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DEVELOPMENT OF RESISTANCE IN THE SPINY BOLLWORM, *EARIAS INSULANA* (BOISD.) TO LAMBDA-CYHALOTHRIN UNDER LABORATORY CONDITIONS

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ABSTRACT

Selection pressure using the pyrethroid insecticide lambda-cyhalothrin was carried out under laboratory conditions against a field strain of the spiny bollworm, *Earias insulana* (Boisd.) collected from cotton fields at Sharkiya Governorate, Egypt, during 2008.The obtained results indicated that the resistance level reached to ca. 97.17-fold in the eleventh generation compared with the unselected strain. In addition, the obtained lambda-cyhalothrin resistant strain exhibited cross-resistance against deltamethrin and methomyl recording ca. 26 and 14-fold, respectively. Slight cross-resistance was found with profenofos, chlorpyrifos, the urea derivative teflubenzuron, the neonicotinoid thiamethoxam and the bioinsecticide *Bacillus thuringiensis* Subspecies *aizawai.* These data may emphasize the possibility of lambda-cyhalothrin rotating with the other groups of insecticides which have no cross-resistance for the control program of the spiny bollworm, *E. insulana* to manage, however, resistance for lambda-cyhalothrin.

Keywords: *Earias insulana,* lambda-cyhalothrin, resistance, cross resistance, insecticide selection.

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INTRODUCTION

The spiny bollworm, *Earias insulana* (Boisd.) is considered one of the most serious pests attacking cotton plants in Egypt and in the most cotton producing countries causing a great damage in the quantity and quality of cotton yield. In the early 1980's pyrethroid insecticides were rapidly substituted for organochlorine and organophosphorus insecticides for the control of cotton pests due to their wide spectrum, low dosage, high killing efficiency and low residues. Unfortunately, resistance of the bollworm to conventional insecticides was developed due to miss use and wide applications (Martinez-Carrillo and Reynolds, 1983; Saini *et al.*, 1988; Hirano *et al.*, 1993; Al-Beltagy *et al.*, 2001; Kranthi *et al.*, 2001 and Suryawanshi and Bhede, 2009). Since resistance of cotton pests to the conventional insecticides took place in a large scale. Therefore, the present investigation aimed to study the development rate of resistance in *E. insulana* to the pyrethroid insecticide, lambda-cyhalothrin as well as cross-resistance to other used insecticides belong to different chemical groups.

MATERIALS AND METHODS

Full grown larvae and pupae of the spiny bollworm, *E. insulana* were collected from the infested cotton plants cultivated at different localities of Sharkiya Governorate of the growing season 2008. Larvae were reared separately on immature cotton seeds till pupation. The newly emerged moths were sexed and gathered in pairs (male and female), each 5-10 pairs were confined in a glass chimney cage for mating and egg deposition. The newly hatched larvae were transferred individually to a semi artificial diet as mentioned by Rashad and Ammar (1984).

Tested Insecticides

1. Lambda-cyhalothrin (kindo 5% EC) supplied by Syngenta Agro Egypt.
2. Deltamethrin (Decis 2.5% EC) supplied by Cairo Chemical Company.
3. Methomyl (Nudrin 90% SP) supplied by Shoura Chemicals Egypt.
4. Profenofos (Cord 72% EC) supplied by El Help Egypt for trade & Agricultural Projects Development.

5. Chlorpyrifos (Pestban 48% EC) supplied by the National Company for Agrochemicals, Agrochem.
6. Teflubenzuron (Nomolt 15% SC) supplied by Shoura Chemicals Egypt.
7. Buprofezin (Applaud 20% SC) supplied by Shoura Chemicals Egypt.
8. Thiamethoxam (Actara 25%WG) supplied by Syngenta Agro Egypt.
9. *Bacillus thuringiensis* Subsp. *aizawai* (Xentari 10.3% WDG) supplied by May Trade Company.

Diet Surface Treatment

A wide range of concentrations of the tested commercial insecticide was prepared in water and used against the first instar larvae of the field population reared in the laboratory to determine the median lethal concentration (LC_{50}). One ml of each prepared concentration was sprayed on ca. 10g of fresh diet poured into glass Petri dish (8 cm diameter) and the treated surfaces were left to dry. Three batches of thirty newly hatched larvae were starved for one hr and transferred gently to Petri dishes using a soft hair brush. Similar three batches of larvae were transferred to other Petri dishes sprayed by distilled water only to be used as a control treatment. The dishes were covered with tissue paper then further covered with their covers and maintained in an incubator adjusted at a temperature of $27\underline{+}\ 2^{\circ}C$ and 65±10% R.H. (Zaki, 2006). Three replicates were used for each concentration as well as for the control.

