Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 97-101

Received: 22-01-2018
Accepted: 23-02-2018

Zunnu Raen Akhtar
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Muhammad Majid
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Usama Irshad
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Zain Saeed
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Jawad Khalid
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Hashim Khan
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Ahmad Ali Anjum
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Aqsa Noreen
Department of zoology, wildlife and fisheries, University of Agriculture, Faisalabad, Pakistan

Easha
Department of zoology, wildlife and fisheries, University of Agriculture, Faisalabad, Pakistan

Correspondence
Zunnu Raen Akhtar
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Resistance development of lepidopteran pests against insecticides in Pakistan: A case study of pink boll worm against different insecticides

Zunnu Raen Akhtar, Muhammad Majid, Usama Irshad, Zain Saeed, Jawad Khalid, Hashim Khan, Ahmad Ali Anjum, Aqsa Noreen and Easha

Abstract
In present experiment, three insecticides including triazophos, bifenthrin and cypermethrin, were used against pink boll worm as compared to control in mortality tests to observe their survival on sprayed cotton leaves under laboratory conditions. Three insecticides were tested for pink boll worm resistance evaluation as compared to control under field conditions. Results showed that among three tested insecticides named triazophos; bifenthrin and cypermethrin, triazophos showed high resistance against pink boll worms followed by bifenthrin and cypermethrin under laboratory and field conditions. Mortality was higher in case of triazophos while it was comparatively low in case of bifenthrin and cypermethrin. Field studies showed that population dynamics were lower in case of triazophos sprayed field while it was higher in bifenthrin and cypermethrin sprayed fields as compared to control. So ultimately it can be asserted that triazophos can show high level of resistance for controlling pink boll worms in cotton.

Keywords: pink boll worm, resistance, cotton, insecticides

1. Introduction
Transgenic crops are superbly grown in 2000 million hectare area from 1996-2015 in the world. Transgenic soybeans, corn, cotton and canola have growing area 1000, 600, 300 and 100 million hectares respectively [1]. Insecticidal crystalline proteins from the bacterium *Bacillus thuringiensis* (Bt) kills some key pests, but is harmless to most non-target organisms including humans [2, 3, 4].

Cotton is Pakistan's important fiber and cash crop. Cotton shows a major part in earning foreign exchange. The cotton crop production has 1.5 percent role in GDP and 7.1 percent in agriculture value addition [5]. Many factors play an important role towards low production of cotton, but most serious one is insect pest attack. Pakistani farmers spend 3 billion dollars annually on different types of pesticides in which more than 80 percent spend on cotton, particularly cotton bollworms [6]. Transgenic cotton is the first non-food crop providing a definite harmless and real tool for controlling Lepidopteran pests [7, 8, 9].

Pink bollworm *Pectinophora gossypiella* (Saunders) (Gelechiidae: Lepidoptera) is one of the most damaging pest of cotton and causes heavy losses in quality and production of cotton. The larvae of *Pectinophora gossypiella* damage the floral outgrowths, bolls, seeds and flowers. *Pectinophora gossypiella* causes the interruption of growth, boll rotting, early or incomplete boll opening, reducing the staple size, strength, and enhances the contents of trash in the lint [10]. Pink bollworm is universal pest and potential host is cotton in China and United States [9].

Chemical control plays an important role in pest management by decreasing risks of yield losses when there are no practical replacements to insecticides to overcome the yield losses [11, 12]. Due to continued use of insecticides, insects started to develop the resistance at high levels. In order to suppress the damage of bollworms, insecticides from different groups are used.

In response to this problem effort to control or suppress bollworm damage to growing cotton frequently involve using insecticides of different groups in rotation program which may be useful tool to delay the resistance problem [13]. Different insecticides combinations gave better result for controlling the larval stage as compared to insecticide used alone [14]. Microbial insecticides derived from *Bacillus thuringiensis* are non-toxic to human beings [15] animals, plants, fishes parasitoids and predators [16]. Use of the microbial insecticides, derived from

Bacillus thuringiensis are non-toxic to human [15], plants, animals, fishes and for parasitoids and predators [16].

