains in El-Behera and Alexandria Governorates by attracticide efficacy monitoring technique (AEMT) which is the modified name of attracticide resistance monitoring technique (ARMT) during two successive seasons (2009 and 2010).

MATERIALS AND METHODS

A-Materials

1- INSECTICIDES USED: Three organophosphorous insecticides were used:

1.1. Chlorpyrifos (Pestban®)
EC 48% provided by Agrochema, Egypt. Application rate: 1 liter/ feddan

1.2. Chlorpyrifos-methyl (Reldan®)
EC 50% provided by Agrochema, Egypt. Application rate: 1 liter/ feddan

1.3. Profenofos (Seleton®)
EC 72% provided by: Syngenta , Agro, Egypt. Application rate: 0.75 liter / feddan.
Formulation: EC 72%
Application rate: 0.75 liter / feddan /400 liter water

2- PHEROMONE USED
Gossyplure.in pink rubber septum containing 1 mg, provided by the Ministry of Agriculture, Egypt.

3- OTHER CHEMICALS USED
Stickum® (sticky adhesive). Acetone.

4- INSECTS USED:
Male moth populations of Pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) field strains from both Kafr-El-Dawar district cotton fields, El-Behera Governorate, and Faculty of Agriculture farm, Alexandria Governorate, Egypt were used locally in the present study.

B- Methods

1 - Procedure of AEMT
The attracticide efficacy monitoring technique (AEMT) is the modified name of attracticide resistance monitoring technique (ARMT) where delta traps were used with sticky adhesive – coated card inserts containing the insecticide concentration placed in the trap bottom. A rubber septa with 1mg gossyplure acted as the pheromone source. This technique provided stable LC_{50}'s with low control mortality. This technique was used as described by Miller (1986) and modified by Shekeban (2000).

2- STATICAL ANALYSIS
2.1. Regression equation and confidence limits:
Regression equation and confidence limits were calculated according to probit analysis computer program (Finney 1971). Regression equations are applied to predict mortality percentages (Y) for any applied concentrations (X), whereas (a) is intercept from (Y) axis and (b) is slope of the linear regression.

2.2. Toxicity index (T.I):
The toxicity index values (T.I) were calculated according to Sun (1950) as follow:

$$\text{Toxicity index (T.I)} = \frac{LC_{50} \text{ of the most toxic insecticide}}{LC_{50} \text{ of the tested insecticide}} \times 100$$

2.3. Relative Toxicity (R.T):
Relative toxicity values were measured according to the equation of Metcalf (1967):

$$\text{Relative Toxicity (R.T)} = \frac{LC_{50} \text{ of the lowest toxic insecticide}}{LC_{50} \text{ of the tested insecticide}} \text{ (Fold)}$$

2.4 Efficacy Ratio (E.R):
Efficacy Ratio value is that parameter which meager the efficacy of the tested insecticide according to this equation:

$$\text{Efficacy Ratio (E.R)} = \frac{\text{Recommended dose of the insecticide}}{\text{Obtained } LC_{95} \text{ of the this insecticide}} \times 100$$

RESULTS AND DISCUSSION

1- Efficacy of organophosphorus insecticides against the field population (male moths) of PBW during 2009 cotton season after 6 hrs of treatment using AEMT in Kafr El-Dawar District and Alex. Faculty of Agriculture Farm cotton fields.

The obtained results which tabulated in table (1) showed that, the LC_{50} values of chlorpyrifos, chlorpyrifos-methyl and profenofos at the 6 hrs duration in early cotton season from Kafr El-Dawar were: 2832.80, 955.10 and 909.10 ppm; respectively, where they were 260.09, 407.84 and 955.93 ppm, respectively from Alex.. The corresponding LC_{95} values were: 284724.2, 66800.78 and 11019.74 ppm, respectively for Kafr El-Dawar population and it was 1929.85, 6661.02 and 85872.7 ppm, respectively for Alex. population. The relative toxicity values showed variation in the efficacy order of these insecticides between the two locations. The efficacy order of Kafr El-Dawar was profenofos > chlorpyrifos-methyl > chlorpyrifos where it was chlorpyrifos > chlorpyrifos-methyl > profenofos for Alex. population.

Table (1): Efficacy Parameters of the selected insecticides against PBW (male moths, field strain) during early season of 2009 after 6 hrs of treatment using AEMT in Kafr- El-Dawar District and Faculty of Agriculture Farm cotton fields

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T.[a] fold	T. I.[b] %
Kafr El-Dawar District	Chlorpyrifos	Y = -2.8 + 0.8X	2832.80 (4717.4 -1709.6)	284724.2	1	32.09
	Chlorpyrifos-methyl	Y = -2.7 + 0.9X	955.10 (1261.2- 723.7)	66800.78	2.97	95.18
	Profenofos	Y = -4.5 + 1.5X	909.10 1073.3 – 769.9)	11019.74	3.12	100
Faculty of Agric. Farm	Chlorpyrifos	Y = -4.6 +1.9X	260.09 (312.8 - 216.1)	1929.85	3.68	100
	Chlorpyrifos-methyl	Y =-3.5 + 1.4X	407.84 (503.5- 330.0)	6661.02	2.34	63.77
	Profenofos	Y =-2.5 + 0.9X	955.93 (1277.5 - 715.5)	85872.7	1	27.21

[a]: Relative Toxicity (R.T.) = LC_{50} of the lowest toxic insecticide / LC_{50} of the tested insecticide.

[b]: Toxicity Index (T.I) = (LC_{50} of the most toxic insecticide / LC_{50} of the tested insecticide) X 100.

The same trend was obtained at the late season treatment but the LC_{50} and LC_{95} values differed from the early season. Data including the regration equation, both LC_{50} and LC_{95} values, the relative toxicity and the toxicity index were tabulated in table (2). For Kafr El-Dawar population the toxicity index and the relative toxicity parameters indicated that profenofos was the most toxic one with LC_{50} of 970.16 ppm and LC_{95} of 16718.8 ppm, followed by chlorpyrifos-methyl (LC_{50} = 1601.18 ppm and LC_{95} = 42651.1 ppm) where chlorpyrifos was the least toxic with LC_{50} = 3025.44 ppm and LC_{95} = 110064 ppm. Also, the toxicity index and the relative toxicity parameters for Alexandria population cleared that chlorpyrifos was the most toxic (LC_{50} =337.24 ppm) followed by chlorpyrifos-methyl (LC_{50}=560.06 ppm) and profenofos (LC_{50}=1092.79 ppm) where the corresponding LC_{95} values were 2628.87, 9094.12 and 73257.6 ppm, respectively.

Table (2): Efficacy Parameters of the selected insecticides against PBW (male moths, field strain) during late season of 2009 after 6 hrs of treatment using AEMT in Kafr- El-Dawar District and Faculty of Agriculture Farm cotton fields

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T. fold	T. I. %
Kafr El-Dawar District	Chlorpyrifos	Y = -3.7+ 1.1X	3025.44 (4582.2 – 2003.7)	110064	1.00	32.07
	Chlorpyrifos-methyl	Y = -3.7 + 1.2X	1601.18 (2078.6 – 1234.8)	42651.1	1.88	60.61
	Profenofos	Y = -3.9 + 1.3X	970.16 (1172.6 – 802.6)	16718.8	3.11	100
Faculty of Agric. Farm	Chlorpyrifos	Y = -4.7 + 1.8X	337.24 (399.4 – 284.5)	2628.87	3.24	100
	Chlorpyrifos-methyl	Y = -3.7 + 1.4X	560.06 (677.6 – 462.7)	9094.12	1.95	60.21
	Profenofos	Y = -2.7 + 0.9X	1092.79 (1444.2 – 827.5)	73257.6	1.00	30.86

Table (3) presented the efficacy ratio of the tested insecticides after 6 hrs of treatment at early and late 2009 cotton season in both locations. For Kafr El-Dawar population, these values were 0.42%, 1.87% and 12.25% at the early season treatment, while they were 1.1%, 2.93% and 8.07% at the late cotton season treatment for chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively. For Alexandria population, the obtained data were differed in both values and order. The highest recorded values were for chlorpyrifos (62.18% and 45.65%) followed by chlorpyrifos-methyl values (18.77% and 13.75%) then the lowest values of 1.57% and 1.84% were recorded for profenofos for both early and late season, respectively.

Table (3) Insecticide efficacy ratios at 2009 cotton season after 6 hr. of treatment.

Location	Insecticide	Early season 2009			Late season 2009		
		LC_{95}	RD[1]	ER[2]	LC_{95}	RD	ER
Kafr El-Dawar District	Chlorpyrifos	284724.2	1200	0.42%	110064	1200	1.10%
	Chlorpyrifos-methyl	66800.78	1250	1.87%	42651.1	1250	2.93%
	Profenofos	11019.74	1350	12.25%	16718.8	1350	8.07%
Faculty of Agric. Farm	Chlorpyrifos	1929.85	1200	62.18%	2628.87	1200	45.65%
	Chlorpyrifos-methyl	6661.02	1250	18.77%	9094.12	1250	13.75%
	Profenofos	85872.7	1350	1.57%	73257.6	1350	1.84%

1- RD= Recommended Dose

2- ER=Efficacy Ratio = (RD/ LC_{95})X100

2- Efficacy of organophosphorus insecticides against the field population (male moths) of PBW during 2009 cotton season after 12 hrs of treatment using AEMT in Kafr El-Dawar District and Alex. Faculty of Agriculture Farm cotton fields.

Results in table (4) showed that, the obtained values of LC_{50} after 12 hrs of treatment for Kafr El-Dawar

population in early 2009 cotton season were in the following descending order: chlorpyrifos-methyl > profenofos > chlorpyrifos where it was chlorpyrifos > profenofos > chlorpyrifos-methyl for Alex. population. The LC_{95} values of the three tested organophosphorus chlorpyrifos, chlorpyrifos-methyl and profenofos were: 7718.66, 2571.21 and 1729.53 ppm, respectively for Kafr El-Dawar population and they were 596.21, 2748.39 and 15339.43 ppm, respectively for Alex. population. The relative toxicity values and the toxicity index parameter showed variation in the efficacy order of these insecticides between the two locations. For Kafr El-Dawar population the efficacy order was chlorpyrifos-methyl > profenofos > chlorpyrifos, where it was chlorpyrifos > profenofos > chlorpyrifos-methyl for Alex. population.

Table (4): Efficacy Parameters of the selected insecticides against PBW (male moths, field strain) during early season of 2009 after 12 hrs of treatment using AEMT in Kafr- El-Dawar District and Faculty of Agriculture Farm cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T. fold	T. I. %
Kafr- El-Dawar district	Chlorpyrifos	Y = -2.6 + 1.1X	246.25 (339.4 – 178.2)	7718.66	1	53.77
	Chlorpyrifos-methyl	Y = -2.7 + 1.3X	132.4 (195.4 – 89.4)	2571.21	1.86	100
	Profenofos	Y = -4.2 + 1.8X	213.57 (268.3- 169.8)	1729.53	1.15	61.99
Faculty of Agric. Farm	Chlorpyrifos	Y = -4.1 + 1.9X	152.31 (199.9 - 115.8)	596.21	1.60	100
	Chlorpyrifos-methyl	Y = -3.7 + 1.6X	243.42 (307.8 - 192.2)	2748.39	1	62.57
	Profenofos	Y = -2.1 + 0.9X	216.00 (337.8 - 137.5)	15339.43	1.13	70.51

The same trend has been occurred at the late 2009 cotton season treatment but the corresponding values of both LC_{50} and LC_{95} were differed than that of the early season. Data including the regration equation, both LC_{50} and LC_{95} values, the relative toxicity and the toxicity index were presented in table (5). For Kafr El-Dawar population the toxicity index and the relative toxicity parameters indicated that profenofos was the most toxic one with LC_{50} of 316.18 ppm and LC_{95} of 7536.21 ppm, followed by chlorpyrifos (LC_{50} = 355.38 ppm and LC_{95} = 9342.40 ppm) where chlorpyrifos-methyl was the lowest toxic one with LC_{50} = 410.08 ppm and LC_{95} = 17877.1 ppm. Also, the toxicity index and the relative toxicity parameters for Alex. population cleared that chlorpyrifos was the most toxic (LC_{50} =199.70 ppm) followed by profenofos (LC_{50}=269.97 ppm) and then the least toxic was chlorpyrifos-methyl (LC_{50}=280.90 ppm) where the corresponding LC_{95} values were 1635.2, 20228.8 and 2743.4 ppm, respectively.

Table (5): Efficacy Parameters of the selected insecticides against PBW (male moths, field strain) during late season of 2009 after 12 hrs of treatment using AEMT in Kafr- El-Dawar District and Faculty of Agriculture Farm cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T. fold	T. I. %
Kafr El-Dawar District	Chlorpyrifos	Y = -2.9+ 1.2X	355.38 (459.5 – 274.4)	9342.40	1.15	88.97
	Chlorpyrifos-methyl	Y = -2.6 + 1.0X	410.08 (545.6 – 307.7)	17877.1	1.00	77.10
	Profenofos	Y = -2.99 + 1.2X	316.18 (418.7 – 238.3)	7536.21	1.29	100
Faculty of Agric. Farm	Chlorpyrifos	Y = -4.1 + 1.8X	199.70 (249.0 - 159.9)	1635.2	1.40	100
	Chlorpyrifos-methyl	Y = -4.1 + 1.7X	280.90 (344.6 - 228.7)	2743.4	1.00	71.09
	Profenofos	Y = -2.1 + 0.9X	269.97 (404.8 - 179.4)	20228.8	1.04	73.97

The efficacy ratios of the tested insecticides after 12 hrs of treatment from both the early and the late 2009 cotton season for the two locations were calculated and presented in table (6). For Kafr El-Dawar population, these values were 15.55%, 48.62% and 78.06% at the early season treatment, while they were 12.84%, 6.99% and 17.91% at the late cotton season treatment for chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively. For Alexandria population, the obtained data were differed in both values and order. The highest recorded values were for chlorpyrifos (201.3% and 73.39%) followed by chlorpyrifos-methyl values (45.48% and 45.56%) then the lowest values of 8.80% and 6.67% were recorded for profenofos for both early and late season, respectively.

