EFFECT OF SOME PESTICIDES WITH DIFFERENT TARGET SITE ON THE PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS)

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ABSTRACT: Laboratory bioassay showed that the conventional pesticide lambda-cyhalothrin was the most effective than thiamethoxam and buprofezin which tested against the newly hatched larvae of the pink bollworm, *Pectinophora gossypiella* (Saunders). The LC50 of thiamethoxam, buprofezin and lambda-cyhalothrin was 5.9, 87.5 and 4.9 ppm, respectively. When the synergism agent, piperonyl butoxide (PBO) was combined with the tested pesticides the toxicity of all pesticides was increased and the LC50 was decreased to 1.4, 15.1 and 2.6, respectively. Some biological aspects (larval duration, pupal stage, number of laid egg per female and percent of hatchability) were affected by buprofezin treatment more than thiamethoxam and lambda cyhalothrin.
Field experiment showed that lambda – cyhalothrin was the most effective than thiamethoxam and buprofezin. The percent of reduction in pink bollworm infestation to cotton bolls by using lambda-cyhalothrin, thiamethoxam and buprofezin was 85.7, 39.3 and 19.5%, respectively, during 2009 cotton season; and 80.1, 64.7 and 39.1%, respectively, during 2010 cotton season. These results suggested that lambda-cyhalothrin is the most effective pesticide against the pink bollworm larvae. And also, buprofezin has a good role in incidence of disturbance in developmental process. In addition, the use of synergistic agent PBO has a good role in increasing toxicity of all the tested pesticides especially with thiamethoxam.

Key words: Pink bollworm, thiamethoxam, buprofezin, lambda cyhalothrin, synergistic agents, mortality percent, biological aspects

INTRODUCTION

Cotton growing and production in Egypt have been faced with several infestations especially lepidopteran insects. Most of them, the pink bollworm, *Pectinophora gossypiella* which causes an enormous damage in cotton yield (El-Aswad and Aly, 2007). Many eggs of the pink bollworm are laid on the sutures or under the bracteoles at the base of the boll, particularly on bolls up to 14 days old, so hatching larvae can penetrate flowers or bolls within 20–30 min (Hutchison et al., 1988) or within 2 h (Ingram, 1994). The larvae of the pink bollworm attack plants at the beginning of the fruiting stage causing huge losses to the cotton green bolls, fibers and seeds and accordingly great reduction in the cotton yield (Khurana and Verma, 1990). Chemical control could be considered one of the most effective methods against eggs and newly hatched larvae which are presented on the outer surface before penetrating cotton bolls (Charmillot et al., 2007)

Thiamethoxam is the first commercial neonicotinoid insecticide from the thianicotinyl subclass. The most prominent member of this class of insecticides is thiamethoxam (Nauen et al., 2003). Thiamethoxam acts by binding to nicotinic acetylcholine receptors of the insect nervous system. It exhibits exceptional systemic characteristics and provides excellent control of a broad range of commercially important pests, such as aphids, jassids, whiteflies, thrips, rice hoppers, Colorado potato beetle, flea beetles and wireworms, as well as some lepidopteran species (Maienfisch et al., 2001). The Neonicotinoid insecticides interfere with the nicotinic acetylcholine receptor of the insect nervous system (Yamamoto 1996).

Buprofezin prevents the adult emergence from the pseudopupa of *Bemisia tabaci* (Valle et al., 2002), and considered a chitin synthesis inhibitor against larvae of Lepidoptera because it interferes with chitin formation by blocking the polymerizations process of N- acetyl glucose amine units (Ishaaya and Horowitz, 1988). In addition, reduced fecundity and egg hatching have been observed after adult females were treated (Uchida et al., 1987)

The synthetic pyrethroid lambda-cyhalothrin has been found to be effective at low application rates against insect pests of many crops (Gogi et al., 2006). Also, it is used worldwide in agriculture, home pest control, protection of foodstuff and disease vector control (Fetouia et al., 2010).

