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RELATIVE TOXICITY OF SOME MODERN INSECTICIDES AGAINST THE PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) AND THEIR RESIDUES EFFECTS ON SOME NATURAL ENEMIES

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Abstract: Toxicity of three modern insecticides (chlorantraniliprole, thiamethoxam and spinetoram) was examined against the first instar larvae of *Pectinophora gossypiella* (Saunders). The field rate and other two concentrations were used. The results showed that thiamethoxam was the most effective insecticide followed by chlorantraniliprole and spinetoram. The LC_{50},s were 8.9, 13.9 and 19 ppm for thiamethoxam, chlorantraniliprole and spinetoram, respectively. The residual effect of these insecticides was tested against some natural enemies such as green lacewing, *Chrysoperla carnea*, seven-spotted ladybug, *Coccinella septempunctata* and trichogramma wasps, *Trichogramma evanescens*. The results showed that all tested insecticides were less toxic to the second instar larvae of *C. carnea* larvae. The percent of mortality with the highest concentrations ranged from 40 to 43.3%. Thiamethoxam and chlorantraniliprole were most toxic to the second instar larvae of *C. septempunctata.* Spinetoram was the most toxic against the larvae of *T. evanescens.* The results recommended that chlorantraniliprole was the most suitable insecticides for control of pink bollworm larvae and less toxic to *C. carnea* and *T. evanescens* larvae. So, it can be used when the population density of *C. carnea* and *T. evanescens* larvae in peak safely. Spinetoram can be used against the pink bollworm larvae during the *C. septempunctata* population in peak safely.
Keywords: chlorantraniliprole, thiamethoxam, spinetoram, *Pectinophora gossypiella.*

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is considered to be one of the most injurious cotton pests, because it is difficult to control with insecticides (Lykouressis *et al*. 2005). The pink bollworm larvae feed on flower buds, flowers, bolls and the seeds within damage to developing seeds, and the termination of growth results in boll rotting, premature or partial boll opening, reduction of staple length, strength, and increases trash content in the lint. With the widespread insecticide resistance of four major chemical classes of insecticides (organophosphates, carbamates, synthetic

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pyrethroids and neonicotinoids), it is necessary to develop some new insecticides with unique modes of action.

Chemical control using synthetic insecticides is the primary method to manage this pest, but it has serious drawbacks, including reduced profits from high insecticide costs, destruction of natural enemy populations (Campbell et al. 1991), build-up of insecticide residues on tomato fruits (Walgenbach et al. 1991) and in the environment, and fundamentally the rapid development of insecticide resistance.

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*., 2001a). The Neonicotinoid insecticides interfere with the nicotinic acetylcholine receptor of the insect nervous system (Yamamoto, 1996).

Spinetoram is provides long lasting control of a broad spectrum of insect pests in a variety of crops. It is applied at low rates and has low impact on most beneficial insects (Mertz and Yao, 1990). Spinetoram causes excitation of the insect nervous system by altering the known binding sites of other classes of insecticides such as of neonicitinoids, fiproles or avermectins (Crouse and Sparks, 1998).

Sattelle *et al.* (2008) reported that chlorantraniliprole (Rynaxypyr), the first commercialised ryanodine receptor insecticide from the anthranilic diamide class, has exceptional insecticidal activity on a range of lepidopteran pests and on other orders such as Coleoptera, Diptera, Isoptera and Hemiptera. Lahm *et al*. (2005) stated that chlorantraniliprole activates the unregulated release of internal calcium stores, leading to Co^{+2} depletion, feeding cessation, lethargy, and muscle paralysis, finally insect death. Cordova *et al*. (2006) reported that chlorantraniliprole activates ryanodine receptors via stimulation of the release of calcium stores from the sarcoplasmic reticulum of muscle cells (i.e. for chewing insect pests) causing impaired regulation, paralysis and ultimately death of sensitive species.

The intensive use of conventional pesticides in agriculture and public health leads to adverse effects such as development of pesticide resistance, frequent pest out breaks and emergence of new pests, so it must be found new and alternative pesticides to overcome of this phenomenon.

This works aim to use new insecticides with new target site of action to insect control and also, less toxic to non target insects such as natural enemies.