Table (6) Insecticide efficacy ratios at 2009 cotton season after 12 hr. of treatment.

Location	Insecticide	Early season 2009			Late season 2009		
		LC_{95}	RD[1]	ER[2]	LC_{95}	RD	ER
Kafr El-Dawar District	Chlorpyrifos	7718.66	1200	15.55%	9342.40	1200	12.84%
	Chlorpyrifos-methyl	2571.21	1250	48.62%	17877.1	1250	6.99%
	Profenofos	1729.53	1350	78.06%	7536.21	1350	17.91%
Faculty of Agric. Farm	Chlorpyrifos	596.21	1200	201.3%	1635.2	1200	73.39%
	Chlorpyrifos-methyl	2748.39	1250	45.48%	2743.4	1250	45.56%
	Profenofos	15339.43	1350	8.80%	20228.8	1350	6.67%

3- <u>Efficacy of organophosphorus insecticides against the field population (male moths) of PBW during 2010 cotton season after 6 hrs of treatment using AEMT in Kafr El-Dawar District and Alex. Faculty of Agriculture Farm cotton fields.</u>

Table (7) shows the efficacy parameters of the tested insecticides against the male moth of pink bollworm both from Kafr El-Dawar and Alexandria cotton fields in early 2010 cotton season after the 6 hrs treatment. Data show that the efficacy of such tested insecticides was different between the two locations. Relative toxicity and toxicity index indicate that, for Kafr El-Dawar, chlorpyrifos was the lowest toxic with LC_{50} of 3100.1 and LC_{95} of 43519.2 ppm while profenofos was

the most toxic with LC_{50} of 1167.03 and LC_{95} of 12304.7 ppm followed by chlorpyrifos-methyl with LC_{50} of 1705.2 and LC_{95} of 20145.6 ppm; while for Alexandria chlorpyrifos was the most toxic insecticide with LC_{50} of 393.51 and LC_{95} of 3163.3 ppm followed by chlorpyrifos-methyl with LC_{50} of 693.54 and LC_{95} of 6624.13 ppm and then the lowest toxic was profenofos with LC_{50} of 1481.23 and LC_{95} of 65143.3 ppm.

Table (7): Efficacy Parameters of the selected insecticides against PBW (male moths, field strain) during early season of 2010 after 6 hrs of treatment using AEMT in Kafr- El-Dawar District and Faculty of Agriculture Farm cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T.[a] fold	T. I.[b] %
Kafr El-Dawar District	Chlorpyrifos	Y = -4.8 +1.4X	3100.1 (3928.6 – 2118.5)	43519.2	1.00	37.64
	Chlorpyrifos-methyl	Y =-4.3 + 1.4X	1705.2 (1556-1048)	20145.6	1.81	68.44
	Profenofos	Y =-4.9 + 1.6X	1167.03 (1375 -989)	12304.7	2.65	100
Faculty of Agric. Farm	Chlorpyrifos	Y =-4.71 + 1.8X	393.51 (462.9 - 334.4)	3163.30	3.76	100
	Chlorpyrifos-methyl	Y =-4.8 + 1.7 X	693.54 (810.8 - 593.2)	6624.13	2.14	56.74
	Profenofos	Y =-3.2 + 1.0 X	1481.23 (1958.6-1121.6)	65143.3	1.00	26.57

The same manner has been occurred in table (8) where the obtained data show that the efficacy of such tested insecticides was differ between the two locations in the late 2010 cotton season. Relative toxicity and toxicity index indicate that, for Kafr El-Dawar, chlorpyrifos was the lowest toxic with LC_{50} of 3196.62 and LC_{95} of 49433.2 ppm while profenofos was the most toxic with LC_{50} of 1332.63 and LC_{95} of 18245.1 ppm followed by chlorpyrifos-methyl with LC_{50} of 1749.2 and LC_{95} of 31436.5 ppm; while for Alexandria chlorpyrifos was the most toxic insecticide with LC_{50} of 494.1 and LC_{95} of 4317.59 ppm followed by chlorpyrifos-methyl with LC_{50} of 908.15 and LC_{95} of 10932.5 ppm and then the lowest toxic was profenofos with LC_{50} of 2344.69 and LC_{95} of 26723.3 ppm.

Table (8): Efficacy Parameters of the selected insecticides against PBW (male moths, field strain) during late season of 2010 after 6 hrs of treatment using AEMT in Kafr- El-Dawar District and Faculty of Agriculture Farm cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T.[a] fold	T. I.[b] %
Kafr El-Dawar District	Chlorpyrifos	Y = -4.9 + 1.4 X	3196.62 (4467.6 – 2291.4)	49433.2	1.00	41.88
	Chlorpyrifos-methyl	Y = -4.3 + 1.3 X	1749.20 (2214.7 – 1382.9)	31436.5	1.82	76.53
	Profenofos	Y = -4.5 + 1.5 X	1338.63 (1611.9 – 1112.1)	18245.1	2.38	100
Faculty of Agric. Farm	Chlorpyrifos	Y = -4.7 + 1.7X	494.10 (576.8 - 423.2)	4317.59	4.74	100
	Chlorpyrifos-methyl	Y = -4.5 + 1.5X	908.15 (1070.5 - 770.5)	10932.5	2.58	54.41
	Profenofos	Y = -5.3 + 1.6X	2344.69 (2940.5 - 1871.1)	26723.3	1.00	21.07

The efficacy ratio data in table (9) show the efficacy loss of the tested insecticides after 6 hrs of treatment at early and late 2010 cotton season in both two locations. The calculated data for Kafr El-Dawar population documented this loss between early and late season as follow: Chlorpyrifos Efficacy values were 2.76% and 2.43% after early and late season treatments; respectively, whereas they were 6.2% and 3.98% for chlorpyrifos-methyl and for profenofos they were 10.97% and 7.4%, respectively. The calculated data for Alexandria population were differed in both values and order. The highest recorded values were for chlorpyrifos (39.93% and 27.97%) followed by chlorpyrifos-methyl values (18.87% and 11.43%) then the lowest values of 2.07% and 5.05% were recorded for profenofos for both early and late season, respectively.

Table (9) Insecticide efficacy ratios at 2010 cotton season after 6 hr. of treatment.

Location	Insecticide	Early season 2010			Late season 2010		
		LC_{95}	RD[1]	ER[2]	LC_{95}	RD	ER
Kafr El-Dawar District	Chlorpyrifos	43519.2	1200	2.76%	49433.2	1200	2.43%
	Chlorpyrifos-methyl	20145.6	1250	6.20%	31436.5	1250	3.98%
	Profenofos	12304.7	1350	10.97%	18245.1	1350	7.40%
Faculty of Agric. Farm	Chlorpyrifos	3163.30	1200	37.93%	4317.59	1200	27.79%
	Chlorpyrifos-methyl	6624.13	1250	18.87%	10932.5	1250	11.43%
	Profenofos	65143.3	1350	2.07%	26723.3	1350	5.05%

4- <u>Efficacy of organophosphorus insecticides against the field population (male moths) of PBW during 2010 cotton season after 12 hrs of treatment using AEMT in Kafr El-Dawar District and Alex. Faculty of Agriculture Farm cotton fields.</u>

The results in table (10) showed that, the LC_{50} values of chlorpyrifos, chlorpyrifos-methyl and profenofos at the 12 hrs duration in early cotton season from Kafr El-Dawar were : 351.93, 385.05 and 268.82 ppm; respectively, where they were 213.77, 303.08 and 406.39 ppm, respectively from Alexandria.. The corresponding LC_{95} values were: 12418.7, 9168.18 and 5735.3 ppm, respectively for Kafr El-Dawar population and they were 2650.1, 4610.29 and 32773.9 ppm, respectively for Alexandria population. The relative toxicity values showed variation in the efficacy order of these insecticides between the two locations. The efficacy order of Kafr El-Dawar was profenofos > chlorpyrifos > chlorpyrifos-methyl, where it was chlorpyrifos > chlorpyrifos-methyl > profenofos for Alexandria population.

Table (10): Efficacy Parameters of the selected insecticides against PBW (male moths, field strain) during early season of 2010 after 12 hrs of treatment using AEMT in Kafr- El-Dawar District and Faculty of Agriculture Farm cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T.[a] fold	T. I.[b] %
Kafr El-Dawar District	Chlorpyrifos	Y = -2.7 + 1.1X	351.93 (465.7 – 265.5)	12418.7	1.09	76.38
	Chlorpyrifos-methyl	Y = -3.1+ 1.2X	385.05 (490.8 – 301.7)	9168.18	1.00	69.81
	Profenofos	Y = -3.1 + 1.2X	268.82 (357.2 – 201.9)	5735.3	1.43	100
Faculty of Agric. Farm	Chlorpyrifos	Y = -3.5 + 1.5X	213.77 (275.1 - 165.9)	2650.10	1.90	100
	Chlorpyrifos-methyl	Y = -3.5 + 1.4X	303.08 (383.9 - 239.0)	4610.29	1.34	70.53
	Profenofos	Y = -2.3 + 0.9X	406.39 (567.9 - 290.2)	32773.9	1.00	52.60

Results in table (11) showed that the obtained values of LC_{50} after 12 hrs of treatment for Kafr El-Dawar population in late 2010 cotton season were in the following descending order: profenofos (LC_{50} = 418.04 ppm) > chlorpyrifos (LC_{50} = 421.82 ppm) > chlorpyrifos-methyl (LC_{50} = 721.45 ppm), where it was chlorpyrifos (LC_{50} = 348.63 ppm) > chlorpyrifos-methyl (LC_{50} = 637.76 ppm) > profenofos (LC_{50} = 1677.16 ppm) for Alexandria population. The LC_{95} values of the three tested organophosphorus chlorpyrifos, chlorpyrifos-methyl and profenofos were: 11085.7, 21173.3 and 8544.23 ppm, respectively for Kafr El-Dawar population and they were 2778.14, 6538.82 and 21943.8 ppm, respectively for Alex. population. The relative toxicity values and the toxicity index parameter showed variation in the efficacy order of these insecticides between the two locations. The efficacy order for Kafr El-Dawar population was profenofos > chlorpyrifos > chlorpyrifos-methyl, whereas it was chlorpyrifos > chlorpyrifos-methyl > profenofos for Alexandria population.

Table (11): Efficacy Parameters of the selected insecticides against PBW (male moths, field strain) during late season of 2010 after 12 hrs of treatment using AEMT in Kafr- El-Dawar District and Faculty of Agriculture Farm cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T.[a] fold	T. I.[b] %
Kafr El-Dawar District	Chlorpyrifos	Y = -3.0 + 1.2X	421.82 (533.1 – 333.5)	11085.7	1.71	99.10
	Chlorpyrifos-methyl	Y = -3.2 + 1.1X	721.45 (894.6 - 581.8)	21173.3	1.00	57.94
	Profenofos	Y = -3.3 + 1.3X	418.04 (525.3 – 332.39)	8544.23	1.72	100
Faculty of Agric. Farm	Chlorpyrifos	Y = -4.6 + 1.8 X	348.63 (411.7 - 295.1)	2778.14	4.81	100
	Chlorpyrifos-methyl	Y = -4.6 + 1.6 X	637.76 (746.3 - 544.9)	6538.82	2.62	54.66
	Profenofos	Y = -4.8 + 1.5 X	1677.16 (2045.3 - 1376.1)	21943.8	1.00	20.79

The efficacy ratios of the tested insecticides after 12 hrs of treatment from both the early and the late 2010 cotton season for the two locations were calculated and presented in table (12). These values were 9.66%, 13.63% and 23.54% for Kafr El-Dawar population at the early season treatment, while they were 10.82%, 5.90% and 15.80% at the late cotton season treatment for chlorpyrifos, chlorpyrifos-methyl and profenofos, respectively. For Alexandria population, the calculated data were differed in both values and order. The highest recorded values were for chlorpyrifos (45.28% and 43.19%) followed by chlorpyrifos-methyl values (27.40% and 19.12%) then the lowest values of 4.12% and 6.15% were recorded for profenofos for both early and late season, respectively.

Table (12) Insecticide efficacy ratios at 2010 cotton season after 12 hr. of treatment.

Location	Insecticide	Early season 2010			Late season 2010		
		LC_{95}	RD[4]	ER[3]	LC_{95}	RD	ER
Kafr El-Dawar District	Chlorpyrifos	12418.7	1200	9.66%	11085.7	1200	10.82%
	Chlorpyrifos-methyl	9168.18	1250	13.63%	21173.3	1250	5.90%
	Profenofos	5735.3	1350	23.54%	8544.23	1350	15.80%
Faculty of Agric. Farm	Chlorpyrifos	2650.10	1200	45.28%	2778.14	1200	43.19%
	Chlorpyrifos-methyl	4610.29	1250	27.11%	6538.82	1250	19.12%
	Profenofos	32773.9	1350	4.12%	21943.8	1350	6.15

The continuous and intensive use of the tested organophosphorus insecticides in the Egyptian programmes for cotton pest control especially from the farmers side lead to many control problems. Lose in the efficacy of the tested insecticides was noticed as which happened hair by several investigators. Miller *et al* 1990 used the same technique to examine the efficacy of some insecticides to the field population of pink bollworm in cotton growing areas and reported that, a gradual decrease in efficacy of the test insecticides was correlated with the high use of carbamate, organophosphorus and pyrethroid insecticides; also, Horowitz *et al* 1993 indicate that the susceptibility of *H. armigera* to the tested compounds was not appreciably altered from 1987 to 1991, although fluctuations during the season were observed in most cases. The efficacy of the tested compounds was fluctuated in a V-shaped curve during the season. Shekeban 2000 studied with the attracticide resistance monitoring technique (ARMT) the increased levels in the efficacy lose of some insecticides from different groups against both pink bollworm and spiny bollworm and reported that, the efficacy of the tested insecticides was decreased from the early season of 1996 to the late season and from early to late season1997 while it was increased with late 1998 where the control program depends on the intensive use of pheromone disruption technique.

