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INFLUENCE OF OVERWINTERING LARVAL DIAPAUSE ON SUSCEPTIBILITY OF *Pectinophora gossypiella* (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE) TO SEVERAL INSECTICIDES

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ABSTRACT

To investigate the effect of overwintering larval diapause on the susceptibility of pink bollworm, *Pectinophora gossypiella* (Saunders) to various insecticidal classes (lambda-cyhalothrin, chlorpyrifos, methomyl, emamectin benzoate and spinetoram), laboratory bioassay tests were achieved on diapaused, rosetted and susceptible larval strains. The two field strains were collected from the infested cotton plants cultivated at different localities of Sharkiya Governorate during 2012 and 2013 seasons. The results indicated that the susceptibility ratios of the rosetted cotton flowers strain/ the diapaused strain (R/D) were varied between the two field strains of *P. gossypiella*, and among the insecticides tested as well, they ranged between 0.58 fold in case of spinetoram and 7.47 fold in case of lambda-cyhalothrin. The data also revealed that the newly hatched larvae descended from rosetted cotton flowers were more resistant to methomyl (121.52 fold) and lambda-cyhalothrin (43.57 fold) than the newly hatched larvae descended from overwintering diapause. The two field strains showed an equal susceptibility ratio in case of chlorpyrifos(28.53 and 28.81). The rosetted larvae were more susceptible to spinetoram than the diapaused larvae. While diapaused strain was more susceptible to emamectin benzoate than other two strains.

Keywords: overwintering diapause; *Pectinophora gossypiella*; susceptibility; insecticides

INTRODUCTION

The pink bollworm *Pectinophora gossypiella* (Saunders) is one of the most serious pests of cotton in much of the tropics and subtropics. It is the key pest in cotton fields, particularly in middle and late season (Ingram, 1994; Korejo *et al*., 2000; Unlu, 2004 and Abd El-Mageed *et al*., 2007). Of the three to five generations produced in a year, the first feeds mainly in squares and flowers; later generations feed in bolls. Characteristic rosetting of blooms occurs when the larvae spin together developing flower petals. Pink bollworm overwinters as a fully developed larva, during this period the pink bollworm is in a state of arrested development called diapause. Overwintering larval diapause is starting in mid-September, pupate in late winter and spring, and produce adults, which emerge over an extended period of time. Those adults that emerge when fruiting cotton is available are the ones that initiate the new year's infestations. Most overwintering occurs in the cotton field, although some may occur wherever cotton debris is deposited. Once diapause is completed, the larva begins to respond to temperature and moisture conditions and ultimately pupates. Adults emerge from the pupae move about searching for cotton. It is capable of traveling long distances in order to reach susceptible cotton. Mating occurs, and a gravid female must lie perish. The laid eggs hatched to newly hatched larvae pentrated the susceptibal squares and fed inside caused the rosetted flowers. The newly hatched larvae are exposed to insecticides for a very short time before they enter flower buds or bolls while fully grown larvae emerging from bolls for pupation are difficult to control with chemicals (Noble, 1969; Rashad *et al.*, 1993; Henneberry and Naranjo, 1998; Attique *et al.*, 2001; Carriere *et al.*, 2001; El-Sayed *et al.*, 2008). Many researchers studied the effect of various insecticides on the pink bollworm (Yang *et al.,* 2000; Zidan *et al.,* 2012; Sabry *et al*., 2014). Components of the population of the pink bollworm of *P. gossypiella* which attack the cotton fields are mainly from the individuals overwintering as diapaused larvae. The emerged moths deposit their eggs on the bud squares, consequently, the eggs hatch to larvae which enter and develop in this host until the full grown larvae that appear in rosetted flowers.

The objective of this study was to evaluate the susceptibility of the newly hatched larvae of *P. gossypiella* descended from overwintering diapausing larvae and that developed in the cotton square buds against the different chemical classes of insecticides, pyrethroids, organophosphates, carbamates, avermectins and spiynosins.

MATERIALS AND METHODS

Insect:

Susceptible strain: The susceptible strain of pink bollworm, *P. gossypiella* was obtained from Bollworm Research Department, Plant Protection Institute, Sharkiya Branch, Agriculture Research Center (ARC). This strain reared for more than ten years without any exposure to pesticides.