Piperonyl butoxide (PBO) is a synergist used in a wide variety of pesticides. The addition of piperonyl butoxide to a pesticide reduces the amount of pesticide required to be effective (Olkowski et al., 1991)

The present study aim to evaluate toxicity of the tested pesticides, lambda-cyhalothrin, thiamethoxam and buprofezin against the pink bollworm under laboratory and field conditions

MATERIALS AND METHODS

Tested pesticides

Thiamethoxam (Neonicotinoid group) - Common name: Actara 25% WDG with recommended rate 40g /400 liters water/ feddan.

Buprofezin (Chitin synthesis inhibitors group) - Common name: Applaud 25% SC with recommended rate 200 ml /400 liters water/ feddan.

Lambda-cyhalothrin (Pyrethroids group) - Common name: Karate 2.5% EC with recommended rate 330 ml /400 liters water/ feddan.

Piperonyl butoxide 90% Sc (synergism)

Laboratory bioassay:

The newly hatched larvae were obtained from Bollworm Division, Plant Protection Research Institute, Agriculture Research Centre. These larvae were reared on semi artificial (Rashad and Ammar, 1985). Three concentrations were used in each tested pesticide (thiamethoxam, buprofezin and lambda – cyhalothrin) as shown in Table 1.

Effect of the tested pesticides combined with PBO to the newly hatched larvae of the pink bollworm.

Piperonyl butoxide 90% was applied at 0.1 ml/ liters (stock solution). This concentration equal 90 ppm. Three stock solutions of PBO were prepared (Astari & Ahmed, 2005) and combined with the tested pesticides (Table 1). One ml of each concentration whether pesticides only or pesticides and synergist was added to 50 g fresh prepared diets. This amount of treated diet was divided into three replicates (Ca.16 g). Each one was poured into a convenient petri dish (12 cm diameter). Twenty healthy newly hatched larvae, starved for approximately 6 hrs were gently transferred to the surface of the diet on each petri dish using a soft brush. Similar numbers of larvae were transferred to untreated diet as a control treatment. The dishes were covered and maintained in an incubator at temperature of 27 ± 1 $^{\circ}$C and 65 – 75 R.H. with a complete dark all daytime. To simulate nature, after ca. one hour, from exposing the first instar larvae to the treated and untreated diet, the healthy larvae were transferred individually into clean and sterile glass tubes (2 x 7 cm) each one containing a 5 g of untreated diet, each tube contained one alive larva. All tubes were inspected after one, two and five day for estimating the mortality percentages. The LC50, s values were calculated by Probit analysis by Proban program. Synergistic ratio (SR) was calculated by dividing the LC50 value of pesticide alone by the LC50 value of pesticide plus synergist.

Effect of the tested pesticides on some biological aspects of the pink bollworm under laboratory conditions

The larvae which survived were taken to determine the effect of the tested pesticides (thiamethoxam, buprofezin and lambda-cyhalothrin) on some biological aspects, the larval durations and pupal stage, adult longevity, number of laid eggs per female and hatchability and compared with the untreated larvae (control).

Field experiment:

Field experiment was carried out in Hehai region, Sharkai Governorate during two successive cotton seasons 2009 and 2010 to evaluate the efficacy of thiamethoxam, buprofezin and lambda-cyhalothrin at the recommended rates against the pink bollworm infesting cotton bolls. Four feddan (feddan = 4200 m^2) cultivated by cotton Giza 86 were used in this experiment sown at 24th April during the two seasons. The normal agriculture practices were applied. Randomly each feddan was divided into three plots and randomly treated by the tested pesticides according to the recommendation of the Ministry of Agriculture program until 31 Augusts. After this date these area were left without any pesticides treatments. At 21st September, three feddan were divided into three plots. These plots were randomly treated by the tested pesticides (thiamethoxam, buprofezin and lambda-cyhalothrin) and the fourth feddan was left without any treatment as a control. Each pesticide was sprayed three times with one week interval. The pesticides were sprayed by using knapsack spryer equipped with one nozzle using 400 liter /feddan. Pesticides treatment was directed at the end of day to avoid the high temperature during spraying. Samples of 100 green bolls (14 – 21 days old) were randomly collected from middle and corners of each replicate one day before the first spray and weekly after spraying. All samples were taken to the laboratory in cloth bags and examined externally and dissected to inspect the percentage of infestation of the pink bollworm.