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EFFICACY OF PYRETHROIDS AGAINST PINK BOLLWORM MALE MOTH FIELD STRAINS USING THE ATTRACTICIDE EFFICACY MONITORING TECHNIQUE

ABSTRACT

El-Behera and Alexandria Governorates, Egypt, were chosen as two different locations in insecticides application for this field study. Experiments were carried out in two successive cotton seasons of 2009 and 2010 in both early and late season. Along the course of this investigation, deltamethrin was the most toxic for both El-Behera and Alexandria populations in all durations. The LC_{50} values of El-Behera population at the 6 hrs assessment from both early and late 2009 and 2010 cotton seasons indicated the following descending order deltamethrin > lambda-cyhalothrin > cypermethrin**.** The LC_{50} values of Alexandria population after 6 hrs of treatment from both early and late 2009 and 2010 cotton seasons showed the same descending order, but pyrethroids in Alexandria were more toxic than they in El-Behera. On the other hand, the relative efficacy ratio data from 6 hrs assessments proved the previous results where these ratios were 8.72% and 44.62% for deltamethrin, 14.07% and 37.99% for lambda-cyhalothrin and 8.36% and 40.62% for cypermethrin after 6 hrs assessment in early 2009 cotton season against both El-Behera and Alexandria populations, respectively. The 12 hrs assessments have been taken closely manner to that of 6 hrs assessment in pyrethroids descending order whereas deltamethrin proved to be the most toxic followed by

lambda-cyhalothrin and cypermethrin was the lowest toxic on except in early 2009 cotton season when cypermethrin was more toxic than lambda-cyhalothrin only for El-Behera population. The efficacy ratios study after 12 hrs of treatment show fluctuation levels for deltamethrin between the two seasons for El-Behera population where they take descending levels for both lambda-cyhalothrin and cypermethrin starting from early 2009 and ending with late 2010. Lambda-cyhalothrin moved in the same fluctuation pathway but cypermethrin ran in the descending way where deltamethrin fluctuated through 2009 and descended in 2010 for Alexandria population.
Key words: Pink bollworm, *Pectinophora gossypiella*, Efficacy, Pyrethroids.

INTRODUCTION

Cotton is a plant that seems to be designated specially to attract a wide range of insect pests. Its green succulent leaves, open flowers, nectaries on every leaf and flower and a vast amount of fruit. All these characteristics attract various insects such as the pink bollworm, spiny bollworm, the tobacco budworm, cotton leaf worm, cotton aphid, boll weevil, cotton fleahopper, spider mites, grass-hoppers, white fly, thrips and many other insects.

The pink bollworm (PBW) Pectinophora gossypiella (Saunders) is considered to be worldwide and sometimes the key regional pest of cotton. Like the boll weevil, the PBW is a well-adapted herbivore of cotton, feeding throughout the growing season on the cotton fruit (square and bolls) and burrowing habits. It has been causing a loss in yield as well as increasing the necessary cost for insect control. Substantial indirect losses occur as a result of the destruction of beneficial insects and the development of resistant insecticides in cotton. It has been extremely difficult to control using pesticides but considerable success has been achieved using alternative control tactics.

Pyrethroids and other formulated insecticides are used in large scale through the world to manage and control pests. For all that, insecticides provide many benefits i.e. increasing the product quantities and quality; lower efficiency is often seen because of the development of pesticide resistance. Therefore the efficacy of several formulated insecticides was studied by several investigators using the attracticide resistance monitoring technique (Haynes *et a*l 1986, Albeltagy et. al. 1996, Al-Beltagy *et al* 2001, Khider et. al. 2002, Shekeban *et al* 2003, Albeltagy et. al. 2010, and Albeltagy 2012).

Therefore, it is important to study efficacy of insecticides against bollworms in Egypt to establish a program to control and reduce the resistance ratio of these pests and increase the efficacy levels of those insecticides. Such a program could be efficiently used to reduce the number of insecticide sprays, cost of controlling progress, and increase the production of cotton per unit.

In our present study, insecticidal efficacy of selected formulated pyrethroids were examined against pink bollworm male moth strains in El-Behera and Alexandria Governorates by attracticide efficacy monitoring technique (AEMT), which is the modified name of attracticide resistance monitoring technique (ARMT), during two successive seasons (2009 and 2010).

MATERIALS AND METHODS

A-Materials

1- FORMULATED INSECTICIDES USED:
Three synthetic pyrethroids were used:

1.1 Cypermethrin (Syper)
EC 10% produced by Dow. Agro Sciences USA.
Application rate 0.6 liter / feddan.

1.2 Deltamethrin (Demthrin)
EC 2.5% provided by Agrochema, Egypt. Application rate: 0.75 liter / feddan

1.3 Lambda-cyhalothrin (Katron)
EC 2.5% provided by Agrochema, Egypt. Application rate: 0.8 liter / feddan

2- PHEROMONE USED
Gossyplure in pink rubber septum containing 1 mg, provided by the Ministry of Agriculture, Egypt.

3- OTHER CHEMICALS USED
Stickum® (sticky adhesive) and Acetone.

4- INSECTS USED
Field male moth populations of pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) from both Kafr-El-Dawar district cotton fields, El-Behera Governorate, and Faculty of Agriculture farm, Alexandria Governorate, Egypt were used locally in the present study.

B- Methods

1 - PROCEDURE OF AEMT
The attracticide efficacy monitoring technique (AEMT) is the modified name of attracticide resistance monitoring technique (ARMT) where delta traps were used with sticky adhesive–coated cards inserts containing the insecticide concentration placed in the trap bottom. Rubber septa with 1mg gossyplure acted as the pheromone source. This technique provided stable LC_{50}'s with low control mortality. This technique was used as described by Miller (1986) and modified by Shekeban (2000).

2- STATICAL ANALYSIS
2.1. Regression equation and confidence limits:

Regression equation and confidence limits were calculated according to probit analysis computer program (Finney 1971). Regression equations are applied to predict mortality percentages (Y) for any applied concentrations (X), whereas (a) is intercept from (Y) axis and (b) is slope of the linear regression.

2.2. Toxicity index (T.I):
The toxicity index values (T.I) were calculated according to Sun (1950) as follow:

$$\text{Toxicity index (T.I)} = \frac{LC_{50} \text{ of the most toxic insecticide}}{LC_{50} \text{of the tested insecticide}} \times 100$$

2.3. Relative Toxicity (R.T):
Relative toxicity values were measured according to the equation of Metcalf (1967):

$$\text{Relative Toxicity (R.T)} = \frac{LC_{50} \text{ of the lowest toxic insecticide}}{LC_{50} \text{ of the tested insecticide}} \text{ Fold}$$

2.4 Efficacy Ratio (E.R):
Efficacy Ratio value is that parameter which meager the efficacy of the tested insecticide according to this equation:

$$\text{Efficacy Ratio (E.R)} = \frac{\text{Recommended dose of the insecticide}}{\text{Obtained } LC_{95} \text{ of the this insecticide}} \times 100$$

RESULTS AND DISCUSSION

1- Pyrethroids efficacy during 2009 cotton season after 6 hrs of treatment against PBW field population using AEMT in El-Behera and Alexandria cotton fields.

Data in table (1) showed that, the LC_{50} values of cypermethrin, deltamethrin and lambda-cyhalothrin at the 6 hrs duration in early cotton season from El-Behera were: 119.7, 22.97 and 33.59 ppm; respectively, where they were 30.68, 10.83 and 17.51 ppm, respectively from Alexandria. The corresponding LC_{95} values were: 1795.16, 537.72 and 354.98 ppm, respectively for El-Behera population and they were 369.31, 105.07 and 131.62 ppm, respectively for Alexandria population. The relative toxicity values showed the same efficacy order of these insecticides in the two locations. This efficacy order was deltamethrin > lambda-cyhalothrin > cypermethrin. In spite of this parallel in the efficacy order, the toxicity values were different where the LC_{50} values for El-Behera were higher than that of Alexandria.

Table (1): Pyrethroids efficacy parameters during early 2009 cotton season after 6 hrs of treatment against PBW (male moths, field strain) using AEMT in El-Behera and Alexandria cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R.T.[a] TT.[a] fold	T.I.[b] %
El-Behera	Cypermethrin	Y = -2.9 + 1.4X	119.70 (143.6– 99.8)	1795.16	23.66	19.18
	Deltamethrin	Y = -1.6 + 1.2X	22.97 (28.4 - 18.6)	537.72	123.32	100
	Lambda-cyhalothrin	Y = -2.5 + 1.7X	33.59 (39.4 - 28.6)	354.98	84.33	68.38
Alexandria	Cypermethrin	Y =-2.3 + 1.5X	30.68 (38.7 - 24.1)	369.31	31.15	35.3
	Deltamethrin	Y =-1.7 + 1.7X	10.83 (13.2 - 8.8)	105.07	88.26	100
	Lambda-cyhalothrin	Y = -2.3+ 1.9X	17.51 (20.4 - 14.9)	131.62	54.59	61.85

[a]: Relative Toxicity (R.T.) = LC_{50} of the lowest toxic insecticide / LC_{50} of the tested insecticide.
[b]: Toxicity Index (T.I) = (LC_{50} of the most toxic insecticide / LC_{50} of the tested insecticide) X 100.

Data including the regration equation, both LC_{50} and LC_{95} values, the relative toxicity and the toxicity index of the tested insecticides at the 6 hrs duration of the late 2009 cotton season were tabulated in table (2). These data pointed out the same trend that occurred at the late season treatment but the LC_{50} and LC_{95} values were higher than that of the early season. For the El-Behera population the toxicity index and the relative toxicity parameters indicated that deltamethrin was the most toxic one with LC_{50} of 27.08 ppm and LC_{95} of 438.42 ppm, followed by lambda-cyhalothrin (LC_{50}=34.76 ppm and LC_{95} = 326.47ppm) where cypermethrin was the least toxic with LC_{50}=141.82 ppm and LC_{95} = 2512.01 ppm. Also, the toxicity index and the relative toxicity parameters for Alexandria population cleared that deltamethrin was the most toxic (LC_{50} =11.41 ppm) followed by lambda-cyhalothrin (LC_{50}=18.41 ppm) and cypermethrin (LC_{50}=48.97 ppm) where the corresponding LC_{95} values were 102.99, 146.13 and 377.23 ppm, respectively.

Table (2): Pyrethroids efficacy parameters during late 2009 cotton season after 6 hrs of treatment against PBW (male moths, field strain) using AEMT in El-Behera and Alexandria cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R.T.[a] fold	T.I.[b] %
El-Behera	Cypermethrin	Y = -2.8 + 1.3X	141.82 (173.34 - 116.15)	2512.01	21.33	19.09
	Deltamethrin	Y = -1.9+ 1.4X	27.08 (32.6 - 22.5)	438.42	111.72	100
	Lambda-cyhalothrin	Y = -2.6 + 1.7X	34.76 (40.4 - 29.8)	326.47	87.03	77.90
Alexandria	Cypermethrin	Y = -3.1 + 1.9X	48.97 (57.5 - 41.6)	377.23	22.31	23.3
	Deltamethrin	Y = -1.8 + 1.7X	11.41 (13.8 - 9.4)	102.99	95.77	100
	Lambda-cyhalothrin	Y = -2.3 + 1.8X	18.41 (21.5 - 15.7)	146.13	59.35	61.97

Table (3) presented the efficacy ratio of the tested insecticides after 6 hrs of treatment at early and late 2009 cotton season in both locations. For El-Behera population, the obtained values at the early season

treatment were 8.36, 8.72 and 14.09 %, while they were 5.97, 10.69 and 15.32 % at the late cotton season treatment for cypermethrin, deltamethrin and lambda-cyhalothrin, respectively. For Alexandria population, the obtained data were differed in their values where these recorded values were 40.62% and 39.76 % for cypermethrin, 44.62% and 45.52% for deltamethrin then the values of 37.99% and 34.22% were recorded for lambda-cyhalothrin in early and late season, respectively.

Table (3) Insecticide efficacy ratios at 2009 cotton season after 6 hr. of treatment.

Location	Insecticide	Early season 2009			Late season 2009		
		LC_{95}	RD[1]	ER[2]	LC_{95}	RD	ER
El-Behera	Cypermethrin	1795.16	150	8.36%	2512.01	150	5.97%
	Deltamethrin	537.72	46.88	8.72%	438.42	46.88	10.69%
	Lambda-cyhalothrin	354.98	50	14.09%	326.47	50	15.32%
Alexandria	Cypermethrin	369.31	150	40.62%	377.23	150	39.76%
	Deltamethrin	105.07	46.88	44.62%	102.99	46.88	45.52%
	Lambda-cyhalothrin	131.62	50	37.99%	146.13	50	34.22%

1- RD= Recommended Dose 2- ER=Efficacy Ratio = (RD/ LC_{95}) X100

2- <u>Pyrethroids efficacy during 2009 cotton season after 12 hrs of treatment against PBW field population using AEMT in El-Behera and Alexandria cotton fields.</u>

Results in table (4) showed that, the obtained values of LC_{50} after 12 hrs of treatment for El-Behera population in early 2009 cotton season were in the following descending order: deltamethrin (LC_{50}=1.19 ppm) > cypermethrin (LC_{50}=6.62 ppm) > lambda-cyhalothrin (LC_{50}=7.93 ppm) where it was deltamethrin (LC_{50}=4.15 ppm) > lambda-cyhalothrin (LC_{50}=9.01 ppm) > cypermethrin (LC_{50}=19.14 ppm) for Alexandria population. The LC_{95} values of the three tested pyrethroids cypermethrin, deltamethrin and lambda-cyhalothrin were: 148.54, 32.93 and 78.46 ppm, respectively for El-Behera population and they were 153.39, 41.59 and 97.51 ppm, respectively for Alexandria population. The relative toxicity values and the toxicity index parameter showed variation in the efficacy order of these insecticides between the two locations.