MATERIALS AND METHODS

Test insects

The pink bollworm adults were obtained from Bollworm Division, Plant Protection Research Institute, Agriculture Research Centre. These adults were laid eggs which incubated at 25 ± 1C^{o} and 70 ± 5% RH in the laboratory. The eggs were hatched after five days and the first instar larvae were used in this work. The newly hatched larvae were reared and treated on semi-artificial diet according to Rashad and Ammar (1985).

The second instar larvae of *Chrysoperla carnea*, *Coccinella septempunctata* and cards of *Sitotroga cerealella* eggs parasitized by *Trichogramma evanescens* adults since three days (to treat the larval stage) were obtained from predator and parasite unit, Plant Protection Research Institute. These insects were tested under laboratory conditions.

Test insecticides

1- Chlorantraniliprole (Coragen® 20% Sc): Chlorantraniliprole belongs to the IRAC chemical group 28: Diamides (Ryanodine receptor modulators). The recommended rate is 50 ml/hectare. Three concentrations were used, the field rate and two lower concentrations. These concentrations were 25, 12.5 and 6.25 ppm, respectively.

2- Spinetoram (Radiant®12% Sc) is a new member of the spinosyn class of insect management tools developed by Dow AgroSciences. The recommended rate is 100 ml/hectare. Three concentrations also, were used, the field rate and two lower concentrations (30, 15 and 7.5ppm, respectively).

3- Thiamethoxam (Neonicotinoid group)–Common name: Actara 25% WDG, Early work indicated that the principal site of action of neonicotinoids was on cholinergic synaptic transmission. The recommended rate is 40 g/hectare. Three concentrations also, were used, the field rate and two lower concentrations (25, 12.5 and 6.25 ppm, respectively).

Toxicity of the tested insecticides against the first instar larvae of the pink bollworm

After the first instar larvae were hatched the larvae starved for about 6 hours and treated with the tested insecticides. About 1ml of each concentration was added to 50 g of freshly prepared diet and mixed very well. All of these diets were divided to three replicates and each one was poured into a convenient Petri dish (12 cm diameter). Twenty healthy newly hatched larvae 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 the temperature of 27±1 °C and 65–75 R.H. with complete darkness during all the daytime. To simulate the nature, after ca. 1h, by exposing the first instar larvae to the treated and untreated diet, the healthy larvae were transferred individually into clean and sterile glass tubes (2x7 cm), each one containing 5g of untreated diet and each tube contained one alive larva. All the tubes were inspected after one, two and five days for estimating the mortality percentages. The LC50s values were calculated according to Finney (1971).

Residual effects of the tested insecticides to the second instar larvae of green lacewing and seven spotted lady beetles.

Three concentrations of the tested insecticides were used. Each concentration includes three replicates. Twenty healthy starved larvae of the second instar larvae of green lacewing and seven spotted lady beetles were put individually in glass tubes (2x7 cm) in each replicate and fed on *Sitotroga cerealella* eggs contaminated with all concentrations of the tested insecticides by dipping the eggs cards for 5 second in insecticide solution and dried it after that. Other three replicates were treated by water only as a control. These tubes were incubated at 26 ± 1°C and 70 ±5% RH. After that these tubes were inspected and LC_{50}s values were calculated according to Finney (1971).

Residual effects of the tested insecticides to the second instar larvae of trichogramma wasp.

The cards of *S. cerealella* eggs parasitized by *T. evanescens* (the eggs acquired a black color) were treated by all concentrations of insecticides used by dipping in insecticides solution for 5 second and dried after that. Each card has a random number of eggs. Three concentrations for any insecticides and each concentration have three replicates. Other three replicates were treated by water only as a control. All treated card were put individually in glass jars and incubated at 26 ± 1°C and 70 ±5% RH. After that these jars were inspected and percents of mortalities were calculated by counting the number of exit hall. LC_{50}, s values were calculated according to Finney (1971).

Statistical analysis

Data were analyzed by the analysis of variance (one ways classification ANOVA) followed by a least significant difference, LSD at 5% (Costat Statistical Software 1990).