Statistical analysis:

Data were analyzed by using Costat Statistical Software (Berkeley, 1990) (one ways classification ANOVA). The percentage of reduction of pink bollworm infestation was calculated (Henderson and Tilton, 1955)

RESULTS

Laboratory experiment

Effect of thiamethoxam, buprofezin and lambda-cyhalothrin on the newly hatched larvae of the pink bollworm under laboratory condition. Table 2 summarize the LC50,s of the tested pesticide. The LC50,s are 5.9, 87.5 and 4.9 ppm for thiamethoxam, buprofezin and lambda-cyhalothrin, respectively. Results indicate that lambda –cyhalothrin is the most effective on the newly hatched larvae of the pink bollworm followed by thiamethoxam and buprofezin. The highest value of slope occurred in lambda-cyhalothrin treatment (1.7) followed by thiamethoxam (1.5) and buprofezin (1.4). The statistical analysis shows that there is a significant difference between all treatments and control, and also significant difference between lambda – cyhalothrin and buprofezin. While no significant difference between lambda – cyhalothrin and thiamethoxam. This result shows that according to the statistical analysis the effect of lambda – cyhalothrin on the newly hatched larvae equal in thiamethoxam treatment. Buprofezin (chitin synthesis inhibitors) was the lowest effective against the pink bollworm larvae.

Synergistic effect of piperonyl butoxide on all tested pesticides

As shown in Table (1) toxicity of all pesticides was increased when the synergistic agent PBO was combined with the tested pesticides. Table 2 represents that the LC50 was 1.4, 15.1 and 2.6 ppm in thiamethoxam, buprofezin and lambda-cyhalothrin, respectively. When the PBO was added thiamethoxam became the most effective pesticides. The results show that LC50 decreased in thiamethoxam, buprofezin and lambda-cyhalothrin combined with PBO from 5.6 to 1.4, 87.5 to 15.1 and 4.9 to 2.6 ppm with the synergistic ratio 4.2, 5.8 and 1.9 –fold, respectively.

Effect of the tested pesticides on some biological aspects

Effect of lambda – cyhalothrin, thiamethoxam and buprofezin on larval duration, pupal stage, adult longevity, number of laid egg per female and hatchability were estimated. Data in Table 3 show that buprofezin has the highest effect on larval duration, while there is no difference between control and other treatments. The larval duration was 17.7, 16.3, 15 and 15.3 days in buprofezin, thiamethoxam, lambda – cyhalothrin treatments and control, respectively. The pupal stage not affected in all treatment compared with control. The adult longevity is reduced to 13.3 days compared to 15.4 days in lambda –cyhalothrin, while, it is 14.3 days in control. The number of laid egg per female is reduced in buprofezin to 58.7 egg /female compared with thiamethoxam (72.1), lambda-cyhalothrin (77.9) and control (85). The percent of hatchability is 62.3, 68.3, 73.7 and 83 % in buprofezin, thiamethoxam, lambda – cyhalothrin and control, respectively.

Field experiment

Lambda-cyhalothrin, buprofezin and thiamethoxam were evaluated during two successive seasons 2009 and 2010 against the pink bollworm larvae.

Effect of the selected pesticides on the pink bollworm infestation during 2009 season

As shown in Table 4 the percent of infestation ranged between 84 and 87, 6% before spraying. After the first treatment the percent of infestation slightly reduced in thiamethoxam and buprofezin. It was 76.7 and 81.3, respectively, compared with 45 and 83% in lambda-cyhalothrin and control, respectively. The percent of infestation did not change in buprofezin treatment after the second and the third treatments. It was 73 and 73.3 %, respectively. The percent of infestation in thiamethoxam gradually reduced after the second and the third treatment. It was reduced to 68.3 and 53%, respectively. The percent of infestation sharply reduced in lambda – cyhalothrin treatment after the second and the third sprats. It was reduced to 20 and 13.3%, respectively.