Table (4): Pyrethroids efficacy parameters during early 2009 cotton season after 12 hrs of treatment against PBW (male moths, field strain) using AEMT in El-Behera and Alexandria cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T. fold	T. I. %
El-Behera	Cypermethrin	Y = -0.9 + 1.2X	6.62 (12.8 - 3.3)	148.54	37.19	17.97
	Deltamethrin	Y = -.08 +1.0X	1.19 (2.99 - 0.45)	32.93	206.93	100
	Lambda-cyhalothrin	Y = -1.5 + 1.7X	7.93 (10.1 - 6.2)	78.46	31.05	15.00
Alexandria	Cypermethrin	Y = -2.3+ 1.8X	19.14 (24.8 - 14.7)	153.39	12.71	21.68
	Deltamethrin	Y = -1.1 + 1.6X	4.15 (5.9 - 2.9)	41.59	58.65	100
	Lambda-cyhalothrin	Y = -1.7 + 1.5X	9.01 (10.4 - 6.0)	97.51	27.01	46.06

The same trend had occurred at 12 hrs duration of the late 2009 cotton season treatment as well as between the early and late season in the 6 hrs duration where the corresponding values of both LC_{50} and LC_{95} differed more than that of the early season for the two locations. Data including the regration equation, both LC_{50} and LC_{95} values, the relative toxicity and the toxicity index were presented in table (5). For El-Behera population, the toxicity index and the relative toxicity parameters indicated that deltamethrin was the most toxic one with LC_{50} of 1.78 ppm and LC_{95} of 81.04 ppm, followed by lambda-cyhalothrin (LC_{50}= 10.10 ppm and LC_{95} = 102.72 ppm) where cypermethrin was the lowest toxic one with LC_{50}= 13.88 ppm and LC_{95} = 297.21 ppm. Also, the toxicity index and the relative toxicity parameters for Alexandria population cleared that deltamethrin was the most toxic with LC_{50} = 4.32 ppm followed by lambda-cyhalothrin with LC_{50} of 8.9 ppm and then the lowest toxic one was cypermethrin with LC_{50} of 22.83 ppm where the corresponding LC_{95} values were 39.20, 91.0 and 236.23 ppm, respectively.

Table (5): Pyrethroids efficacy parameters during late 2009 cotton season after 12 hrs of treatment against PBW (male moths, field strain) using AEMT in El-Behera and Alexandria cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T. fold	T. I. %
El-Behera	Cypermethrin	Y = -1.4 + 1.2X	13.88 (21.4 - 8.8)	297.21	29.54	12.82
	Deltamethrin	Y = -0.3 + 0.99X	1.78 (3.9 - 0.8)	81.04	230.38	100
	Lambda-cyhalothrin	Y = -1.6 + 1.6X	10.10 (12.5 - 8.0)	102.72	40.60	17.62
Alexandria	Cypermethrin	Y = -2.2 + 1.6X	22.83 (29.5- 17.6)	236.23	12.30	18.92
	Deltamethrin	Y = -0.98 + 1.7X	4.32 (5.7 - 2.7)	39.20	65.02	100
	Lambda-cyhalothrin	Y = -1.5+ 1.6X	8.90 (11.2 - 6.9)	91.00	31.56	48.53

Pyrethroid insecticides efficacy ratio parameter after 12 hrs of treatment from both early and late 2009 cotton season of the two locations were calculated and tabulated in table (6). For El-Behera population, the efficacy ratios were 101, 142.4 and 63.73 % at the early season treatment, while the corresponding values were 50.47, 57.85 and 48.78 % at the late cotton season

treatment for cypermethrin, deltamethrin and lambda-cyhalothrin, respectively. The obtained data show that, Alexandria population has some differences in values but still has the same order. The highest recorded values were for deltamethrin (112.7 and 119.6 %) followed by cypermethrin which recorded values of (97.45 and 63.5 %) then the lowest values of 51.28 and 54.95% were calculated for lambda-cyhalothrin in both early and late season, respectively.

Table (6) Insecticide efficacy ratios at 2009 cotton season after 12 hr. of treatment.

Location	Insecticide	Early season 2009			Late season 2009		
		LC_{95}	RD	ER	LC_{95}	RD	ER
El-Behera	Cypermethrin	148.54	150	**101%**	297.21	150	**50.47%**
	Deltamethrin	32.93	46.88	**142.4%**	81.04	46.88	**57.85%**
	Lambda-cyhalothrin	78.46	50	**63.73%**	102.72	50	**48.78%**
Alexandria	Cypermethrin	153.39	150	**97.45%**	236.23	150	**63.5%**
	Deltamethrin	41.59	46.88	**112.7%**	39.20	46.88	**119.6%**
	Lambda-cyhalothrin	97.51	50	**51.28%**	91.00	50	**54.95%**

3- <u>Pyrethroids efficacy during 2010 cotton season after 6 hrs of treatment against PBW field population using AEMT in El-Behera and Alexandria cotton fields.</u>

Table (7) presented the efficacy parameters of the tested insecticides against the pink bollworm, male moth population from both El-Behera and Alexandria cotton fields in early 2010 cotton season after 6 hrs of treatment. Data show that the efficacy of such tested insecticides was differed between the two locations only on their toxicity values where they have the same toxicity order. Relative toxicity and toxicity index indicate that, for El-Behera, cypermethrin was the lowest toxic with LC_{50} of 151.6 and LC_{95} of 1527.7ppm while deltamethrin was the most toxic with LC_{50} of 29.1 and LC_{95} of 309.7ppm followed by lambda-cyhalothrin with LC_{50} of 40.4 and LC_{95} of 394.1ppm; Also, for Alexandria deltamethrin was the most toxic insecticide with LC_{50} of 11.67 and LC_{95} of 164.50 ppm followed by lambda-cyhalothrin with LC_{50} of 21.86 and LC_{95} of 218.80 ppm and then the lowest toxic was cypermethrin with LC_{50} of 59.37 and LC_{95} of 650.38 ppm.

Table (7): Pyrethroids efficacy parameters during early 2010 cotton season after 6 hrs of treatment against PBW (male moths, field strain) using AEMT in El-Behera and Alexandria cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T. fold	T. I. %
El-Behera	Cypermethrin	Y =-3.4 + 1.6X	151.6 (164.9-118.3)	1527.7	20.44	19.19
	Deltamethrin	Y =-2.4 + 1.6X	29.1 (34.1 - 24.9)	309.7	106.53	100
	Lambda-cyhalothrin	Y =-2.7 + 1.7X	40.4 (47.2 -34.6)	394.1	76.73	72.02
Alexandria	Cypermethrin	Y = -2.2 + 1.4 X	59.37 (50.6 - 32.1)	650.38	24.94	19.65
	Deltamethrin	Y = -1.5 + 1.4 X	11.67 (14.5 - 9.3)	164.50	126.92	100
	Lambda-cyhalothrin	Y = -2.2 + 1.6 X	21.86 (25.7 - 18.6)	218.80	67.75	53.38

Data which derived from late 2010 cotton season after 6 hrs of treatment and tabulated in table (8) demonstrated that, the same manner of results has been occurred when the efficacy of the tested insecticides was differed in their values and not differed in their order between the two locations. Deltamethrin was the most toxic with LC_{50} of 29.1 and 11.97 ppm followed by lambda-cyhalothrin with LC_{50} of 40.4 and 21.86 ppm while cypermethrin was the lowest toxic with LC_{50} of 151.6 and 59.37 ppm for El-Behera and Alexandria, respectively. The corresponding LC_{95} values were 309.7 and 164.5 ppm for deltamethrin, 394.1 and 218.8 ppm for lambda-cyhalothrin while they were 1527.7 and 650.38 ppm for cypermethrin, respectively.

Table (8): Pyrethroids efficacy parameters during late 2010 cotton season after 6 hrs of treatment against PBW (male moths, field strain) using AEMT in El-Behera and Alexandria cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T. fold	T. I. %
El-Behera	Cypermethrin	Y = -3.5 + 1.6 X	165.51 (197.4 - 138.9)	1807.33	19.31	19.57
	Deltamethrin	Y = -2.4 + 1.6 X	32.40 (38.0 - 27.6)	364.13	98.66	100
	Lambda-cyhalothrin	Y = -2.8 + 1.7 X	45.80 (53.5 - 39.2)	425.83	69.79	70.74
Alexandria	Cypermethrin	Y = -2.9 + 1.6X	66.48 (78.5 - 56.3)	745.92	35.26	21.04
	Deltamethrin	Y = -1.7 + 1.5X	13.99 (17.1 - 11.4)	188.04	167.59	100
	Lambda-cyhalothrin	Y = -3.5 + 2.2X	36.19 (40.8 - 32.1)	197.38	64.78	38.65

The efficacy ratio data in table (9) show the efficacy loss of the tested insecticides after 6 hrs of treatment between early and late 2010 cotton season in both two locations. The calculated data for El-Behera population appeared this loss between early and late season as follow: the cypermethrin efficacy ratios were 9.82% and 8.3%, deltamethrin efficacy ratios were 15.14% and 12.87% and lambda-cyhalothrin efficacy ratios were 12.69% and 11.74% after early and late season treatments; respectively, whereas the corresponding values from Alexandria were 23.06% and 20.11% for cypermethrin, 28.5% and 24.93% for deltamethrin and

finally they were 9.82% and 8.3% for lambda-cyhalothrin, respectively.

Table (9) Insecticide efficacy ratios at 2010 cotton season after 6 hr. of treatment.

Location	Insecticide	Early season 2010			Late season 2010		
		LC_{95}	RD	ER	LC_{95}	RD	ER
El-Behera	Cypermethrin	1527.7	150	**9.82%**	1807.3	150	**8.3%**
	Deltamethrin	309.7	46.8	**15.14%**	364.13	46.88	**12.87%**
	Lambda-cyhalothrin	394.1	50	**12.69%**	425.83	50	**11.74%**
Alexandria	Cypermethrin	650.38	150	**23.06%**	745.92	150	**20.11%**
	Deltamethrin	164.50	46.8	**28.5%**	188.04	46.88	**24.93%**
	Cypermethrin	1527.7	150	**9.82%**	1807.3	150	**8.3%**

4- Pyrethroids efficacy during 2010 cotton season after 12 hrs of treatment against PBW field population using AEMT in El-Behera and Alexandria cotton fields.

The results in table (10) showed that, the LC_{50} values of cypermethrin, deltamethrin and lambda-cyhalothrin at the 12 hrs duration in early cotton season from El-Behera were: 15.85, 5.44 and 10.03 ppm; respectively, where they were 26.52, 5.78 and 10.24 ppm, respectively from Alexandria.. The corresponding LC_{95} values were: 304.97, 64.35 and 143.53 ppm, respectively for El-Behera population, while they were 264.59, 98.58 and 185.7 ppm, respectively for Alexandria population. The relative toxicity values showed the same efficacy order of these insecticides between the two locations (deltamethrin > lambda-cyhalothrin > cypermethrin). Moreover, the toxicity of the same insecticide was greater for El-Behera population than that for Alexandria population.

Table (10): Pyrethroids efficacy parameters during early 2010 cotton season after 12 hrs of treatment against PBW (male moths, field strain) using AEMT in El-Behera and Alexandria cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T. fold	T. I. %
El-Behera	Cypermethrin	Y = -2.6 + 1.7X	15.85 (41.2 - 13.7)	304.97	24.29	34.32
	Deltamethrin	Y = -1.2 + 1.5X	5.44 (7.4 - 3.9)	64.35	70.78	100
	Lambda-cyhalothrin	Y = -1.4 + 1.4X	10.03 (12.9 - 7.8)	143.53	38.38	54.23
Alexandria	Cypermethrin	Y = -2.3 + 1.7X	26.52 (33.4 - 21.0)	264.59	15.32	21.79
	Deltamethrin	Y = -1.1 + 1.3X	5.78 (8.1 4.1)	98.58	70.30	100
	Lambda-cyhalothrin	Y = -1.3 + 1.3X	10.24 (13.4 - 7.8)	185.70	39.68	56.44

Table (11) show that, the obtained LC_{50} values for El-Behera population after 12 hrs of treatment in late 2010 cotton season were in the following descending order: deltamethrin (LC_{50} = 6.15 ppm) > cypermethrin (LC_{50} = 17.96 ppm) > lambda-cyhalothrin (LC_{50} = 30.79 ppm), where they were deltamethrin (LC_{50} = 9.19 ppm) > lambda-cyhalothrin (LC_{50} = 15.7 ppm) > cypermethrin (LC_{50} = 43.61 ppm) for Alexandria population. The LC_{95} values of the three tested pyrethroids cypermethrin, deltamethrin and lambda-cyhalothrin were: 791.33, 117.0 and 174.63 ppm, respectively for El-Behera population while they were 469.03, 140.39 and 119.07 ppm, respectively for Alexandria population.

Table (11): Pyrethroids efficacy parameters during late 2010 cotton season after 12 hrs of treatment against PBW (male moths, field strain) using AEMT in El-Behera and Alexandria cotton fields.