Field strains: The two field strains of pink bollworm*, P. gossypiella*, were collected from the infested cotton plants cultivated at different localities of Sharkiya Governorate during 2012 and 2013 seasons. The first, diapaused strain that descended from diapaused larvae were collected from dried cotton bolls in cotton stalks after 2012 cotton season. The larvae, which developed to diapause, were individually kept in glass tubes (2X7 cm), closed with a piece of absorbent cotton wool and left under the natural conditions of the laboratory until pupation and adult emergence. The tubes were examined every two days starting from January to collect the emerged moths. The second, rosetted flowers strain, descended from the larvae in rosetted flowers, were collected from the infested cotton plants, flowers through the end of June and the beginning of July of the 2013 cotton season. The rosetted flowers were kept in glass jars (3kg) covered with muslin cloth by rubber band; each jar contained about 50 rosetted flowers. The jars were left under the natural conditions of the laboratory. Jars were examined weekly and the pink bollworm pupae were separated individually in glass tubes until moth's emergence. 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 (1985).

Insecticides:

Insecticides used belong to different groups, pyrethroids, organophosphates, carbamates, avermectins and spiynosins.

1. Lambda-cyhalothrin, [mixture of (S)-α-cyano-3-phenoxybenzyl-(Z)-(1R,3R)-3-(2- chloro-3,3,3-trifluoroprop-1-enyl) -2,2-dimethylcyclopropane carboxylate and(R)-α-cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2- chloro-3,3,3-trifluoroprop-1-enyl) -2,2-dimethylcyclopropane carboxylate (IUPAC)] (lambda star 5% EC) supplied by starchem company, pyrethroids group.

2. Chlorpyrifos, [O,Odiethyl O3,5,6trichloro2pyridyl phosphorothioate (IUPAC)] (Pestban 48% EC) supplied by the National Company for Agrochemicals, Agrochem, organophosphates group.

3. Methomyl, [S-methyl N- (methylcarbamoyloxy)thioacetimidate (IUPAC)] (Neomyl 90% SP) supplied by Kafr El Zayat Pesticides and Chemicals, carbamates group.

4.Emamectin benzoate, [A mixture containing 90% of (10 *E* ,14 *E* ,16 *E* ,22 *Z*) - (1 *R* ,4 *S* ,5′ *S* ,6 *S* ,6′ *R*,8 *R* ,12 *S*, 13 *S* ,20 *R* ,21 *R*, 24 *S*)-6′- [(*S*)- *sec* - butyl]-21,24- dihydroxy - 5′,11,13,22- tetramethyl – 2 – oxo -3,7,19- trioxatetracyclo [15.6.1 .1 4,8 .0 20,24] pentacosa- 10,14,16,22- tetraene – 6 – spiro - 2′ - (5′,6′-dihydro-2′ *H* -pyran) -12- yl 2,6 - dideoxy-3- *O* - methyl- 4- *O* - (2,4,6- trideoxy-3- *O* – methyl - 4 - methylamino - a- L- *lyxo* - hexopyranosyl) - a - L- *arabino* -hexopyranoside and 10% of (10 *E* ,14*E* ,16 *E* ,22 *Z*)- (1 *R* ,4 *S* ,5′ *S,* 6 *S* ,6′ *R* ,8 *R* ,12 *S* ,13 *S* ,20 *R* ,21 *R* ,24 *S*) - 21, 24 dihydroxy - 6′- isopropyl - 5′,11,13,22- tetramethyl-2-oxo-3,7,19- trioxatetracyclo [15.6.1.1 4,8 .0 20,24] pentacosa-10,14,16,22-tetraene-6- spiro-2′-(5′,6′-dihydro-2′ *H* -pyran)-12-yl 2,6-dideoxy-3- *O* - methyl-4- *O* -(2,4,6- trideoxy-3- *O* –methyl – 4 - methylamino – a - L- *lyxo* - hexopyranosyl) – a - L- *arabino* -hexopyranoside (IUPAC)] (proclaim 5%) supplied by Syngenta Agro Egypt, avermectin group.