The statistical analysis shows that there are significant differences between lambda-cyhalothrin and other pesticides, while, there is no significant difference between thiamethoxam and buprofezin after the fist and second treatments. But there is significant difference between the treated plots and untreated control. After the third treatment significant difference between buprofezin and thiamethoxam was found. The statistical analysis cleared that lambda–cyhalothrin is the most effective pesticide followed by thiamethoxam and buprofezin. The percent of reduction in infestation was 85.7, 39.3 and 19.5% in lambda-cyhalothrin, thiamethoxam and buprofezin, respectively, after the third treatment.

Effect of the selected pesticides on the pink bollworm infestation during 2010 season

During 2010 season the percent of pink bollworm infestation was ranged between 91 to 86.7% before spraying Table 5. After the first spray by thiamethoxam and buprofezin, the percentage of infestation slightly reduced from 86.7 to 78.3% and 90.7 to 83.7, respectively. These percents were decreased to 44.3 and 53.3 after the third treatment by thiamethoxam and buprofezin, respectively. In case of lambda-cyhalothrin treatment, the percent of infestation sharply decreased from 89 to 56% after the first treatment and reached 17% after the third treatment. The percent of reduction by thiamethoxam and buprofezin after the third treatment was 64.7 and 39.1%, respectively, and 80.1 by lambda–cyhalothrin. The statistical analysis shows that there is no significant difference between the effectiveness of thiamethoxam and buprofezin after all treatments, but there is a significant difference between these pesticides (thiamethoxam and buprofezin) and lambda cyhalothrin.

DISCUSSION

The previous results show that lambda-cyhalothrin was the most effective pesticides followed by thiamethoxam and buprofezin. The same result was found Brickle et al. (2001). The authors found that lambda-cyhalothrin was highly effective in dryland *Bacillus thuringiensis* cotton compared with spinosad and thiodicarb. Deng et al. (2008) stated that buprofezin had low toxicity to the wolf spider, *Pirata piratoides*. Gough and Wilkinson (1984) found that buprofezin was not effective against the cotton bollworm, *H. armigera* at any tested dose for any time of treatment in any spray. Torres et al. (2003) stated that thiamethoxam was low to moderate toxicity against *Aphis gossypii*. On the other hand, another results found that found that buprofezin was proved to be effective against the nymphs of whitefly, *Bemisia tabaci* (Ali et al., 2005).

According to the mechanism of action of these pesticides, it is logically PBO increases the toxicity of both lambda-cyhalothrin and thiamethoxam, but the new result it is increase the toxicity of buprofezin which interfere with chitin formation and not considered nervous poison.

These results show that piperonyl butoxide has a good role in lambda-cyhalothrin, thiamethoxam and buprofezin synergism. This means that PBO play a major role in inhibition of cytochrome P450 monooxygenase (Wang et al., 2009). Cottage & Gunning (2006) found that buprofezin is an inhibitor of AChE in B-biotype *B. tabaci*. The authors also, stated it is possible that buprofezin might disrupt AChE function by methods such as steric hindrance, dynamically inhibiting the widening fluctuation of the gorge by changing the opening behavior of alternative passages and disrupting the active site conformation.

The present results also, cleared that buprofezin is the most effective on the biological aspects of the pink bollworm compared with the other pesticides. This may be because buprofezin is a chitin synthesis inhibitor and interfere with the molting process. The same result was found by Smith (1995). The author found that buprofezin caused significant larval mortality and reduced egg production in the scale-feeding coccinellid *Chilocorus circumdatus* Gyllenhal. Valle et al. (2002) recorded that buprofezin prevents the adult emergence from the pseudopupa of *Bemisia tabaci*. Deng et al. (2008) found that buprofezin significantly reduced the percent hatching of spiders' eggs but had only a slight effect on egg production. Hoffmann et al. (2009) found that thiamethoxam reduced larval emergence rates by > 90% for all life stage targets in *Plum curculio*.