After one hour of exposing the first instar larvae to the insecticide-treated diet or to the untreated one, the larvae of each replicate were transferred individually into clean and sterile glass tubes (2x7cm). These tubes contained a small piece (about 2 g) of the untreated artificial diet (for each tube), covered with cotton piece and kept under the previous constant conditions. Twenty-four hrs later all tubes were inspected for mortality. Mortality percentages were corrected according to Abbott's formula (Abbott, 1925). LC-p lines were drown according to EPA analysis program 1.3, according to Finney (1972).

Selection Pressure

Newly hatched larvae, obtained from the field populations and reared for three generations in the laboratory, were divided into two groups of about 1000 larvae each. One of the two groups was exposed to selection pressure with lambda-cyhalothrin for 11 generations (selected strain). Selection was carried out with all generations until F_{11} except F_5 and F_{10} (Unselected generations). The other group was reared in the laboratory away of any insecticidal contamination under the same conditions and for the same period to be used as unselected strain. The concentration used for selection was chosen to kill 40 – 60 % of the tested larvae of any generation subjected to the insecticidal selection using the same previous technique of bioassay. The survived larvae were maintained until pupation and adult emergence of the next generation. Selection was thus continued up to the eleventh generation. Concentrations used for selection were increased from a generation to another during the selection period to attain the percentage of mortality 40-60% of the treated larvae.

Cross-Resistance

Eleventh generation larvae of the lambda-cyhalothrin resistant strain as well as the susceptible one were exposed to seven insecticides belonging to different groups of chemicals namely, deltamethrin, methomyl, profenofos, chlorpyrifos, teflubenzuron, thiamethoxam and the bioinsecticide, *Bacillus thuringiensis*, Subspecies *aizawai* (Xentari). The newly hatched larvae of F_{11} of both strains were exposed to serial concentrations of each of the preceding, as mentioned before. In case of bioinsecticide, newly hatched larvae were exposed to artificial diet free of sorbic acid, formaldehyde and methyl-p-hydroxy benzoate which have antibacterial effect. In case of *B. thuringiensis,* and teflubenzuron, the holding period was extended for other five days after which the cumulative mortality was recorded. The acute toxicity of the used insecticide (expressed as mortality percentages) was assessed. The corrected mortality percentages were statistically analyzed to find LC_{50} values. The resistance ratio of the tested insecticides (LC_{50} of R-strain / LC_{50} of S-strain) was estimated according to Wu *et al.* (1994).

RESULTS AND DISCUSSION

Efficacy of Lambda-Cyhalothrin to the First Instar Larvae

The susceptibility of newly hatched larvae of F_1 and F_3 of *Earias insulana* field population to lambda-cyhalothrin is shown in Fig. 1. It is obvious that larval susceptibility increased (ca. 2-fold) during rearing in the laboratory for three generations;