5. Spinetoram, [Major component (XDE-175-J) (2R,3aR,5aR, 5bS,9S ,13S ,14R,16aS, 16bR)-2-(6-deoxy-3-Oethyl-2,4-di-O-methyl-α-L-mannopyranosy-loxy)-13- [(2R,5S,6R)-5-(dimethylamino) tetrahydro-6-methylpyran-2- yloxy]-9-ethyl-2,3,3a,4,5,5a,5b,6,9,10,11,12,13,14,16a,16b hexadecahydro-14-methyl-1H-as-indaceno[3,2- d]oxacyclododecine-7,15-dione IUPAC: Minor component (XDE-175-L) (2R,3aR,5aS,5bS,9S,13S,14R,16aS, 16bS)-2-(6-deoxy-3-Oethyl-2,4-di-O-methyl-α-L-mannopyranosyloxy)-13- [(2R,5S,6R)-5-(dimethylamino) tetrahydro – 6 – methylpyran – 2 - yloxy] – 9 – ethyl - 2,3,3a,5a,5b,6,9,10,11,12,13,14, 16a ,16btetradecahydro-4,14-dimethyl-1H-as-indaceno[3,2- d]oxacyclododecine-7,15-dione (IUPAC)] (Radiant 12% SC) supplied by Dow AgroSciences, spinosin.

Bioassay tests:

Evaluation of the susceptibility for the susceptible and field strains against the previous insecticides as follows: Diet surface treatment, a wide range of concentrations of the tested insecticides was prepared in water and used against the newly hatched larvae of *P. gossypiella* from the different colonies. One ml of each prepared concentration was sprayed on ca. 10g of fresh diet poured into a 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 hour and transferred gently to Petri dishes using a soft hair brush. Similar three batches of larvae were transferred to other Petri dishes sprayed by 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 in a temperature of 27± 1°C and 65±5% 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 hours later all tubes were inspected for mortality.

Data analysis:

The dosage mortality response was determined by probit analysis (Finney 1971) using a computer program of Noack and Reichmuth (1978). Toxicity index according to Sun's equation of 1950 as follows:

Toxicity index = LC_{50} of the compound A/ LC_{50} of the compound B X 100

Where A: is the most effective compound

B: is the other tested compound

The susceptibility ratio was calculated from the following equation (Sabry and Abdel-Aziz 2013):

Susceptibility ratio (SR) = LC_{50} of the field strain/ LC_{50} of the susceptible strain.

RESULTS And DISCUSSIONS

Data presented in Table (1) showed that the toxicity of lambda-cyhalothrin (pyrethroids) was the most potent on the susceptible strain of *P. gossypiella* with LC_{50} 0.03 µg/ml on the newly hatched larvae. The least effectiveness compound was chlorpyrifos (organophosphates) with 0.91 µg/ml as LC_{50} values and its toxicity index was 3.65%. The toxicity index values ranged between 13.43% for methomyl to 20% for spinetoram. As for the slope values of the toxicity lines ranged between 0.96 to 1.29.

Table (1): Toxicity of the newly hatched larvae of *P. gossypiella* (the susceptible larvae) to different tested insecticides

Insecticide	LC_{50} (µg/ml)	Toxicity index (%)	Confidence Limits	Slope
Lambda-cyhalothrin	0.03	100.00	0.01-0.07	1.13
Chlorpyrifos	0.91	3.29	0.50-1.59	1.29
Methomyl	0.23	13.43	0.10-0.49	0.96
Emamectin benzoate	0.21	14.28	0.10-0.72	1.06
Spinetoram	0.15	20	0.067-0.35	0.96

Table (2) revealed that the toxicity of the a forementioned insecticides against the newly hatched larvae of the diapaused strain of *P. gossypiella*, the LC_{50} values ranged between 0.16 µg/ml for 25.92 µg/ml for emamectin benzoate and Chlorpyrifos, respectively . In addition to the toxicity index showed that the emamectin benzoate followed by lambda-cyhalothrin were most potent with 100 and 84.21 %, respectively. Chlorpyrifos was the lowest efficacy. Regarding the slope values of the toxicity lines, they ranged between 0.83 to 1.63.