To through some light on the differences between 2009 and 2010 seasons the results showed that the percents of reduction which caused by buprofezin and thiamethoxam in 2010 season were more than 2009 season. The percents of reduction were 19.5 and 39.3; and 39.1 and 64.7% in 2009 and 2010 season, respectively. Lambda-cyhalothrin caused the highest percent of reduction in both season, it was 85.7 and 80.1% in 2009 and 2010 season. Mourad et al. (1991) found that lambda-cyhalothrin was the most effective pesticides against the pink bollworm followed by organophosphors pesticides. Gogi et al. (2006) found that buprofezin caused a slight effect against the whitefly, *Bemisia tabaci*. This may be due to these pesticides specific mainly on hemipteran pest species (Nauen et al., 2003). El-Metwally et al. (2003) found that lambda-cyhalothrin and fenpropathrin gave the highest reduction of the pink bollworm infestation. On the other hand, the authors fond that chlorpyrifos gave moderate, while, flufenoxuron and hexaflumuron (chitin synthesis inhibitors, play the same role of buprofezin) gave the least reduction of infestation. Younis et al. (2007) stated that synthetic pyrethroids exhibited the greatest reduction in pink bollworm infestation compared with chlorpyrifos. The same result also was found by El-Aswad and Aly (2007). The authors found that the tested pyrethroids were more effective in reducing cotton bollworm infestation than that of the tested organophosphors insecticides. Torres et al. (2003) recorded that thiamethoxam has low to moderate toxicity against *Aphis gossypii*.

Finally, the present results cleared that thiamethoxam is more effective on the larvae of pink bollworm than buprofezin in both laboratory and field. Toxicity of all tested pesticides was increased when PBO used. Toxicity of buprofezin greatly increased when the PBO was added. So, this result may explain a new mode of action for buprofezin in addition to molt disruption. Buprofezin is more effective on biological aspects (larval duration, pupal stage, adult longevity, number of laid egg per female and hatchability) compared with thiamethoxam and lambda-cyhalothrin. Thiamethoxam has a moderate toxicity on the first instar larvae of pink bollworm compared with lambda-cyhalothrin when it uses alone, but it is more effective when it uses combined with PBO. Buprofezin can be used against the pink bollworm when the population density was low because this pesticide has a low toxicity on the natural enemies and effective on the larvae during development processes. Liu & Chen (2000) found that buprofezin at the higher concentrations (500 and 1,000 mg [ai]/liter) reduced survival rates to 13–47% and prolonged the overall development from first instars to adult emergence by 2 or 3 d when first instars of *Chrysoperla rufilabris* were treated.

On the other hand, some authors stated that the fecundity was highly reduced in *Trichogramma pretiosum* by pyrethroids (Bastos et al., 2006, Bayram et al., 2010).

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Table 1

Concentrations of the tested pesticides and mixture of pesticides combined with piperonyl butoxide

Concentrations / Pesticides	Pesticides only (ppm)			Pesticides + PBO (ppm)		
	[1]C1	[2]C2	[3]C3	C1	C2	C3
Thiamethoxam	25	12.5	6.25	25+90	12.5+45	6.25+22.5
Buprofezin	125	62.5	31.25	125+90	62.5+45	31.25+22.5
L-cyhalothrin	20	10	5	20+90	10+45	5+22.5

1-C1: first concentration
2-C2: second concentration
3-C3: third concentration

Table 2

Toxicity of the tested pesticides and tested pesticides combined with PBO to the first instar larvae of the pink bollworm, *Pectinophora gossypiella*, under laboratory conditions