Location	Formulated Insecticides	Reg. Equation Y= a + b x	LC_{50} (ppm) [Conf. Limits]	LC_{95}	R. T fold	T. I %
El-Behera	Cypermethrin	Y = -1.3+ 1.0X	17.96 (28.19 - 11.3)	791.33	40.16	34.24
	Deltamethrin	Y = -1.0 + 1.3X	6.15 (8.6 - 4.3)	117.00	117.30	100
	Lambda-cyhalothrin	Y = -1.5 + 1.4X	30.79 (14.9 - 9.3)	174.63	23.43	19.97
Alexandria	Cypermethrin	Y = -2.6 + 1.6 X	43.61 (52.6 - 36.1)	469.03	38.45	21.10
	Deltamethrin	Y = -1.3 + 1.4 X	9.19 (11.9 - 7.1)	140.39	182.49	100
	Lambda-cyhalothrin	Y = -2.2 + 1.9 X	15.70 (18.4 - 13.4)	119.07	106.82	58.53

The efficacy ratios of the tested insecticides after 12 hrs of treatment from both the early and the late 2010 cotton season for the two locations were calculated and presented in table (12). For El-Behera the efficacy ratios were 49.19, 72.85 and 34.84 % at the early season treatment, while they were 18.96, 40.07 and 28.63 % at the late cotton season treatment for cypermethrin, deltamethrin and lambda-cyhalothrin, respectively. For Alexandria population, the calculated efficacy ratios were 56.69, 47.56 and 26.93% at the early season treatment, while they were 31.98, 33.39 and 41.99% at late season treatment for cypermethrin, deltamethrin and lambda-cyhalothrin, respectively.

Table (12) Insecticide efficacy ratios at 2010 cotton season after 12 hr. of treatment.

Location	Insecticide	Early season 2010			Late season 2010		
		LC_{95}	RD^1	ER^2	LC_{95}	RD	ER
El-Behera	Cypermethrin	304.97	150	**49.19%**	791.33	150	**18.96%**
	Deltamethrin	64.35	46.88	**72.85%**	117.00	46.88	**40.07%**
	Lambda-cyhalothrin	143.53	50	**34.84%**	174.63	50	**28.63%**
Alexandria	Cypermethrin	264.59	150	**56.69%**	469.03	150	**31.98%**
	Deltamethrin	98.58	46.88	**47.56%**	140.39	46.88	**33.39%**
	Lambda-cyhalothrin	185.70	50	**26.93%**	119.07	50	**41.99%**

Comparing the LC_{50} values in early and late season also from season to the next season observed that, there is an increase in the LC_{50} values of selected formulated pyrethroids and increasing in their toxicities after 12 hrs of treatment compared to 6 hrs after treatment this refer to the extension in exposure period.

Moreover, for El-Behera population; the toxicity of the tested pyrethroids was higher than that of Alexandria population; this may refer to the difference in pyrethroid applications between the two locations where in El-Behera pyrethroids were used one time / season and also one type / season while in Alexandria pyrethroids were used more than one time / season and also many types / season where it's an experimental

farm and insecticides application run under experimental rules and previous prepared program.

The use of sex pheromone traps to monitor the response of adult insect populations to insecticide formulations is a novel idea first described by Riedl *et al* (1985), while Haynes *et al* (1986 a, b) mentioned the first use of yellow sticky cards incorporating with various dosages of selected insecticide formulations in pheromone traps. Also, Haynes *et al* (1987) summarized the advantages of using the pheromone sticky traps in monitoring insecticide resistance in different insect field populations. They mentioned that, the new method eliminates handling of insects that is involved in other methods of assessing population with pheromone traps. Schouest and Miller (1988) measured toxicity of fenvalerate and permethrin on a laboratory strain of adult PBW by the attracticide bioassay. Brewer and Trumble (1989) mentioned that this attracticide resistance monitoring technique (ARMT) provided stable LC_{50} 's with low control mortality, they also mentioned that, slopes of probit regression for male were similar for topical and ARMT bioassay indicating a parallel response of the insects to the two methods.

Assaying insecticidal efficacy of selected formulated insecticides against pink bollworm *Pectinophora gossypiella* (Saunders) male moths by attracticide resistance monitoring technique in laboratory and field tests showed that deltamethrin was the most toxic insecticide than the other tested pyrethroids.

These results are in good agreement with those reported by Kassem and Zeid (1987), Kassem *et al* (1988), Shekeban (1989), Marei *et al*. (1991), Shekeban et al (2003) and Shekeban (2007 and 2008). Also, El-Bassiony (2001) obtained the same trend of results when testing these compounds against the laboratory and field strains of pink bollworm using ARMT.

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OBSERVATIONS ON COMPARATIVE EFFICACY OF INSECTICIDES AGAINST *CAMPOLETIS CHLORIDEAE AND CHRYSOPA SP.*

ABSTRACT
A recent study was carried out to determine the relative toxicity of nine commonly used insecticides viz. cypermethrin, endosulfan, carbaryl, dimethoate, phosalone, parathion, monocrotophos, trichlorphos and chlorpyriphos to the natural enemies *Campoletis chlorideae* and *Chrysopa sp*, under the laboratory conditions. Insecticides were tested at field recommended doses according to the standard method for testing side effects of pesticides on natural enemies of insect pests. The study evaluated the accurate toxicity of nine insecticides to natural enemy populations, based on mortality at 48 h after treatment. Endosulfan and phosalone appeared to have less detrimental effect on *Campoletis chlorideae* and *Chrysopa sp,*. We need to examine its effect on other predators and parasitoids. The results obtained in the present study showed that all the nine insecticides were not equally effective to both the bio-agents. Thus, a potential approach for true compatibility of pesticides and biological control agents with broad implications has been demonstrated.
KEY WORDS: *Heliothis armigera, Campoletis chlorideae, Chrysopa sp*., pesticides, bio-agent, relative toxicity, mortality.

INTRODUCTION

Naturally occurring predators and parasites (natural enemies) are important in regulating populations of *Heliothis*. Conservation of beneficial arthropods is a fundamental principle of the integrated pest management (IPM) concept. Conserving natural enemies can provide economic benefit to growers, as natural enemies help to reduce pest populations. Studies on natural enemies of insect pests of chickpea have identified key species and outlined the role played by them in pest population dynamics (Verma et. al.1995). However, their full potentiality to provide the control of pests in nature needs to be exploited particularly when integrated with the use of limited need-based insecticidal applications. The use of some insecticidal applications may be unavoidable, considering the increased demand for pulses, cotton, oil seeds, and cereals in the country, but the insecticides to be used should be selected with care so that they do minimal harm to predators and parasites. Testing of pesticides on natural enemies is important before field application and their selectivity depending upon the abundance or inundative release of natural enemies is imperative for conserving bio-agents and for maintaining a healthy agro-eco system (Brahman 1998**).** Most of the insecticides (about 44%) are used against Lepidoptera. One in depth study conducted (II

BC Kenya, now CABI-ARC) in late 1980s, demonstrated the potential importance of parasitoids and particularly of predators in regulation of a key pest, the highly polyphagous noctuid moth *H. armigera*. Among the larval parasitoids *Campoletis chlorideae* (Ichneumonid) is very common. Several arthropod predators including *Chrysopa sp.* have been observed to feed on eggs and early stage larvae of *Heliothis* (Manjunath et. al.1998). Divakar and Pawar (1982, 1987) also reported the efficacy of natural enemies in biocontrol of crop pests in India. Roach' s (1975) survey in north-eastern South Carolina revealed that only *Campoletis chlorideae* and *Chrysopa sp.*, occurred in sufficient numbers to affect *Heliothis* populations. A major problem with pesticides, even modern selective bio-insecticides, is that they can cause disruptions to the natural enemy complex by removing the food/host resource required by parasitoids and predators. Information on the relative toxicity of different pesticides against a range of natural enemies is available from a variety of sources including the long- term IOBC-WPRS working group research programme. The increasing indiscriminate use of pesticides adversely affects such potential natural enemies (Jones et al., 1998). Therefore, selectivity of pesticides is important in Integrated Pest Management. To manage the pest effectively research efforts have been made during last two decades under All India Coordinated Pulses Improvement Project (AICPIP). This research was conducted to evaluate the toxicity of the nine insecticides to the predominant species of natural enemies (noted above) using the laboratory initial toxicity test.

MATERIAL AND METHODS

This study was carried out during 2011, at Research Station, Bilaspur Chhattisgarh, India to evaluate the comparative efficacy of insecticides against natural enemies *Campoletis chlorideae* and *Chrysopa sp.* The following technical grade insecticides were used in laboratory initial toxicity test: cypermethrin, endosulfan, carbaryl, dimethoate, phosalone, parathion, monocrotophos, trichlorphos and chlorpyriphos. Insecticides were purchased from M/S Lupin Agro-chemicals (India) Ltd Bombay. Samples of *Campoletis chlorideae* and *Chrysopa sp.* for experiment purposes were obtained from Sisco Research Laboratory, Mumbai, India and M\S Biocontrol Research Lab., Banglore, India respectively. *H. armigera* insects were collected from all chickpea growing areas of Bilaspur, Chhattisgarh and were used in present study**.** The larval parasitoid *Campoletis chlorideae* and the egg predator *Chrysopa sp.* were regularly reared in the laboratory using the pod borer *Heliothis armigera* as host. Insecticides were tested at field recommended dose according to the standard method for testing side effects of pesticides on natural enemies suggested by Hassan et, al., (1985). While mortality of the parasitoid 48 hrs after treatment was considered for grading the pesticides, the efficiency index based on the oviposition of surviving females was also taken into consideration in case of the predator.

RESULTS AND DISCUSSION

The insecticides exhibited a range of toxicity to the natural enemies screened after 48 hrs of exposure (Table 1 & Table 2). Results presented in Table 1 & Table 2 indicated that endosulfan and phosalone are least toxic to both the natural enemies. Phosalone and endosulfan both had very little toxicity to both of the species tested, indicating that these products could likely be used with very little impact on natural enemy populations in the field. The present observations on the effect of phosalone and endosulfan are in agreement with those of Reddy et al., (1998) on *Bracon kirkpatricki* and *C. carnea*, Krishnamorthy, (1986) on C. scelestes, Ruberson, et al., (1994) on *Microplitis croceipes* and Lewis (1972) on *Cardiochiles nigriceps*. Sukhoruchenko et al. (1977) have also reported that phosalone is harmless to natural enemies and is therefore suitable for use in integrated control programs. Endosulfan is frequently considered to be safe to natural enemies. In earlier evaluations endosulfan was found to be safe to *B. hebetor* and *B. brovicornis (*Sharma and Sarup, 1982) but was reported to be toxic to *B. kirkpatricki (*Hamilton and Attia, 1976, Mani and Nagarkatti 1982). It is apparent from the Table 1 & Table 2 that insecticides viz parathion and chlorpyriphos were found to be slightly harmful to the parasitoid as well as to the predator in the present study. Cypermethrin and dimethoate were slightly harmful to *Campoletis chlorideae but* harmless to *Chrysopa sp*.. Trichlorphos was highly toxic to *Campoletis chlorideae* but was moderately harmful to *Chrysopa sp*. Carbaryl and monocrotophos were found to be most toxic to both the natural enemies. These results are in agreement with earlier finding of Dulmage et al., (1998) who also reported highly toxicity of Carbaryl and monocrotophos to *Chrysoperla carnea*. Previous laboratory and field research studies have shown that major lepidopteran pests are currently being controlled by the application of broad-spectrum insecticides such as monocrotophos or carbaryl four times at weekly intervals during the growing season. However, these broad-spectrum materials are highly toxic to insect natural enemies (Hamilton and Attia, 1976). The results obtained in the present study showed that all the nine insecticides were not equally effective to both the bio-agents. Reduction in pest population and increase in yield following inundative release of *Clubiona sp.* has been reported (Ridgway et. al., 1970). The activity of natural enemies is often hampered by the high insecticidal pressure throughout the crop growth and indiscriminate use of

pesticides adversely affects such potential natural enemies (Armstrong et al., 1996). Pesticides because of their selectivity are well suited to being key components in an agro-ecosystem, because they lack direct activity on natural enemies (Rote, et. al., 1981). A major problem with pesticides, even modern selective bio-insecticides, is that they can cause disruptions to the natural enemy complex by removing the food/host resource required by parasitoids and predators. Scientists have suggested that sub-lethal or slow-killing doses could potentially provide immediate control of crop damage by a pest while stimulating the buildup of its natural enemies. The availability of insecticides that are less toxic to insect natural enemies will permit growers to conserve natural enemies and limit problems with secondary pests. Ridgway et al. (1967) demonstrated the impact of a presumably selective insecticide, on predator populations, and the role of these predators in suppressing *Heliolhis* sp populations. Others have also shown the detrimental effect of insecticides on natural enemy populations.

Table 1: Relative toxicity of nine insecticides to *Campoletis chlorideae*.

S No	Insecticides	Concentration tested	Effect on C. chlorideae		
			Mortality	Evaluation category	Efficiency index
1.	Cypermethrin	0.01%	61.9	2	24.7
2.	Endosulfan	0.07%	20.7	1	11.7
3.	Carbaryl	0.15%	100.0	4	100.0
4.	Dimethoate	0.02%	60.5	2	47.4
5.	Phosalone	0.05%	23.9	1	18.6
6.	Parathion	0.1%	53.5	2	49.9
7.	Monocrotophos	0.04%	100.0	4	99.9
8.	Trichlorphos	0.05	96.7	4	88.5
9.	Chlorpyriphos	0.05	72.4	2	55.8

** Efficiency index (E) is calculated as per the formula E=100%-(100-M) x R where M is larval mortality and R= Egg laying in comparison to control. *Evaluation category: 1=Harmless (less than 50%) 2=Slightly harmful (50-79%). 3=Moderately harmful (80-90%). 4=Harmful (above 90%).