RESULTS AND DISCUSSION

As mentioned in Table 1 the percent of mortalities in all tested insecticides are 63.3, 83.3 and 73.3% for spinetoram, thiamethoxam and chlorantraniliprole, respectively, with the first concentrations used of all tested insecticides. This means that thiamethoxam (neonicotinoid) is the most toxic insecticide followed by chlorantraniliprole and spinetoram. This result is also, with the second and third concentrations. The slope values are 1.7, 2.1 and 2.6 for spinetoram, thiamethoxam and chlorantraniliprole, respectively. The LC_{50},s are 19, 8.9 and 13.9 ppm. The statistical analysis shows that there is a significant difference among all treatments. Significant difference between all treatments and control also found. The LSD values are 14.12, 11.85 and 9.4 for first, second and third concentrations. This result may be due to the systemic activity of thiamethoxam compared the other insecticides (contact). These results were agreement with Maienfisch *et al.* (2001b). The use of the systemic neonicotinoid, thiamethoxam, has been widely adopted to prevent attack by early-season pests in cotton and other crops. The authors cleared that thiamethoxam was the first commercially available second-generation neonicotinoid and belongs to the thianicotinyl sub-class. It was marketed under the trademarks Actara for foliar and soil treatment and Cruiser for seed treatment. The compound has broad-spectrum insecticidal activity and offers excellent control of a wide variety of commercially important pests in many crops. Low use rates, flexible application methods, excellent efficacy and the favourable safety profile make this new insecticide well-suited for modern integrated pest management programmes in many cropping systems. Kilpatrick *et al.* (2005) found that acetamiprid and thiamethoxam exhibited significant mortality against field-deposited eggs of bollworm, *Helicoverpa zea* (Boddie). Dhawan *et al.* (2009) evaluated that the efficacy of chlorantraniliprole against *Earias sp*. and *Helicoverpa armigera* Hübner. On the basis of pooled analysis of the damage due to bollworm complex, chlorantraniliprole 30 g a.i./ha resulted in significantly lowest floral shedding, boll damage and loculi damage as compared with the standard checks i.e. deltamethrin, quinalphes, chlorpyriphos and indoxacarb. The seed cotton yield was significantly higher in chlorantraniliprole than in standard checks. Less number of sucking pests was observed in chlorantraniliprole compared with standard checks and it did not cause any resurgence in sucking pests. Thus, chlorantraniliprole 30 g a.i./ha was the most effective treatment for the control of bollworm complex on cotton.

Residual effect of the tested insecticides to the second instar larvae of green lace, *C. carnea*

Results in Table 2 show that all tested insecticides are harmless less toxic against the second instar larvae of green lace, *C. carnea.* The percents of mortalities ranged between 40 to 43.3% with the highest concentrations. The LC_{50},s were 38.8, 31.9 and 36.5 ppm for spinetoram, thiamethoxam and chlorantraniliprole, respectively. The statistical analysis shows that there is no significant difference among all treatments, but there is a significant difference between treatments and control. According to the recommendation of the International Organization for Biological Control, West Palaearctic Regional Section (IOBC/ WPRS) working group (Hassan, 1989), harmless pesticides caused less than 50% mortality, slightly harmful caused 50 - 79% mortality, moderately harmful caused 80–89% mortality and harmful caused more than 90%. According to this recommendation all tested insecticides considered harmless insecticides. On the other hand Hussain *et al.* (2012) found that spinosad, lufenuron, chlorantraniliprole and emamectin benzoate were found as intermediately toxic to *C. carnea* larvae. Nasreen *et al*. (2005) found that thiamethoxam was moderately harmful to *C. carnea* larvae at lower concentration (C1) whereas were toxic at recommended and higher concentrations. Vivek *et al.* (2012) found that based on the LC_{50}, the descending order of toxicity to *C. carnea* larvae was acetamiprid (0.005)>, thiamethoxam (0.006)>, imidacloprid (0.013)>buprofezin (0.241)> and neembaan (< 50% mortality at highest concentration).