Tested pesticides	Percent of mortality with pesticides only				Slope±SE	LC50 and confidence limits	Percent of mortality with pesticides and PBO				Slope±SE	LC50 and confidence limits	Synergistic ratio
	C_1	C_2	C_3	Mean ± SE			C_1	C_2	C_3	Mean ± SE			
Thiamethoxam	80	70	50	^{bc}66.7±15.3	1.4 ± 0.3	5.9 (3.2 –7.99)	90	80	75	^{b}81.7±7.6	0.97±04	1.4 (0.008-3.5)	4.2
Buprofezin	60	40	25	[(1)] ^{b}41.7±16	1.5 ± 0.3	87.5 (70.1–121.8)	85	70	65	^{b}73.3±10.4	1.1 ± 0.3	15.1 (1.7 –26.9)	5.8
L - cyhalothrin	85	70	50	^{c}68.3±17.6	1.7 ± 0.3	4.9 (3.2– 6.4)	95	85	70	^{b}83.1± 12.6	1.8 ± 0.4	2.6 (1.1 – 3.9)	1.9
Control	10	5	5	^{a}6.7±2.9	-----	------	5	10	5	^{a}6.7 ± 2.9	-----	------	--

[(1)]Means in a column followed by the same letter do not significantly differ at the 5% level according to Fisher's LSD test

Table 3

Effect of buprofezin, thiamethoxam and lambda-cyhalothrin on some biological aspects of the pink bollworm, *Pectinophora gossypiella*

Aspects/ pesticides	Larval Duration/day	Pupal stages/day	Adult Longevity/day	No. laid eggs/ female	Hatchability Percentage
Buprofezin	[(1)] ^{b}17.7± 0.6	^{a}7.7±1.2	^{a}13.3± 0.6	^{c}58.7±8.1	^{b}62.3 ± 7.1
Thiamethoxam	^{a}16.3 ± 0.8	^{a}7.3 ± 0.9	^{a}13.7 ± 0.6	^{b}72.1 ± 11.3	^{ab}68.3 ± 9.6
L- cyhalothrin	^{a}15 ± 1	^{a}6.3 ± 0.6	^{b}15.4 ± 0.6	^{b}77.9 ± 10.3	^{ab}73.7 ± 4.1
Control	^{a}15.3 ± 0.6	^{a}7.3 ± 0.6	^{a}14.3 ± 0.6	^{a}85 ± 5.0	^{a}83 ± 1.7

[(1)]Means in a column followed by the same letter do not significantly differ at the 5% level according to Fisher's LSD test

Table 4

Percent of infestation by the pink bollworm larvae in cotton crop after treatment by tested pesticides during 2009 season

Pesticides / Treatments	Percent of infestation ± SE			
	Control	Buprofezin	L-cyhalothrin	Thiamethoxam
Before treatment	86.6± 7.1	87.6± 2.5	85.7±5.7	84± 7
After first treatment	[(1)] ^{a}83± 7.9	^{a}81.3 ±7.1	^{b}45 ±7	^{a}76.7± 3.5
After second treatment	^{a}84 ± 8.7	^{b}73 ± 7	^{c}20 ±2	^{b}68.3 ± 2.5
After third treatment	^{a}90 ± 4.3	^{b}73.3± 6.1	^{d}13.3±2.9	^{c}53 ± 11.1
[(2)]Percent of reduction	-------	19.5	85.7	39.3

[(1)]Means in a column followed by the same letter do not significantly differ at the 5% level according to Fisher's LSD test

[(2)]Percentage of reduction after the third treatment

Table 5

Percent of infestation by the pink bollworm larvae in cotton crop after treatment by tested pesticides during 2010 season

Pesticides/ Treatments	Percent of infestation ± SE			
	Control	Buprofezin	L- cyhalothrin	Thiamethoxam
Before treatment	91 ±2.6	90.7± 3.5	89± 4	86.7± 3
After first treatment	[1] ^{a}88.7± 6.5	^{a}83.7± 6.8	^{b}56 ±6.6	^{a}78.3±1.5
After second treatment	^{a}86 ± 6.2	^{ab}69.3 ±8	^{b}38.3± 11.9	^{b}60.7± 10.5
After third treatment	^{a}87.3± 5.8	^{b}53.3± 3.6	^{c}17 ± 6.1	^{b}44.3 ±9
[2]Percent of reduction	----	39.1	80.1	64.7

[1]Means in a column followed by the same letter do not significantly differ at the 5% level according to Fisher's LSD test

[2]Percentage of reduction after the third treatment