Table (2): Toxicity of the newly hatched larvae of *P. gossypiella* (the diapaused larvae) to different tested insecticides

Insecticide	LC_{50} (µg/ml)	Toxicity index (%)	Confidence Limits	Slope
Lambda-cyhalothrin	0.19	84.21	0.09-0.31	1.39
Chlorpyrifos	25.92	0.62	9.06-55.07	0.83
Methomyl	10.36	1.54	4.16-19.43	1.11
Emamectin benzoate	0.16	100.00	0.04-0.29	1.04
Spinetoram	0.74	21.62	0.45-1.13	1.63

In case of rosetted cotton flowers strain of *P. gossypiella*, Table (3) displayed that the toxicity of the tested insecticides against the newly hatched larvae. The LC_{50} values ranged between 0.35 / 27.82 µg/ml. The toxicity index indicated that the emamectin benzoate insecticide was the most potent, followed by spinetoram (100 and 81.4%) compared with methomyl (1.26%) was the lowest one. The least one was the lambda-cyhalothrin (24.13%). With regard to the slope values of the toxicity lines, they ranged between 1.71/ 3.33 for emamectin benzoate and spinetoram. It was clear that the populations of the rosetted cotton flower's strain were heterogeneous for their sensitivity to emamectin benzoate, compared with the other tested insecticides.

Table (3): Toxicity of the newly hatched larvae of *P. gossypiella* (the rosetted flower larvae) to different tested insecticides

Insecticide	LC_{50} (µg/ml)	Toxicity index (%)	Confidence Limits	Slope
Lambda-cyhalothrin	1.45	24.13	1.16-1.81	2.72
Chlorpyrifos	26.17	1.33	19.7-32.97	2.64
Methomyl	27.82	1.26	22.31-33.76	3.12
Emamectin benzoate	0.35	100.00	0.21-0.51	1.71
Spinetoram	0.43	81.4	0.33-0.52	3.33

A great variation was found in the susceptibility ratios among different strains of *P. gossypiella*, and among the insecticides tested as well (Table 4). It was much cleared that the susceptibility ratios of the diapaused strain/ the susceptible strain (D/S) ranged between 0.76 fold with emamectin benzoate to 45.25 fold with methomyl. The diapaused larval strain showed the highest resistance to methomyl and chlorpyrifos with 45.25 and 28.53 fold, respectively. Whereas, it was highly susceptible to emamectin benzoate, spinetoram and lambda-cyhalothrin with 0.76, 4.78 and 5.83 fold, respectively. While the susceptibility ratios of the rosetted cotton flowers strain/ the susceptible strain (R/S) ranged between 1.66 fold in case of emamectin benzoate to 121.52 fold in case of methomyl. The rosetted larvae strain was highly resistant to methomyl, lambda-cyhalothrin and chlorpyrifos with 121.52, 43.57 and 28.81 fold, respectively. However, it was highly susceptible to emamectin benzoate and spinetoram with 1.66 and 2.76 fold, respectively. The susceptibility ratios of the rosetted cotton flowers strain/ the diapaused strain (R/D) ranged between 0.58 fold in case of spinetoram and 7.47 fold in case of lambda-cyhalothrin. The susceptibility ratios between different colonies of *P. gossypiella* show that the newly hatched larvae descended from rosetted cotton flowers were less likely to acquire resistance which can be arranged as follows: spinetoram, chlorpyrifos, emamectin benzoate, methomyl and lambda-cyhalothrin, the susceptibility ratios were at 0.58, 1.01, 2.17, 2.69 and 7.47 fold, respectively, compared to the larvae descended from diapaused larvae.