Table 2: Relative toxicity of nine insecticides to *Chrysopa sp.*

S No	Insecticides	Concentration tested	Effect on Chrysopa sp		
			Mortality	Evaluation category	Efficiency index
1.	Cypermethrin	0.01%	49.7	1	27.8
2.	Endosulfan	0.07%	23.9	1	13.9
3.	Carbaryl	0.15%	98.5	4	99.2
4.	Dimethoate	0.02%	48.8	1	41.7
5.	Phosalone	0.05%	30.3	1	23.8
6.	parathion	0.1%	57.9	2	46.8
7.	Monocrotophos	0.04%	97.9	4	91.8
8.	Trichlorphos	0.05	87.8	3	79.4
9.	Chlorpyriphos	0.05	68.5	2	51.3

** Efficiency index (E) is calculated as per the formula E=100%-(100-M) x R where M is larval mortality and R= Egg laying in comparison to control. *Evaluation category: 1=Harmless (less than 50%) 2=Slightly harmful (50-79%). 3=Moderately harmful (80-90%). 4=Harmful (above 90%).

Based on the above observations, it may be concluded that the endosulfan and phosalone are not harmful to both the bio-agents tested in the present evaluation. Extensive screening of insecticides readily available in India should be under taken with the objective of utilizing only those that have a demonstrably higher degree of safety to natural enemies. Unless this is done and recommendations of insecticidal applications are based on such studies, it would be meaningless to consider augmenting natural enemy populations. We should analyze data to interpret the effects of pesticides on natural enemies. Further, to select appropriate pesticides and establish an optimal method of pesticide use, we need to quantify the role of natural enemies in pest control.

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EFFICACY OF FOUR SYNTHETIC PYRETHROIDS AND CHLORPYRIFOS 20 EC TO CUT WORM, *AGROTIS IPSILON (*HUFNAGEL*).*

ABSTRACT

Relative toxicity of four synthetic pyrethroids and chlorpyrifos were evaluated against 1 to 2 days and 8 to 9 days old larvae of *Agrotis ipsilon* (Hufn.) using dry film technique. The order of toxicity to 1-2 day old larvae with LC_{50} values in parentheses was: cypermethrin (0.00000212) > delthamethrin (0.00000296) > alphamethrin (0.00002031) > fenvalerate (0.00002295) > chlorpyrifos (0.00006851). The former four insecticides were respectively 33.91, 23.32, 2.98 and 2.76 times more toxic than Chlorpyrifos. The order of toxicity against 8-9 days old larvae was: cypermethrin > deltamrthrin > alphamethrin > chlorpyrifos. The synthetic pyrethroids were 23.94, 22.36, 3.82 and 3.34 times more toxic than Chlorpyrifos respectively. The LC_{50} values of cypermethrin, deltamethrin, alphamethrin, fenvalerate and Chlorpyrifos against 8-9 days old larvae were 0.00001210, 0.00001346, 0.00008241, 0.00009321 and 0.00024561 per cent, respectively. Among synthetic pyrethroids, cypermethrin and deltamethrin were found superior to others regarding protection against larvae of *Agrotis ipsilon* (Hufn.). The studies indicated that the population of *Agrotis ipsilon* (Hufn.) to the insecticides tested was quite susceptible.

INTRODUCTION

Cauliflower (*Brassicae oleracea* var. Botrytis) and cabbage (*Brassicae oleracea* var. *capitata*) is widely grown both in hills as well in plains (Ahmad *et al.,*2007). High incidence of cutworm, *Agrotis ipsilon* (Hufn.) is one of the limiting constrains (Rai *et al.,*1985, Mishra and Ram, 1997). Several insecticides have been tested for the effective management of this pest, but synthetic pyrethroids are the most potent insecticides against a wide variety of crop pests (Elliot *et al.,* 1978). So, there is a need to determine the relative toxicity of four synthetic pyrethroids *viz.*, cypermethrin, deltamethrin, fanvalerate and Alphamethrin along with Chlorpyrifos by bioassay method using *P. brassicae* larvae.

MATERIAL AND METHODS

To determine the LC_{50} values, the stock solutions of known strength of the insecticides were prepared in acetone from the technical grades. The desired insecticidal concentrations were applied in the form of a dry film, deposited on the inner surface of the tube (20x25 cm dia) by following Gupta and Rawlin's (1966) technique. Thin and uniform film of insecticide was prepared by taking one ml of insecticide solution in a test tube and rotating with the help of a hand rotating machine till dryness. Toxicity of insecticidal film was determined against the laboratory reared 1-2 day and 8-9 day old larvae of cutworm, *Agrotis ipsilon* (Hufn.) reared on an artificial diet (Baruah, 1989). Then, 1 to 2 day old larvae were released into each tube which served as one replicate. Four replications of each insecticidal concentration were maintained. Simultaneously, a control set with dry film of acetone only was also run.

Simultaneously, another set of experiments were carried out for LC_{50} values where 8-9 days old larvae were used as test insects. In this case, one larva in each tube was kept and such ten tubes constituted one replication. The tubes were then kept in an incubator maintained at $28^0 \pm 2^0$C. After 24 hrs, mortality counts were made and moribund larvae were counted as dead. The mortality in control, if any, was corrected by using Abbot's formula (1925). The dosage mortality data obtained were subjected to Probit analysis (Finney, 1971) to find out LC_{50} values of a particular insecticide as unity.

RESULT AND DISCUSSION

On the basis of LC_{50} values, it is evident from Table 1 that cypermethrin was the most toxic insecticides and Chlorpyrifos the least to *Agrotis ipsilon* (Hufn.). The order of toxicity with respect to LC_{50} values is as cypermethrin > deltamethrin > Alphamethrin > fenvalerate > Chlorpyrifos.

Table 1: Relative toxicity of some insecticide to 1-2 day old larvae of *Agrotis ipsilon* (Hufn.)

Insecticide	Heterogeneity*	Regression equation	LC_{50}(%)	Fiducial limits LC_{50} ±	Relative toxicity
Deltamethrin	0.436	1.65841+2.49840x	0.00000296	0.000000112	23.32
Cypermethrin	0.596	1.71152+2.45612x	0.00000212	0.000000106	33.91
Alphamethrin	1.431	1.85254+1.92547x	0.00002031	0.000001015	2.98
Fenvalerate	1.395	1.54278+2.81231x	0.00002295	0.000001141	2.76
Chlorpyrifos	1.131	0.63254+2.24698x	0.00006851	0.000001231	1.00

* In none of the cases, the data were found to be significantly heterogenous at P =0.05%
Y= Probit kill
X= Log concentration

From the comparison of LC_{50} values, it is very clear that all the synthetic pyrethroids tested were more toxic than Chlorpyrifos. Vaissayre and Renou (1978) reported LC_{50} value ranging from 5.44 to 6.19 mg/larvae for three synthetic pyrethroids (deltamethrin, fenvalerate and cypermethrin) as compared to 28.68 mg/larvae for DDT against the larvae of *H.armigera* as determined by topical application method. Baruah and Chauhan (1996) reported the order of toxicity against *H.armigera* as deltamethrin > cypermethrin > fenvalerate>Chlorpyrifos.

Comparison of LC_{50} values of different insecticides revealed that cypermethrin, deltamethrin, alphamethrin and fenvalerate were 33.91, 23.32, 2.98 and 2.76 times more toxic than chlorpyrifos against the 1-2 day old larvae of *Agrotis ipsilon* (Hufn.). The relative toxicity observed during the present study is in conformity with that of Baruah and Chauhan (1996) who reported that

deltamethrin, cypermethrin and fenvalerate were 34.93, 20.13 and 5.28 times more toxic than chlorpyrifos.

On the basis of LC_{50} values, it is clear from Table 2 that cypermethrin is the most toxic insecticide and chlorpyrifos the least to 8-9 day old larvae of *Agrotis ipsilon (Hufn.)*. The order of toxicity with respect to LC_{50} values is cypermethrin > deltamethrin > Alphamethrin > fenvalerate > chlorpyrifos. From the comparison of LC_{50} values, it is clear that all the synthetic pyrethroids tested are more toxic than chlorpyrifos. Elliot *et al.,* (1974) stated that pyrethroids were considerably more active than any other available compound. Ahmad and Sharma (1985) reported the order of toxicity as decis > Ripcord > Ambush > Cymbush > sumicidin > chlorpyrifos against maize stem borer *Chilo partellus.* Similar order of toxicity, *i.e.* deltamethrin > cypermethrin > fenvalerate > permethrin > BHC, against *S. litura* was reported by Gupta *et.al.* (1985).

Table 2: Relative toxicity of some insecticide to 8-9 day old larvae of *Agrotis ipsilon* (Hufn.)

Insecticide	Heterogeneity*	Regression equation	LC_{50}(%)	Fiducial limits $LC_{50\pm}$	Relative toxicity
Deltamethrin	1.586	0.45896+1.65241x	0.00001346	0.000000156	22.36
Cypermethrin	0.792	0.96854+2.86547x	0.00001210	0.000000142	23.94
Alphamethrin	0.612	0.26847+1.24568x	0.00008241	0.000001236	3.82
Fenvalerate	0.761	0.23458+2.98654x	0.00006321	0.000001221	3.34
Chlorpyrifos	0.896	1.65821+2.12547x	0.00024561	0.000001292	1.0

* In none of the cases, thedata were found to be significantly heterogenous at P =0.05%
Y= Probit kill
X= Log concentration

Comparison of relative toxicity of different insecticide with chlorpyrifos as standard indicate the cypermethrin, deltamethrin, Alphamethrin and fenvalerate are 23.94, 22.36, 3.82 and 3.34 times more toxic than chlorpyrifos. The present findings are in conformity with that of Peter and Sundararajan (1990) who reported that deltamethrin was the most toxic insecticide against 4thinstar larvae of *H. armiger*a by topical application method. It was 28.4 times more toxic than chlorpyrifos insecticide. The pyrethroids were more toxic than the organophosphate and organochlorine insecticides.

In the present investigation, younger larvae are more susceptible than the older ones and the LC_{50} values for synthetic pyrethroids increased approximately by a factor of 3.8 times to 8-9 day old larvae of P. brassicae as compared to 1-2 day old larvae. The present findings are in conformity with that of Kohli (1982) who also noted that younger larvae were generally more susceptible and the LD_{50} for synthetic pyrethroid increased approximately by a factor of 2 per instar.

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Influence of diet on pesticide detoxification ability of *Spodoptera litura* exposed to organophosphate and synthetic pyrethroid insecticides

ABSTRACT
Spodoptera litura is a polyphagous pest of several agricultural crops. Present study was designed to understand the impact of food quality on the pesticide detoxifying ability of *S. litura*. Larvae were maintained on four types of diet. Results show a difference in the performance of esterase, superoxide dismutase and cytochrome P450 activity among different food regimen. Among the two pesticides tested, detoxification enzymes activity significantly differed organophosphates exposure. The results suggest that an insecticide dose should be based on the type of crops rather than selecting a single insecticide dose for all crops.
Keywords: Host Plant; esterase; Cyt P450; superoxide dismutase; common cutworm.

INTRODUCTION

Spodoptera litura (Lepidoptera: Noctuidae) is polyphagous, damaging numerous vegetables and field crops worldwide (Hill, 1975). It has a large host range of more than 120 host plants (Ramana *et al*., 1988). Insects show preference for some types of food over other. This is mainly based on the food quality which includes the presence of nutrients and also absence/scanty levels of secondary metabolites (Behemer *et al*., 2002). Chemical insecticide has been used for the control of *S. litura*. This has led to the development of insecticide resistance in *S. litura* (Huang, 2007; Cho *et al*., 1999). Different host plants have been known to influence the pesticide susceptibility in insects (Ghidiu *et al*., 1990; Sharma and Yadav, 2001). Also the presence of secondary metabolites has been known to alter the functioning of detoxification enzymes in insects (Yu *et al*., 1979). Insecticide resistance is mainly contributed by metabolic resistance involving mixed function oxidases (MFO), esterases and glutathione-S-transferases (Wang *et al*. 2010). The present study is designed to understand how diet (Cabbage, Cotton, Castor, and artificial diet) modulates the metabolic detoxification mechanism in *S.litura larvae*.

MATERIALS AND METHODS

Insect Culture and Treatment Protocol

First instar *Spodoptera litura* was obtained from the Insectary (Molecular Entomology Lab, Department of Biotechnology, Periyar University). The larvae were divided into four groups based on the type of food administered. Group I: Castor (*Ricinus communis*); Group II: Cabbage: *(Brassica oleracea*), Group III: Cotton (*Gossypium* spp.) and Group IV: artificial diet. 5^{th} instar of *Spodoptera litura* larvae were treated with field recommended dosage of monocrotophos (175 g/a.i) and deltamethrin (12.5g/a.i) insecticides in different host plant using leaf dip method recommended by Insecticide Resistance Action Committee (IRAC; www.irac-online.org/resources/methods.asp).

Enzyme preparation

Twenty 5^{th} instar larvae were taken from each group and were exposed to different insecticides at field recommended doses using leaf dip method. 24 hours post exposure the live larvae were separated, starved, chilled on ice and dissected to remove midgut tissue. The tissues were homogenized on ice in homogenization buffer (0.1 M phosphate buffer, pH 7.2). The homogenate was centrifuged at 4°C, 10, 000 g for 15 min, and the solid debris and cellular material were discarded. The supernatant was decanted into a clean eppendorf tube, placed on ice and used for detoxification enzyme assays.