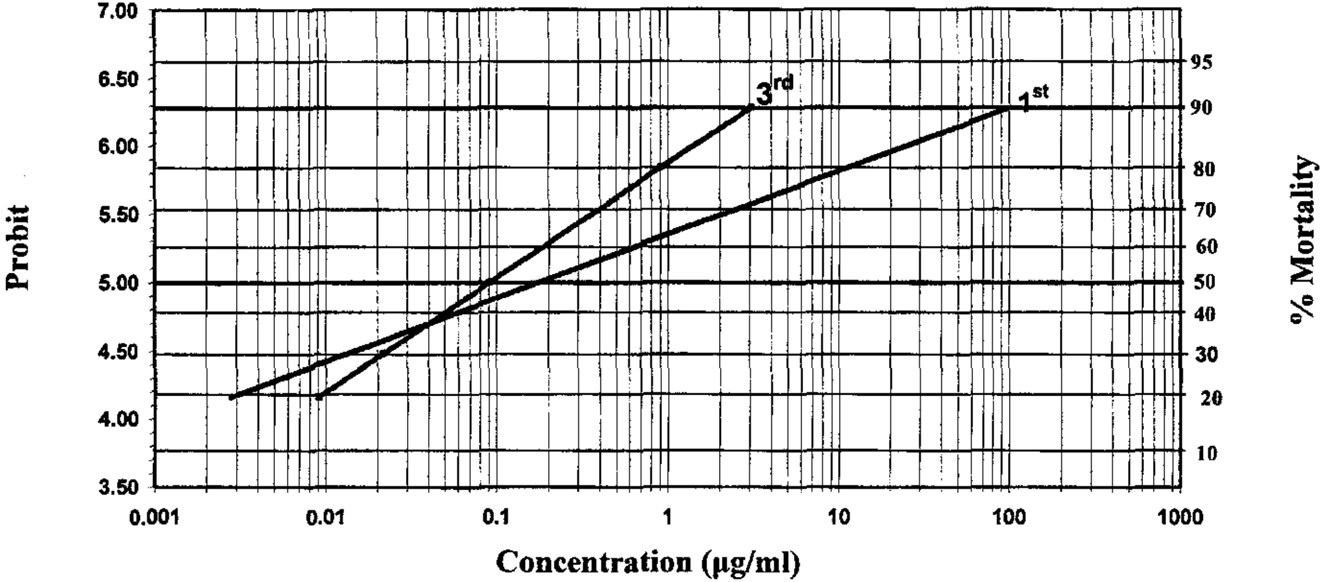

Fig. 1. Toxicity lines of lambda-cyhalothrin tested against the first instar larvae of first and third generations of *Earias insulana* field strain

LC_{50} values decreased from 0.178 µg/ml in F_1 to 0.091 µg/ml in F_3. This may be due to elimination of some tolerant individuals. LC_{90} values were sharply decreased from 97.167 µg/ml in F_1 to 3.919 µg/ml in F_3 indicating a pronounced increase in homogeneity of the population. Increasing the slope values from 0.468 for the toxicity line of F_1 to 0.833 for the toxicity line of F_3 generation may elucidate this phenomenon.

Resistance Development

Data in Table 1 show that the development of resistance in *E. insulana* to Lambda-cyhalothrin was slow during the first four generations recording resistance ratio of 2.822-fold at LC_{50} level and 1.840-fold at LC_{90} level for the fourth generation. The fifth generation was left without insecticidal selection to maintain the vigorous of the strain; the obtained individuals were slightly decreased. The rate of resistance build up to lambda-cyhalothrin in the selected strain was clearly increased during the next four generations (F_6-F_9) recording 20.06-fold in F6. Abrupt increase took place at the low level of toxicity (LC_{50}) in F_9 when the resistance level was measured (63.244-fold).

To continue maintaining vigorous in the selected individuals, exposure to lambda-cyhalothrin was avoided during the tenth generation. A sharp increase of resistance took place after this relaxation period, recording 97.17-fold during the eleventh generation, at the LC_{50} level. The corresponding levels of resistance ratio at the high level were lower than those at the low level.

Slope values of toxicity lines for lambda-cyhalothrin selected strain were relatively high in the first two generations (2.22 and 2.25, respectively) then decreased gradually to reach 1 only in F_7 and increased again to reach 2.24 in F_{11} (Table 1). Such irregularity pattern of slope values of the selected may be due to changing of heterogeneity of the individuals during the selection period.

Data presented in Table 1 show that the susceptibility of the unselected strain to lambda-cyhalothrin was slightly increased during its rearing in the laboratory; LC_{50} of the F_1 was 0.078 then decreased gradually to reach 0.036 in F_6 and then increased again to record 0.060 µg/ml in F_{11}. The corresponding LC_{90} values were 0.295, 0.261 and 0.318 µg/ml in

Table 1. Development of resistance in *Earias insulana* larvae to lambda-cyhalothrin during selection for 11 generations

Generations	Selected strain		slope	Unselected strain		slope	Resistance ratio (RR) at	
	LC_{50} (µg/ml)	LC_{90} (µg/ml)		LC_{50} (µg/ml)	LC_{90} (µg/ml)		LC_{50}	LC_{90}
F1	0.078	0.295	2.224	0.078	0.295	2.224	1.000	1.000
F2	0.099	0.368	2.247	0.050	0.290	1.68	1.980	1.269
F3	0.116	0.509	1.995	-	-	-	-	-
F4	0.127	0.611	1.877	0.045	0.332	1.469	2.822	1.840
F5	**Unselected generation**							
F6	0.722	5.015	1.522	0.036	0.261	1.484	20.060	19.215
F7	0.668	12.781	1.000	0.045	0.307	1.532	14.844	41.652
F8	1.353	8.241	1.633	-	-	-	-	-
F9	2.593	15.760	1.635	0.041	0.288	1.52	63.244	54.722
F10	**Unselected generation**							
F11	5.830	21.640	2.240	0.060	0.318	1.761	97.17	68.05

F_1, F_6 and F_{11}, respectively. Slope values of the reference population toxicity lines were slightly decreased from 2.224 in F_1 to 1.761 for F_{11} indicating a degree of heterogeneity.