Natural products of plant origin are new trend that preserves the environment from contamination with harmful toxicant. Azadirachtin particularly shows low acute mammalian toxicity and was found effective against many insect orders [17]. Spinosad is a soil dwelling bacterium (Saccharopolyspora spinosa) plays an important role in pest control. Spinosad was used to control Lepidopteran pests [18, 19, 20]. The present work was conducted to evaluate the resistance development in Pink bollworm *Pectinophora gossypiella* against different commercially used insecticides under laboratory and field conditions.

2. Materials and Methods
2.1 Laboratory studies
2.1.1 Insect rearing
Pink bollworms (*pectinophora gossypiella*) population was collected from University of Agriculture, Faisalabad field for rearing during year of 2016. Bolls infested with pink bollworm were detached from the plant and kept in plastic bags. These infested bolls were placed in rearing cages and adults were emerged from pupae. Adults were separated from the old cages and shifted to new cages for mating. Sugar solution and new green bolls were provided to the adults for feeding and egg laying, respectively. After hatching, first instar larvae fed on non-Bt bolls till 3rd instar.

2.1.2 Insecticides concentration
As shown in table 1, three insecticides which are used in present study were cypermethrin (Arrivo, 10% EC), bifenthrin (Talstar, 10% EC) and triazophos (Trizone, 40% EC). Field recommended concentrations of these pesticides were tested in laboratory. Three solutions: triazophos @ 1ml/100ml H_2O, cypermethrin @ 0.25ml/100ml H_2O and bifenthrin @ 0.25ml/100ml H_2o, respectively were prepared and used.

2.2 Mortality Test
Mortality test was conducted with third instar larvae of pink bollworms. Four treatments were used in laboratory as in the field, each containing three pesticides and one control treatment. In each treatment thirty petri-dishes were used. Bt bolls were first cut into pieces and dipped into pesticides solution and kept about 20 minutes for air drying. After that, bolls were placed into the petri-dishes and released one larva in each petri-dish. Petri-dish was closed with parafilm and three holes with pin were made on each side for circulation of air. Mortality data was recorded after every 24 hours for consecutive 7 days for each treatment.

2.3 Field preparation
The present study was conducted at Entomological Research Area as well as post graduate laboratory department of entomology, University of Agriculture Faisalabad. In field, randomized complete block design (RCBD) and Completely Randomized Design (CRD) were used in the field studies. Land was prepared for cotton sowing by following the local agronomic practices. Seed Beds of 5ft width and 6-8" height was prepared for sowing and proper flow of water. Main plot size was 100x142 sq. ft. and further divided in to four subplots, size of each was 25x142 sq. ft. length wise. Three plots for pesticides application and one was kept as control treatment. A Bt cotton variety of (FH-LALAZAR) was sown by manual hand method maintaining 6-9" plant to plant distance and row-to-row distance 2.5 ft. All standard agronomic practices were applied equally in all treatments throughout the whole duration of crop.

2.3.1 Pesticides concentrations applied
Three commercially used pesticides were selected to check their efficacy against the pink bollworm (*pectinophora gossypiella*). Among these three pesticides two were pyrethroids and one was from organophosphate group. Active ingredients in these pesticides were cypermethrin, bifenthrin and triazophos. Field recommended doses of these pesticides were used following the labeled information. Pesticides concentration for the experimental area was calculated from the recommended doses. Manual spray method was used for the pesticides application. Hand operated knap sack sprayer machine was used to apply the pesticide in the field.

2.3.2 Sampling
On the emergence of pink bollworm, first spray was applied in the field. After ten days interval second spray was applied against pink bollworm populations in the field. In data recording, four number of sampling were performed S1, S2, S3 and S4. After two days of spray, first three sampling were conducted in consecutive three days and fourth sampling was performed after two days interval. Data was recorded by randomly selecting ten plants from each plot. From each plant, three bolls were selected for observing of pink bollworm from upper, middle and lower parts. Pink bollworms population was counted by opening the randomly selected boll. Mean population of the pink bollworm per plant was calculated from the recorded data.

2.4 Statistical analysis
All of the data of population dynamics was analyzed by t-test assuming unequal variances using software Statistix version 8.1. While of mortality tests, all data were in percentage.