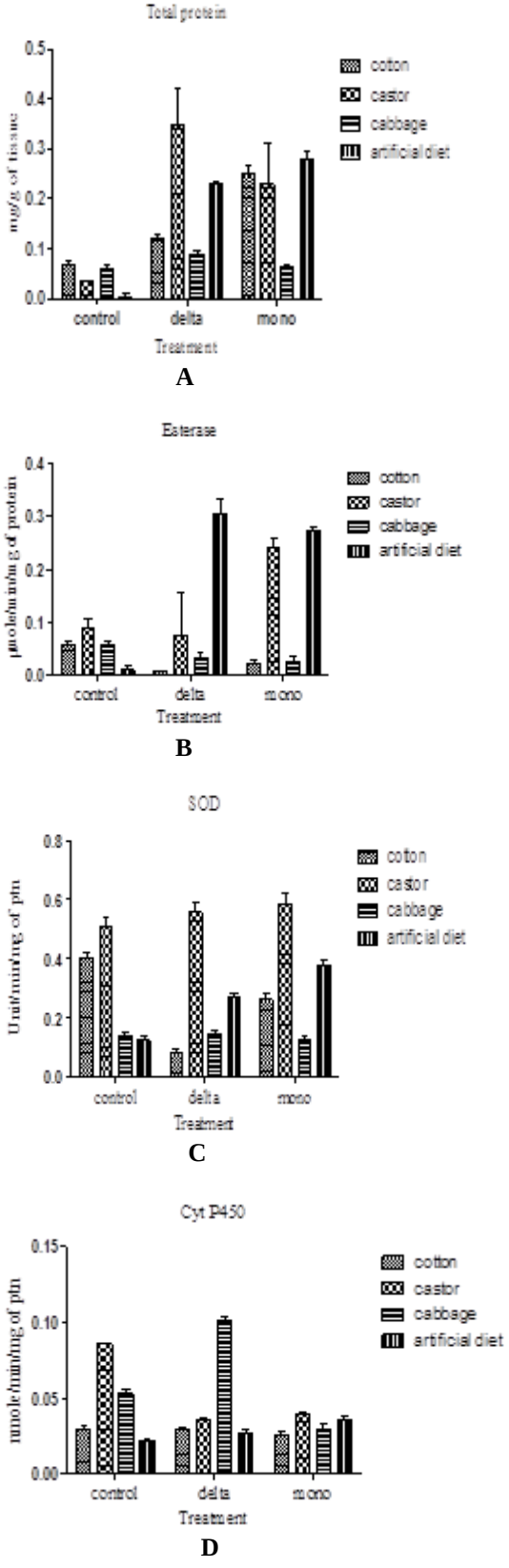
Enzyme assays

Following protocols were used for enzyme assay (i) Total Protein (Lowry *et al*., 1951); (ii) Carboxylesterase (Kranthi *et al*., 2005); (iii) Cytochrome P450 (Brogdon *et al*., 1998) and (iv)Superoxide Dismutase(Marklund and Marklund, 1974).

RESULTS AND DISCUSSION

The activity of Cytochrome P450 in *Spodoptera litura* larvae that fed on cabbage were the highest activity followed by the deltamethrin treatment as compared to the control as well as other diets. SOD activity was higher in artificial diet followed by both treatments (deltamethrin and monocrotophos). Esterase activity was higher in castor by the monocrotophos as well as artificial diets showing significantly higher levels of activity in both treatment as compared to control and other diets. However, the larvae fed on castor and cabbages were similar and lower than on artificial diet and cotton. The approximate food quality and detoxifying enzymes ability of *S. litura* larvae on the four foods significantly differed. Several studies on insect nutrition quality interactions have focused on the impact of diets on insect performance. Food efficiencies on different host plants vary considerably by *S. litura* larvae (Balasubramanian *et al*. 1985) and by insects in general (Scriber and Slansky. 1991). CarE activity when they fed on different host plants found the activity of CarE in larvae of *H.armigera* was inhibited by monocrotophos and quinalphos and induced by fenvalerate (Wang *et al*. 2010). However the castor fed on larvae in CarE activity was higher monocrotophos inhibit by deltamethrin.

Figure 1(A- D) represent detoxification enzyme activities of *Spodoptera litura* reared on different diet.



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ROLE OF GLUTATION-S-TRANSFERASES IN THE RESISTANCE FORMING TO INSECTICIDES OF THREE DIFFERENT CLASSES IN HOUSEFLY (*MUSCA DOMESTICA*)

ABSTRACT
The role of glutathione-S-transferases in the process of resistance forming to insecticides from three chemical classes: organophosphates, pyrethroids and chitin synthesis inhibitors in the housefly were researched in this investigation. It was revealed that an increase in activity of this enzyme during selection was in all selected strains in 1.2-1.4 times.

INTRODUCTION

Glutathione-S-transferases (GST) catalyze conjugation of glutathione with various xenobiotics, including insecticides. Then conjugates are metabolized to mercapturon acid and excreted from the insect.

Glutathione-S-transferases are involved in the detoxication of organophosphorus (OP) compounds in insects and mites (Lu et al., 2003). Distillation of diazinon and paraoxon in resistant houseflies occur only at the expense of this enzyme (Huang et al., 1998). Increased activity of the transferases had been found in resistant to azinphosmethyl resistant houseflies (Oppenoorth et al., 1977), butterflies *Epiphyas postvittana* (Suckling et al., 1990), a malathion-resistant *Nilaparvata lugens* (Liu, Han, 2004).

At the same time, high transferase activity was not a factor contributing to the resistance of flies to diazinon (Motoyama, Dautenman, 1977). Decay of malaoxon by transferases was similar in OP-resistant and susceptible strains of houseflies (Welling et al., 1983). There was no significant difference in the activity of GST in larvae and adults of the beetle *Oryzaephilus surinamensis* (Rose, Wallbank, 1986) or the mites *Tetranychus urticae* (Tsagkarakou et al., 2002).

There are data of the role of glutathione-S-transferases in the detoxification of pyrethroids. S. Nedelkina et al. (1988) showed an increase of activity of these enzymes in houseflies resistant to tetrametrin and permethrin. In the larvae of *Cydia pomonella* a positive correlation between the increase in GST activity and resistance to deltamethrin was observed (Bouvier et al., 2002). Examination of 16 populations of maize weevil beetle in Brazil by the correlation analysis revealed that the conjugation cypermethrin, deltamethrin and permethrin, carried glutathione-S-transferase, plays a role in resistance to pyrethroids in these insects (Fragoso et al., 2003).

T. Sparks and B. Hammock (1983) conducted studies of the biochemical mechanisms of resistance to chitin synthesis inhibitors (CSI) on example diflubenzuron with synergists in the housefly. The use of inhibitor transferase diethylmaleate (DEM) showed the absence of a strong synergism. This suggests that the transferases do not take a great role in diflubenzuron detoxication.

MATERIAL AND METHODS

The objects of the studies were imago of one sensitive strain and six selected strains of housefly. For selection were used following insecticides: from class OP - phosmet (phtalophos, 20% e.k.), phoxim (volaton, 50% e.k.); from class pyrethroids - deltamethrin (decis, 2.5% e.k.), fenvalerate (sumicidin, 20% e.k.), ethophenprox (trebon, 30% e.k.); from class chitin synthesis inhibitors - chlorfluazuron (eim, 12% e.k.). The determination of resistance index (RI) to selectants was conducted, as it is described earlier (Sokolyanskaya, 2007).

GST activity was determined in post-microsomal fraction by the method W. Habig (1974). The incubation medium contained 0.5 ml of 30 mM glutathione (red), 2.5 ml of 0.1 M K-phosphate buffer pH=7.5; 50 ml of homogenate and 20 ml 0.175 M substrate (1.2-dichloro-4-nitrobenzene). Measurement of activity was carried out at 37°C on a differential spectrophotometer Spekord M40, molar extinction coefficient - 8.500 $M^{-1}cm^{-1}$.

RESULTS AND DISCUSSION

From the six studied preparations, referring to three different chemical insecticide classes, resistance in the houseflies is formed quicker to pyrethroids (fenvalerate and deltamethrin) and slower to derivate of benzylphenylurea chlorfluazuron (tab. 1). The intermediate position is occupied by the pyrethroid ethophenprox and organophosphates phoxim and phosmet.

Table 1: The resistance forming in selected strains of housefly

Strain and Insecticide	Index	Generation				
		F_6	F_{12}	F_{18}	F_{24}	F_{30}
R-v, phoxim	RI	0.8	1.75	3.1	4.32	4.8
R-pht, phosmet	RI	0.9	1.88	1.83	2.1	2.07
R-d, deltame-thrin	RI	1.6	5.6	6.7	18.6	42.6
R-tr, etho-phenprox	RI	1.0	1.1	3.9	4.8	5.0
R-fv, fenvalerat	RI	2.5	21.4	24.5	31.4	32.6
R-e, chlorflu-azuron	RI	0.74	0.97	1.14	1.87	1.55

Changing of the activity of glutathione-S-transferases in selected strains of house fly is shown in Table 2. In the sixth generation of phoxim -selected strain (R-v) activity of these enzymes increases almost 2-fold and remained so until the 12th generation. Then, in the 18th generation, it decreases slightly and remains almost unchanged in the 24th generation. In the strain, selected phosmet (R-pht), in the 6th generation of a decrease in GST activity compared with the sensitive strain. To the 12th generation their activity increased slightly exceeded the level of enzyme activity in S strains, and remains the same until the 24th generation. It is obvious that in this strain transferases did not play a significant role in the mechanism of resistance. Similar results were obtained for houseflies strain Hokota resistant to diazinon (Shono, 1974). The activity of these enzymes slightly increased in resistant to phoxim *Helicoverpa armigera* (Tang et al., 2000).

These enzymes were more important in the resistance forming in housefly strain R-v: their activity was significantly higher than that of the sensitive strain throughout the selection. Many studies show the involvement of these systems in the metabolism of organophosphorus compounds. Thus, in multi-resistant flies GST activity significantly increased, detailed products of diazinon, parathion and diazoxon were found (Lewis, Sawicki, 1971). Transferases play an important role in the metabolism of azinphosmethyl (Motoyama, Dauterman, 1975), parathion and etrimphos (Oppenoorht, 1979) in houseflies. However, it is difficult to assess the contribution of glutathione-S-transferases in the mechanism of resistance to the OP, because dealcil and dearil metabolites may also be formed by oxidative or hydrolytic reactions.

Table 2: The activity of glutathione-S-transferases in selected strains of housefly (nmol/ min mg protein)

Generation	Strain R-v	Strain R-pht	Strain R-tr	Strain R-d	Strain R-fv	Strain R-e
F_0 (strain S)	28.1±3.8					
F_6	46.9±7.9	18.6±5.5	18.9±1.4	26.9±3.5	29.6±8.8	29.9±2.4
F_{12}	52.5±2.8	32.9±2.9	24.9±5.2	33.9±2.6	33.2±2.4	35.8±3.2
F_{18}	37.2±3.1	28.1±4.2	34.5±6.1	39.2±7.3	33.2±2.6	24.0±3.0
F_{24}	41.9±2.1	35.7±3.2	35.4±3.0	40.7±2.3	39.7±2.9	37.4±4.4
F_{30}	47.8±3.3	38.4±3.2	35.6±2.1	37.4±3.9	41.4±2.9	41.4±2.2

Note –in color checks difference of selected strain with sensitive is reliably, P>0.95.

The activity of glutathione-S-transferases in the strain of R-tr changed similar to that in the strain R-v. In strains, selected deltamethrin and fenvalerate, the activity of these enzymes in the 6th generation was at the level of the sensitive strain, and then it slightly increased and in the 24th generation of 1.4 times higher compared with the base strain value. There are data about the role of GST in the detoxication of pyrethroids. G. Ivanova et al. (1989) found that in houseflies resistant to tetrametrin (RI=10) and permethrin (RI=9) activity of these enzymes increased in 1.5 and 1.8 times respectively. Increased activity of these enzymes was observed in, selected by cyfluthrin, beetles *Tribolium castaneum* (Reidy et al., 1990). In resistant to deltametrin strains cutworm *Spodoptera littoralis* activity of glutathione-S-transferases was in 1.5 times higher compared with the sensitive strain (Pinchard, Vaissayre, 1994).

The activity of glutathione-S-transferases in the 6th generation strain, selected chlorfluazuron (R-e) did not change, in the 12th generation it slightly increased, in the 18th generation is reduced, and then successively increased. Our data suggests that the transferases do

not take a great role in detoxication of chitin synthesis inhibitors.

Thus, the activity of GST in the early stages of selection in all versions, except the strain selected phoxim did not differ from that of the sensitive strain. However, during further selection was an increase in activity of this enzyme in all selected strains in 1.2-1.4 times (Fig. 1). Typically, this enzyme is not the main cause of insect resistance to various insecticides (Motoyama, Dauterman, 1977; Pimprikar, Georghiou, 1979; Welling et al., 1983), and the conjugation reaction is usually treated as secondary detoxification mechanisms. On the other hand, established a significant role for GST in the mechanism of resistance in the mosquito *Anopheles gambiae* to DDT (Prapanthadara et al., 1995), the diamondback moth to methyl parathion (Huang et al., 1998), *Aedes aegipty* to permethrin and DDT (Grant, Matsumura, 1989), the diamondback moth to teflubenzuron (Ku et al., 1994).

Glutathione-S-transferases brings the contribution to resistance forming to used selectants, but as a whole plays in detoxication processes less significant role, than monooxygenases and esterases.