Residual effect of the tested insecticides to the second instar larvae of seven spotted ladybeetle, *C. septempunctata*

Results in Table 3 show that the second instar larvae of *C. septempunctata* are more susceptible to the tested insecticides especially thiamethoxam and chlorantraniliprole. The percents of mortalities with the highest concentrations (recommended rate) are 58.3, 88.3 and 86.7%, for spinetoram, thiamethoxam and chlorantraniliprole, respectively. With lower concentrations chlorantraniliprole is more toxic than thiamethoxam. The percents of mortalities are 71.7, 50% and 73.3, 58.3 with the second and third concentrations for thiamethoxam and chlorantraniliprole, respectively (Table 3). So the LC_{50} of the tested insecticides are 22.8, 6.3 and 4.7 ppm for spinetoram, thiamethoxam and chlorantraniliprole, respectively. Statistical analysis shows that there is no significant difference between thiamethoxam and chlorantraniliprole treatments. But there is a significant difference between chlorantraniliprole and spinetoram. On the other hand, Misra (2011) reported that chlorantraniliprole at doses ranging from 20- 50 g a.i./ha was safe to natural enemies. Yang et al. (2012) found also the field rate of chlorantraniliprole (40 mg/L) is a safe for natural enemies. Rahmani *et al.* (2013) found that, although thiamethoxam is approximately safe for the ladybird, more care should be taken when it is used in IPM programs. According to Dow AgroSciences (2008) spinetoram was classified by the U.S. Environmental Protection Agency (EPA) as an environmentally and toxicologically reduced risk product.

Residual effect of the tested insecticides to the larval duration of trichogramma wasps, *Trichogramma evanescens*

Data show that spinetoram is the most toxic insecticides against *T. evanescens* larvae. The numbers of exit halls in *Sitotroga cerealella* eggs (the parasite host of *T. evanescens*) are decreased with spinetoram treatment. The percents of mortalities are 93.3, 76.7 and 53% with the first, second and third concentrations of spinetoram, respectively. The numbers of exit halls with thiamethoxam and chlorantraniliprole treatments are approximately similar. The statistical analysis shows that there is a significant difference between spinetoram and other treatments. No significant difference between thiamethoxam and chlorantraniliprole. The $LC_{50,}$s are 7.1, 67.2 and 43.7 ppm for spinetoram, thiamethoxam and chlorantraniliprole, respectively. Wasps of the genus *Trichogramma* have several advantages as biological control agents, including relative ease of rearing and fact that they kill their host in the egg stage before it causes feeding damage (Bigler, 1984). Most of the foliar pesticide applications are toxic to many nontarget organisms, including predators and parasitoids. Low rates of parasitism have been reported in several agricultural regions and this may be due, partly, to the extensive use of pesticides (Gullan and Cranston, 1992). Pesticides may limit the efficiency of the biological control agents and plant growth regulators can have deleterious effects on parasitoids *Trichogramma*. Although pesticides do not have a specific site of action in arthropods, they can induce sublethal effects on reproduction or on the biological functions (Stefanello *et al*. 2008).

The present data cleared that thiamethoxam was the highest toxic against the first instar larvae of the pink bollworm and also less toxic against the natural enemies, green lacewing and trichogramma wasps compared the other two insecticides spinetoram and chlorantraniliprole. So, thiamethoxam was suitable for integrated management of the pink bollworm larvae and relatively safe to green lacewing and trichogramma wasps. On the other hand, spinetoram was less toxic against the second instar larvae of the seven spotted lady beetle compared the other two pesticides (chlorantraniliprole and thiamethoxam). So, it was suitable for pest management when the population density of the seven spotted lady beetle high. Data also, showed that spinetoram was the most toxic insecticides against the trichogramma wasps. The percent of mortality was 93.3% compared 30.7 and 36.7% with chlorantraniliprole and thiamethoxam. So, dada recommended that never use of spinetoram during the high density of trichogramma population. But it can be used thiamethoxam safely in pink bollworm control.