Table (4): Susceptibility ratios of the newly hatched larvae of *P. gossypiella* of different strains against the tested insecticides

Insecticide	Susceptibility ratio		
	D^1/S^3	R^2/S^3	R^2/D^1
Lambda-cyhalothrin	5.83	43.57	7.47
Chlorpyrifos	28.53	28.81	1.01
Methomyl	45.25	121.52	2.69
Emamectin benzoate	0.76	1.66	2.17
Spinetoram	4.78	2.76	0.58
Diapaused strain (1); Rosetted strain (2); Susceptible strain (3)			

The intensive use of pesticides in agriculture leads to adverse effects such as development of pesticide resistance. The obtained results showed that lambda-cyhalothrin (pyrethroids) was the most potent on the susceptible strain. The tested insecticides emamectin benzoate (avermectins), spinetoram (spiynosins) and lambda-cyhalothrin were the most potent on the diapaused strain. As the rosetted flowers strain, emamectin benzoate and spinetoram were the most potent. On the other hand, the chlorpyrifos (organophosphates) and methomyl (carbamates) were the lowest potent on the three tested strains, susceptible, diapaused and rosetted.

The results of the present study and those of other investigators (Rashad *et al.*, 1993; Henneberry and Naranjo, 1998; Attique *et al.*, 2001; El-Sayed *et al.*, 2008) support the variation in the susceptibility between the two field strains and among the different chemical classes of insecticides. Moreover, Schmutter (1985) reported that as a result of continued massive use of certain synthetic insecticides against the cotton pest, tolerant and resistant strains have been developed.

Also, Zidan *et al.* (2012) found that lambda-cyhalothrin (pyrethroids) was more potent on *P. gossypiella* followed by methomyl (carbamates), and chlorpyrifos, (organophosphates). Sabry *et al.* (2014) showed that thiamethoxam was the most effective insecticide followed by chlorantraniliprole and spinetoram (spiynosins) against *P. gossypiella*. On the other hand, Sabry and Abdel-Aziz (2013) and Sabry *et al*. (2014) reported that the rate of resistance in pink bollworm increased gradually in the beginning of the selection and sharply increased after the F_4. Their results recommended that spinosad (spiynosins) can be used safely against the pink bollworm twice during the same season without any building up of resistance. No cross resistance was occurred between pink bollworm spinosad resistant colony and some insecticides from different groups of pesticides. Their results also confirmed that enzyme detoxification mechanism is considered one of the main mechanism of resistance to insecticides and the use of pesticides rotation play an important role in pesticide resistance management.

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تـأثير السـكون اليرقـى الشـتوى لـدودة اللوزالقرنفليـة علـى حساسـيتها للعديـد مـن المبيدات

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لدراسة تأثير سكون اليرقات فى الشتاء على حساسية دودة اللوز القرنفلية لعديد من مجاميع المبيدات الحشرية المختلفة أجريت إختبارات حيوية معملية على اليرقات المنحدرة من السلالات الساكنة وأزهارالقطن النجمية والحساسة و مبيدات لمبدا- سيهالوثرين، كلوربيريفوس، ميثوميل، إيما ميكتين بنزوات و سبينتورام. و قد تم جمع السلالاتين الحقليتين من نباتات القطن المصابة والمنزرعة فى أماكن مختلفة من محافظة الشرقية أثناء موسمى القطن ٢٠١٢ و٢٠١٣.

أوضحت النتائج أن نسبة الحساسية بين السلالة المنحدرة من الأزهار النجمية و السلالة المنحدرة من اليرقات الساكنة (R/D) قد إختلفت بين المبيدات المختبرة حيث تراوحت بين ٠.٥٨ ضعف فى حالة سبينتورام و ٧.٤٧ ضعف فى حالة لمبدا- سيهالوثرين. كذلك أظهرت النتائج أن اليرقات حديثة الفقس المنحدرة من أزهار القطن النجمية كانت أكثر مقاومة لكل من الميثوميل (١٢١.٥٢ ضعف) ولمبدا- سيهالوثرين (٤٣.٥٧ ضعف) أكثر من اليرقات حديثة الفقس المنحدرة من اليرقات الساكنة. بينما أظهرت السلالتين الحقليتين نسبة حساسية متساوية فى حالة مبيد كلوربيريفوس(٢٨.٥٣ و ٢٨.٨١ ضعف). و كانت اليرقات حديثة الفقس المنحدرة من أزهار القطن النجمية أكثر حساسية لمبيد سبينتورام عن اليرقات المنحدرة من اليرقات الساكنة. بينما كانت السلالة المنحدرة من اليرقات الساكنة أكثر حساسية لمبيد إيما ميكتين بنزوات مقارنة بالسلالتين الأخرتين.