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Abstracts in Resistance Management

THE PROBLEM OF CROSS-RESISTANCE OF INSECTS AND MITES TO INSECTICIDES AND ACARICIDES

With intensive use of insecticides and acaricides in agriculture, the problem is the emergence of cross-resistance, that is, resistance to multiple drugs. As a rule, within the same class of insecticides, there is some degree of cross-resistance to related compounds. Depending on the mechanism of resistance cross-resistance is possible and among classes. Emphasis is made on the cross-resistance between DDT and pyrethroids, due to the gene kdr. For example, resistant to DDT cockroaches *Blatella germanica* showed a high cross-resistance to all types of pyrethroids in their topical application on the abdomen (Scott et al., 1986). In the laboratory, highly resistant to DDT (only 800) populations of houseflies showed cross-resistance to six synthetic pyrethroids (PI=75-150): resmethrin, permethrin, decamethrin, cypermethrin, fenvalerate and S-3206 (Malinowski, 1980). Houseflies resistant to resmethrin had high resistance to DDT (Funaki, Motoyama, 1986). Selection of houseflies of pyrethrin has also led to a high cross-resistance to DDT (Fine, 1961). At the same time, El-Daham and Saad (1981) not found in laboratory strain *Spodoptera littoralis*, a slightly resistant to DDT had a cross-resistance to cibolt, cypermethrin and fenvalerate.

Insects resistant to DDT, as a rule, do not show resistance to organophosphorus (OP) compounds. For example, the already mentioned strain of houseflies with the level of resistance to DDT 800x showed no cross-resistance to OP, as well as to the related γ-HCCG (Malinowski, 1980). DDT-resistant flies *Musca domestica* did not show cross-resistance to the related compounds in India (Ansari, 1976). At the same time, predatory mites *Amblyseius fallacis*, despite the use of organophosphorus insectoacaricides for 10 years, maintained a high resistance to DDT (Croft et al., 1982).

There are also conflicting reports about the presence of cross-resistance to drugs of the same or another class with respect to arthropods which are processed by OP in the laboratory or field conditions. Flies *Delia antiqua*, resistant to parathion, showed resistance to chlorpyriphos, naled and chlorfenvinphos (PI=15; 9.3 and 7.7 respectively) (Carrol et al., 1983). In Egyptian cotton leafworm *S. littoralis*, resistant to sumition (PI=12), a higher resistance to dimetoxiderivate OP (malathion, methyl parathion, dipterex, PI=8-18.9) than to dietoxiderivate (ciolan and parathion, PI=1.6-7)

were found (Klingauf et al., 1977). K. Ozaki and T. Kassai (1984) had found in leafhoppers *Nilaparvata lugens*, resistant to malathion (PI=93), more than 10-fold cross-resistance to monocrotophos, fenthion, fenitrothion, diazinon, disulfoton, fentoat, mekarbam and metomil, less than 10-fold cross-resistance to a number of other organophosphorus compounds. Leafhoppers resistant to fenitrothion (RI=289) were highly resistant to fenthion (271x), diazinon (71x), izoxation (270x), piridafention (412x). malathion (421 x), fentoat (157x). Processing of peach aphid by organophosphorus compounds lead to the development of resistance to pirimifos-methyl, malathion, geptenofosu but left effective diazinon and etafos (Zilbermints 1988). Resistant to methamidophos strain of *N. lugens* (PI = 44) had also cross-resistance to malathion and diazinon (Liu et al., 2002). Dildrin resistant houseflies from Danish farms were also resistant to dimethoate (22x), malathion (15x), trichlorfon (14x), tetrachlorvinphos (5x) (Farnham, Sawicki, 1976). Tetrachlorvinphos resistant houseflies showed cross-resistance to dichlorvos, paraoxon and diazoxon (PI=9.17) and carbamates propoxur and dimethylan (PI=9 and 7, respectively) (Iripathi, 1976). According C.R. Harris et al. (1982) houseflies of natural populations were resistant to seven organochlorine compounds, eleven of the OP, and almost all carbamates. And laboratory strains of houseflies resistant to phoxim and phosmet, showed no cross-resistance to related compounds (Amirhanov, Arzhavitina, 1990).

Quite often, arthropods affected by processing of the OP shown cross-resistance to pyrethroids. For example, resistance to organophosphorus compounds in peach aphids was higher in resistance to pyrethroids, although contact was never made with these insecticides (Zilbermints, 1988). According to T. Brown et al (1982) resistance to permethrin in *Heliotis virescens* increased parallel with an increase of resistance to methyl parathion, although to a lesser extent. In beetles, *Tribolium castaneum*, multi-resistant to organophosphorus insecticides cross-resistance to natural pyrethrins (34x) and eight synthetic pyrethroids was detected. The maximum level of cross-resistance was observed to tetramethrin - 338x, the lowest to resmethrin - 2, 2x (Cloud, Ruczkowski, 1980).

At the same time, the sawtoothed grain beetle *Oryzaephilus surinamensis*, resistant to malathion, did not have cross-resistance to permethrin (Saleem, Wilkins, 1984), and the cabbage moth *Plutella xylostella*, resistant to malathion, did not have resistance to fenvalerate (Noppun et al., 1987). Beetles of the natural population of *T. castaneum*, resistant to malathion (56x), no had cross-resistance to permethrin and cypermethrin (Saleem, Shakoori, 1989). In resistant to methamidophos strain of *N. lugens* (PI=44) cross-resistance was found to etofenproxs, but it retained sensitivity to fenvalerate (Liu et al., 2002). Resistant to malathion and fenitrothion strain of leafhoppers *Laodelphax striatellus* demonstrated no cross resistance to pyrethrin, allethrin, permethrin and fenpropathrin, but a weak resistance was noted to furamethrin, resmethrin and tetramethrin (Ozaki, Kassai, 1984). OP-resistant strains of houseflies detected low level of cross-resistance to pyrethroids too (Roslavtseva et al., 1982).

In pyrethroid-resistant strains of arthropods the presence and absence of intra- and inter-group cross-resistance can also be observed. So, the selected permethrin strain of predatory mite *A. fallacis* showed cross-resistance to a wide range of pyrethroids, a synthetic (decamethrin, cypermethrin, fenvalerate, etc.) and natural (allethrin, unpeeled pyrethrins, S-bioallethrin) (Croft et al., 1982). In selection by permethrin females mosquito *Aedes aegypti* increased resistance to other pyrethroids and DDT (Chadwick et al., 1984). Derived Shanghai strain of mosquito *Culex pipiens pallens* resistant to resmethrin also exhibited cross-resistance to natural pyrethrin and fenvalerate (PI=7.0 and 7.34, respectively) (Zhang, 1986). Another strain *C.p. pallens*, selected by deltamethrin, had cross-resistance to permethrin, resmethrin, sumithrin and DDT. At the same time cross-resistance to organophosphates - malathion and sumithion was not observed (Chen, 1990). Resistant to DDT and pyrethroids *A. aegypti* did not show cross-resistance to OP too (Chadwick et al., 1984). Selection in vitro of houseflies by deltamethrin, cypermethrin, fenvalerate and DDT has led to cross-resistance to all studied pyrethroids, natural pyrethrin, DDT and methoxychlor. In this case, cross-resistance to the five studied OP and lindane and propoxur was not observed (Malinowski, 1988). At the same time, the selection of green peach aphid by pyrethroids showed intragroup resistance (up to 400 times in ambush, decis, ripcord and sumitsidin, 6-9 times a izatrin and rovicurt), and intergroup resistance to organophosphates (PI=20-40) (Zilbermints, 1988). *P. xylostella*, resistant to pyrethroids, had cross-resistance to DDT (130x) and diazinon (15x) (Tabashnik et al., 1987). Pyrethroid-resistant houseflies also had a small cross-resistance to OP (Amirkhanov, Arzhavitina, 1990).

It was already noted the presence of cross-resistance between DDT and pyrethroids. But there are data that contradict this rule. For example, in Australia from 1974 to 1978 natural population of bollworm *H. armigera* was highly resistant to DDT (PI=500), but fully susceptible to pyrethroids. Since 1979, due to the use of pyrethroids the resistance formed to these drugs in pests. In this case, resistance to DDT significantly

decreased at first, but after 1983 had increased again (Gunning et al., 1990).

There is literature data of cross-resistance to inhibitors of chitin synthesis. So, houseflies, selected by drugs from the classes of chlorine-and phosphorus compounds have shown, when processing white predpuparium, clear cross-resistance to diflubenzuron (Cerf, Georghiou, 1974). Cross-resistance to dimilin was detected by topical treat of housefly larvae resistant to a number of OP (Rupes et al., 1977), and the introduction of dimilin in an environment where were developing larvae of flies that are resistant to OP, carbamates, lindane, DDT and cyclodien compounds, PI=3 - 10 depending on the strain of flies (Oppenoorht, Van der Pas, 1977), and resistant to OP and pyrethroids (Ivanova, 1980). Populations of moth *Cydia pomonella* in orchards in Switzerland formed a cross-resistance to diflubenzuron for several years (Charmilot, Pasquier, 2002). In France, *C. pomonella* larvae of different ages, selected on resistance to deltamethrin, showed cross-resistance to teflubenzuron, what indicates the presence of non-specific metabolic pathways of resistance (Bouvier et al., 2002).

But at the same time has not been found cross-resistance to diflubenzuron in diazinon-resistant field populations of *L. cuprina* (Hughes, Levot, 1987). W. Guyer and R. Neumann (1988) pointed the lack of cross-resistance to inhibitors of chitin synthesis chlorfluazuron and diflubenzuron in caterpillars *S. littoralis*, resistant to organophosphorus compounds, and caterpillars of the same species, insignificantly resistant to diflubenzuron, showed no cross-resistance to cypermethrin, but showed it to related chitin synthesis inhibitors on the basis of benzilfenilurea (Ahmed et al., 1987). Similar results were noted for houseflies, multiresistant to various insecticides, in relation to diflubenzuron (Keiding, 1987), as well as the Colorado potato beetle in the USA, with multiple resistance to most insecticides, which were sensitive to triflumuron (Schroder, 1991). Slight resistance to this compound (PI=5) showed caterpillars of natural populations of *S. littoralis*, who for years were treated intensive with insecticide classes of OP and pyrethroids, and have developed considerable resistance to them (PI>100) (Ishaaya, Klein, 1990). House flies, selected organophosphate (phoxim and phosmet) and pyrethroid (deltamethrin, fenvalerate, etofenprox) insecticides showed no cross-resistance to chlorfluazuron and flufenoxuron too (Sokolyanskaya, 2007). Resistant to tebufenozid strain *S. exigua* (PI = 47) also differed by lack of cross-resistance to chlorfluazuron (Huang et al., 2005).

Despite the fact that the avermectins and neonicotinoids are used relatively recently, there are instances of cross-resistance to these drugs. Reliable cross-resistance to abamectin was detected in two strains of houseflies resistant to pyrethroids. One strain was selected with permethrin in laboratory conditions had up to 6000-fold resistance. Another strain drawn from the natural population had slight resistant to permethrin - 5. Topical processing of abamectin acetone solution to 3-5-day-old females of both strains showed cross-resistance to the insecticide (Scott, 1989).
At the same time, other strains of houseflies detected no cross-resistance to abamectin. Six laboratory strains of *Musca domestica*, resistant to various insecticides were used. Capacities with adults were sprayed with abamectin and registration was carried out in 48 hours (Roush, Wright, 1986). Other member of the order Diptera – drosophila showed no cross-resistance to insecticides in this class too. Colorado potato beetle in Massachusetts, which has multiple resistances, showed no cross-resistance to abamectin (Argentine, Marshall, 1990). Populations of fruit flies *Drosophila melanogaster* and *D. simulans*, caught in different areas of North America and have varying degrees of resistance to malathion (2-118), showed no cross-resistance to avermectin (Windelspecht et al., 1998). Resistant to tebufenozid strain of *S. exigua* (PI=47) also differed by lack of cross-resistance to emamectin (Ishaaya, Klein, 1990). Resistant to butenfipronil caterpillars of *P. xylostella* showed no cross-resistance to abamectin (Niu et al., 2007).

The high level of cross-resistance to neonicotinoids was detected in the whitefly *Bemisia tabaci*, Q-type whiteflies in Spain and B-type in Italy and Spain (Nauen et al., 2002). Strong cross-resistance to neonicotinoids was established in individuals of B-type in Israel too (Roush, Nauen, 2003). Strain of peach aphid with a 14-fold resistance to imidacloprid showed low, but multiple resistances to pyrethroids and organophosphates: lambda-cyhalothrin (PI=12), deltamethrin (PI=10); ometoat (PI=8), phoxim (PI=5), methamidophos (PI=5) (Chen et al., 2005). In strain *N. lugens*, resistant to methamidophos, (PI=44) cross-resistance to imidacloprid was found (Liu et al., 2002). Strain of aphid *A.gussypii*, resistant to fenvalerate, showed no cross-resistance to imidacloprid, at the same time strain resistant to imidacloprid, was highly resistant to fenvalerate (PI=109) and slightly resistant to methomyl, endosulfan and ometoat (PI=3.8) when powered by cotton. On cucumbers all indicators of cross-resistance was less - to fenvaleoat PI=33.5; to methomyl - 7, ometoat - 3, cross-resistance to endosulfan was absent (Wang et al., 2001).

In addition to the phenomenon of cross-resistance also the presence of negative cross-resistance is noted, which is reflected in the fact that the strains resistant to

one class of drugs have a greater sensitivity to drugs of another class. G. Georhiou et al. (1983) noted the presence of a negative cross-resistance of OP-resistant mosquitoes to permethrin. Activity of fenvalerate increased with increasing of levels of resistance to malathion in leafhoppers *N. lugens, L. striatellus, Nephotettis cincticeps* (Ozaki, Kassai, 1984). Negative resistance was found in houseflies resistant to permethrin in relation to OP (Li et al., 1983), as well as in the strains houseflies resistant to organophosphates and pyrethroids, in relation to chlorfluazuron (Amirkhanov, Arzhavitina, 1990). Triazophos was more toxic to pyrethroid-resistant larvae *H, armigera*, than for the sensitive caterpillars (Martin et al., 2003).

Thus, data on intra- and inter-group cross-resistance are very contradictory and always take extra special study. Apparently, the mechanism of resistance and cross-resistance depends on the chemical structure of the insecticide, rather, on the particular mechanism of action and detoxification of insecticides due to their chemical structure and the type of insect.

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