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Table 1. Toxicity of spinetoram, thiamethoxam and chlorantraniliprole to the first instar larvae of the pink bollworm, *P. gossypiella*

Pesticides	Percents of mortalities			Slope values ± SE	LC_{50} and confidence limits/ ppm
	C_1 (mean±SE)	C_2 (mean±SE)	C_3 (mean±SE)		
Spinetoram	63.3±10.4^{b}	43.3±7.6^{b}	25.0± 5^{b}	1.7 ± 0.3	19.0 (15.6 – 24.8)
Thiamethoxam	83.3±2.9^{a}	61.7±7.6^{a}	36.7±7.6^{a}	2.1 ± 0.3	8.9 (7.2 – 10.6)
Chlorantraniliprole	73.3±10.4^{ab}	48.3±5.8^{b}	16.7±2.9^{bc}	2.6 ± 0.3	13.9 (12.2 – 16.2)
Control	10^{c}	11.7±2.9^{c}	8.3±2.9^{c}	------	------
F values	57.03***	34.01***	23.2***	------	------
LSD (5%)	14.12	11.85	9.4	-------	-------

C_1: first concentration (field rate)
C2: Second concentration
C3: Third concentration

Table 2. Residual effect of spinetoram, thiamethoxam and chlorantraniliprole to the second instar larvae of green lacewing, *C. carnea*

Pesticides	Percents of mortalities			Slope values ± SE	LC_{50} and confidence limits/ ppm
	C_1 (mean±SE)	C_2 (mean±SE)	C_3 (mean±SE)		
Spinetoram	41.7±5.8^{a}	18.3±5.8^{a}	8.3±5.8^{a}	2.1 ± 0.4	38.8(29.9 – 62.6)
Thiamethoxam	43.3±10.4^{a}	25±5^{a}	11.7±2.9^{a}	1.7± 0.3	31.9(23.6 – 59.1)
Chlorantraniliprole	40 ±5^{a}	18.0±7.6^{a}	10±5^{a}	1.8±0.4	36.5(25.5 – 71.1)
Control	3.3 ± 5.8^{b}	0.0^{b}	0.0^{b}	------	------
F values	20.48***	11.62**	4.8*	-----	------
LSD (5%)	13.3	10.17	7.69	-----	------

C_1: first concentration (field rate) C2: Second concentration C3: Third concentration

Table 3. Residual effect of spinetoram, thiamethoxam and chlorantraniliprole to the second instar larvae of the seven spotted ladybeetle, *C. septempunctata*

Pesticides	Percents of mortalities			Slope values± SE	LC_{50} and confidence limits/ ppm
	C_1 (mean±SE)	C_2 (mean±SE)	C_3 (mean±SE)		
Spinetoram	58.3±7.6^b	38.3 ± 2.9^b	20.0 ± 5.0^b	1.73±0.32	22.8(18.9 – 31.2)
Thiamethoxam	88.3 ± 2.9^a	71.7 ± 2.9^a	50.0 ± 5.0^a	1.95± 0.34	6.3(4.3 – 7.8)
Chlorantraniliprole	86.7 ± 7.6^a	73.3 ± 2.9^a	58.3 ± 7.6^a	1.52± 0.33	4.7(2.3 – 6.6)
Control	10.0 ± 0.0^c	6.7 ± 5.8^b	8.3 ± 7.6^b	---------	--------
F values	128.4***	205.9***	40.87***	---------	--------
LSD (5%)	10.52	7.19	12.15	---------	--------

C_1: first concentration (field rate)
C2: Second concentration
C3: Third concentration

Table 4. Residual effect of spinetoram, thiamethoxam and chlorantraniliprole to the larval stage of trichogramma wasps, *T. evanescens*

Pesticides	Percents of mortalities			Slope values ± SE	LC_{50} and confidence limits/ ppm
	C_1 (mean±SE)	C_2 (mean±SE)	C_3 (mean±SE)		
Spinetoram	93.3±1.5^a	76.7±1.5^a	53±3.6^a	2.3±0.4	7.1(5.1 – 8.6)
Thiamethoxam	30.7±4.1^b	22.0± 2.6^b	12.0± 1.0^b	1.1±0.3	67.2(34.9 – 782.8)
Chlorantraniliprole	36.7±6.7^b	25.3± 2.5^b	13.3± 1.5^b	1.3±0.3	43.7(27.8 – 148.9)
Control	10 ± 1.1^c	6 ± 1.0^c	7 ± 1.3^c	--------	--------
F values	235.9***	545.7***	259.5***	--------	--------
LSD (5%)	7.73	4.21	4.27	--------	--------

C_1: first concentration (field rate)
C2: Second concentration
C3: Third concentration