Resistance against the pyrethroid insecticides in *Earias* spp. obviously cleared in the second half of the eighties, since Saini *et al.* (1988) studied the development of resistance to fenvalerate and cypermethrin in the first instar larvae of *E. vittella* (Fab.) for 15 successive generations and found that the noctuid developed 7.8-fold resistance to fenvalerate and no resistance to cypermethrin. Also, Hirano *et al.* (1993) reported that *Pectinophora gossypiella* (Saund.) exhibited more resistance than *E. insulana* to fenvalerate and fenpropathrin. While, Kranthi and Kherde (1998) reported that *P. gossypiella* had a very high level of resistance to cypermethrin, similarly *E. vittella* and *Helicoverpa armigera* (Hub.). Al-Beltagy *et al.* (2001) mentioned that both *P. gossypiella* and *E. insulana* exhibited high levels of resistance to the pyrethroid fenvalerate (34.84-fold) in pink bollworm, but the spiny bollworm developed a tolerance of 3.31-fold only. Suryawanshi *et al.* (2008) studied resistance of *H. armigera* to the synthetic pyrethroid cypermethrin and found that the insect become more resistant (279.80-fold). Suryawanshi and Bhede (2009) cleared that *H. armigera* had developed a high level of resistance to all the tested pyrethroids especially to cypermethrin.

The present results indicated that the development of resistance in *E. insulana* to lambda-cyhalothrin was weak and slow during the first four generations, then the resistance build up was clearly increased during the next four generations (F_6-F_9) to reach 63.244-fold in F9. A sharp increase of resistance level was noticed in F_{11} representing with 97.17-fold when compared with the unselected strain.

Cross-Resistance of Lambda-Cyhalothrin Resistant Strain to other Insecticides

The first instar larvae of the eleventh generation of the selected and unselected population were exposed to eight insecticides; namely lambda-cyhalothrin, deltamethrin, profenofos, chlorpyrifos, methomyl, teflubenzuron, thiamethoxam and *Bacillus thuringiensis*. Data in Table 2 show that the selected strain larvae were highly sensitive to the pyrethroid insecticide deltamethrin and highly tolerant to the carbamate insecticide methomyl and the organophosphorus insecticide profenofos. The LC_{50} values of deltamethrin, methomyl and profenofos of selected strain were 1.369, 19.06 and 17.642 µg/ml, respectively. Concerning the slope values of the toxicity lines for the selected strain, deltamethrin showed the steepest toxicity line (1.916), while teflubenzuron had the flattest toxicity line (0.832).

Strange to note that cross resistance data showed that the lambda-cyhalothrin resistant strain exhibited the highest level of cross resistance (ca. 26-fold only) to deltamethrin compound which includes the α-cyano-3-phenoxybezyl moiety which is also included in lambda-cyhalothrin. It is known that both compounds are included in category II of pyrethroids. Also, wide range of cross resistance took place with the other three insecticides methomyl, chlorpyrifos and profenofos which are categorized under the anticholinesterase inhibitors; the corresponding cross resistance ratio were 13.792, 3.919, and 0.866 indicating a relatively low

Table 2. Cross resistance of F_{11} *Earias insulana* lambda-cyhalothrin resistant strain to different insecticides

Tested insecticides	Unselected strain				Selected strain F_{11}				Cross resistance ratio
	LC$_{50}$ (µg/ml)	Confidence limits		Slope	LC$_{50}$ (µg/ml)	Confidence limits		Slope	
		Lower	Upper			Lower	Upper		
lambada-cyhalothrin	0.060	0.046	0.084	1.762	5.829	4.670	7.159	2.240	97.170
deltamethrin	0.053	0.000	0.074	1.389	1.369	1.034	1.732	1.916	25.830
profenofos	20.363	16.396	24.759	2.345	17.642	7.426	25.032	1.774	0.866
chlorpyrifos	2.589	1.878	3.210	2.122	10.147	6.381	13.823	1.497	3.919
methomyl	1.382	1.111	1.648	2.600	19.060	10.914	31.752	0.901	13.792
teflubbenzuron	1.276	0.889	1.857	1.302	3.973	2.098	6.934	0.832	3.114
thiamethoxam	2.123	0.938	3.536	0.969	6.417	3.969	10.135	1.025	3.023
B. thuringiensis* Subsp. *aizawai	1.953	1.287	2.743	1.358	9.333	5.096	13.848	1.258	4.779

level of cross resistance, respectively. The selected strain displayed, however, a low level of cross resistance to the chitin synthesis inhibitor teflubenzuron (ca. 3-fold) and the neonicotinoid thiamethoxam (ca. 3-fold). It is known that the targets of these two compounds are completely different, being chitin polymerization and disturbance in opening and closing of myofibril gates. Also, a low level of cross resistance was noticed for the bioinsecticide *B. thuringiensis* (ca. 5-fold).