Table 1: Triazophos, bifenthrin and cypermethrin concentrations used for experiment

Sr. #	Treatments	Dose rate/acre
T1 1000ml/acre	Triazophos	1000ml/acre
T2 250ml/acre	Bifenthrin	250ml/acre
T3	Cypermethrin	250ml/acre
T4	Control	-

3. Results
3.1 Mortality test
In laboratory studies, (Fig.1) mortality tests showed that percentage of mortality was 100% for triazophos as compared to control after 24 hours.

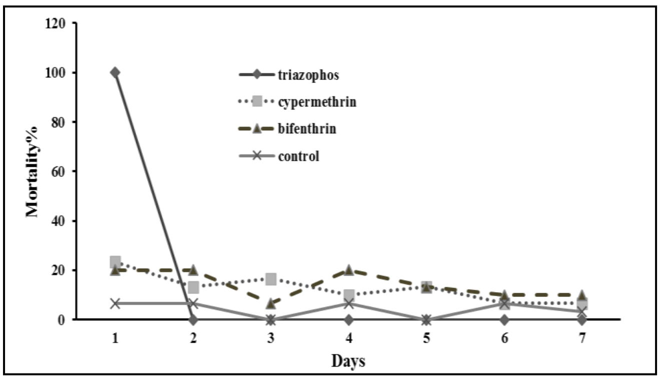

Fig 1: 1st mortality test of pink boll worms with insecticides named triazophos, cypermethrin, bifenthrin. Total number of samples per test (*n=120*)

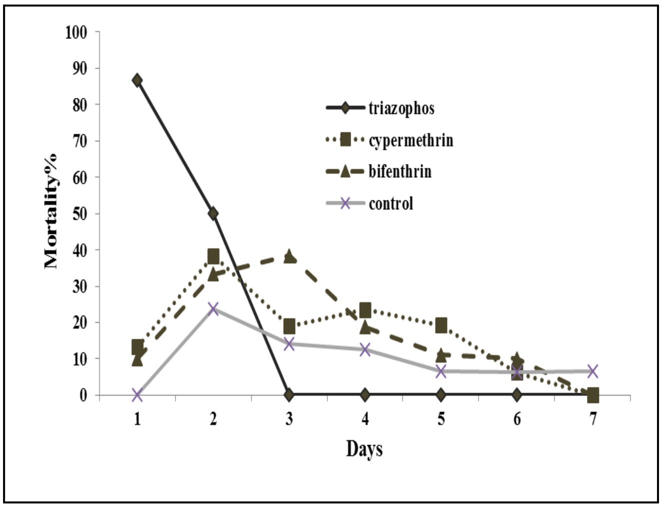

Fig 2: 2nd mortality test of pink boll worms with insecticides named triazophos, cypermethrin, bifenthrin. Total number of samples per test (*n=120*)

But in cypermethrin and bifenthrin percentage of mortality was 23.66% as compared to control in which mortality percentage was 6.66%. After 48 hours the 13.33% mortality was observed but in bifenthrin treated population mortality was 20% as compared to control treatment. After 72 hours, in control no mortality was observed as compared to the cypermethrin and bifenthrin where mortality was 16.33% and 6.66%, respectively. On fourth day, bifenthrin treated population shows higher mortality percentage as compared to cypermethrin. In bifenthrin treated population was 20% as compared to cypermethrin 10% but in control treatment 6.66%. On day five, similar percentage of mortality was observed that was 13.33% for bifenthrin and cypermethrin but in control was 0%. Toxicity of triazophos was higher as compared to bifenthrin and cypermethrin. But in cypermethrin and bifenthrin comparison both shows almost similar results. Cypermethrin and bifenthrin was more effective as compared to control treatment (Fig. 2).