It could be concluded generally that lambda-cyhalothrin resistant strain of *E. insulana* with 97.170-fold of resistance exhibited a low level of cross resistance against two of the tested compounds namely; deltamethrin and methomyl being 25.830 and 13.792-fold, respectively, but slight or no cross-resistance was found against the other tested compounds.

The obtained results are, however, in agreement with that obtained by Leonard *et al.* (1988) who showed that fenvalerate-resistant strain of the noctuid *H. virescens* exhibited a broad spectrum of cross-resistance against different insecticides, namely, permethrin (16.0-fold), cypermethrin (9.4-fold), fluvalinate (15.5-fold), bifenthrin (11.9-fold), esfenvalerate (10.4-fold), deltamethrin (6.7-fold), lambda-cyhalothrin was consistently low (1- to 2-fold and (parathion-methyl and profenofos exhibited 2.0 to 15.8-fold), respectively. Parathion-methyl resistance was correlated with increasing levels of fenvalerate resistance. Wu *et al.* (1994) found that no cross-resistance was detected to deltamethrin (5.2-fold), cypermethrin (2.5-fold), cyhalothrin (0.66-fold), permethrin (0.87-fold), methomyl (0.74-fold), and monocrotophos (1.5-fold) in a fenvalerate resistant strain of *H. armigera.* Tang (1992) Studied insecticide resistance in *H. armigera* and suggested that the use of pyrethroids against this pest should be stopped and rotated with insect growth regulators and *Bacillus thuringiensis*.

Ramasubramanian and Regupathy (2004) showed that *H. armigera* populations selected for resistance to one pyrethroid was positive cross-resistance to all other pyrethroids, but no cross-resistance to endosulfan and thiodicarb. YiHua *et al.* (2008) showed that the selected field strain of *H. armigera* with a mixture of phoxim and cyhalothrin for 33 generations developed 17.4-

fold resistance to phoxim and 144.7-fold resistance to cyhalothrin and high cross-resistance to two other pyrethroids (deltamethrin 86.2-fold and fenvalerate 23.4-fold).

These data may be emphasizing the possibility of rotating lambda-cyhalothrin with the other tested insecticides which had no cross-resistance such as chlorpyrifos, profenofos, teflubenzuron, thiamethoxam as well as the bioinsecticide *B. thuringiensis* to manage resistance of *E. insulana* to lambda-cyhalothrin.

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تطور المقاومةِ في دودة اللوز الشوكية إرياس إنسيولانا بويسد لمبيد لمبدا-سيهالوثرين تحت الظروف المعملية

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أُجرى ضغط انتخابى بمبيد لمبدا-سيهالوثرين تحت الظروف المعملية على سلالة حقلية من دودة اللوز الشوكية، التى جمعت من حقول القطن فى محافظة الشرقية، مصر، موسم ٢٠٠٨. أشارت النتائج المتحصل عليها إلى أن مستوى المقاومة وصل إلى حوالى ٩٧,١٧ – ضعف فى الجيل الحادى عشر مقارنة بالسلالة الغير منتخبة المرباه فى المعمل تحت نفس الظروف.بالإضافة إلى ذلك تبين أن السلالة المقاومة لمبيد لمبدا-سيهالوثرين إكتسبت مستويات مختلفة من المقاومة المشتركة ضد مبيدى الدلتاميثرين والميثوميل مسجلة حوالى ٢٦ و١٤- ضعف على التوالى. أظهرت السلالة المنتخبة مقاومة مشتركة ضعيفة ضد مبيدات البروفينوفوس، الكلوربيروفوس، تيفلوبنزيورون، ثياميثوكسام والمبيد الحيوى باسيلس ثيورينجنسيس. تفيد هذه النتائج فى إمكانية إستبدال مبيد لمبداسيهالوثرين مع المجموعات الأخرى من المبيدات التى لا يوجد بينها مقاومة مشتركة فى برنامج مكافحة دودة اللوز الشوكية، والتى أظهرت مقاومة لمبيد لمبدا-سيهالوثرين.