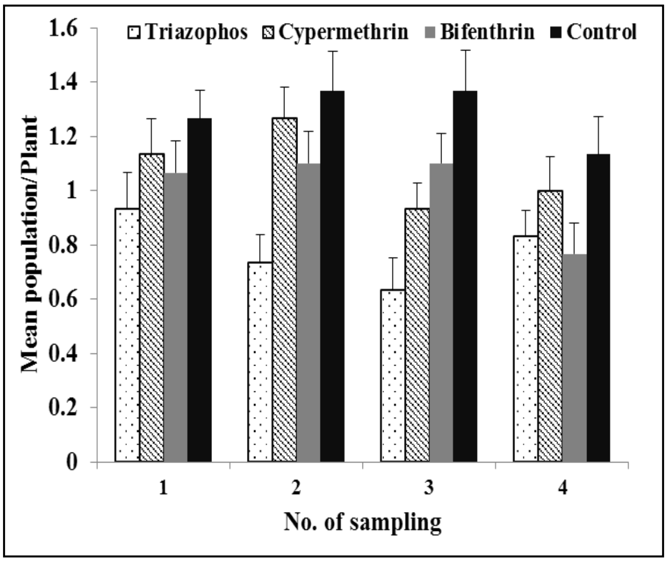

Fig 3: Population dynamics of pink boll worms on cotton crop sprayed with insecticides named triazophos, cypermethrin, bifenthrin with recommended concentration under field conditions. Total number of samples per day (*n=120*)

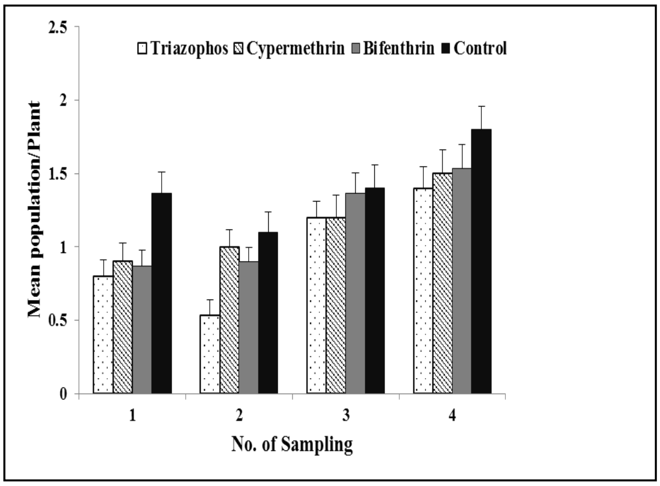

Fig 4: Population dynamics of pink boll worms on cotton crop sprayed with insecticides named triazophos, cypermethrin, bifenthrin with recommended concentration under field conditions. Total number of samples per day (*n=120*)

3.2 Field studies

In field studies, from (Fig 3) after the application of first spray, first sampling results showed that triazophos was most effective as compared to control treatment in which mean population of pink bollworm per plant was observed 0.497 and 1.265, respectively. A significant difference was observed between triazophos and control (*df*=4; *p*=0.01). Triazophos was also effective in comparison with cypermethrin and bifenthrin. Bifenthrin was more effective as compared to the triazophos and cypermethrin where the mean population of pink bollworm larvae per plant was 0.363 and 0.496, respectively. A significant difference was observed between bifenthrin and control treatment (*df*=4; *p*=0.04). Mean population of pink bollworm larvae observed for triazophos was 1.131 as compared to highest population for bifenthrin 1.461. But no significant difference was observed between cypermethrin and control (*df*=4; *p*=0.05). In overall comparison triazophos was more effective as compared to bifenthrin and cypermethrin. In 2nd spray, results shows that in triazophos treated plot pink bollworm mean population per plant was 0.75 as compared to bifenthrin and cypermethri that was, 1.03 and 0.89, respectively. But in control treatment was maximum 1.112.After 2nd spray a significant difference was observed between triazophos and control treatment where (*df*=4;*p*=0.04). Mean population per plant for triazophos, cypermethrin, bifenthrin and control was 0.61, 0.698, 0.65 and 1.163, respectively. Bifenthrin was better as compared to control treatment. But no significance difference was observed between these two treatments (*df*=4; *p*=0.16). Cypermethrin was more effective in controlling pink bollworm population as compared to the control treatment. But there was no significant difference was observed as (*df*=4; *p*=0.13) (Fig 4).

4. Discussion

In Pakistan, Lepidopterans pests have already developed the resistance against different mode of insecticides in cotton. In Lepidopteran pests cytochrome P450 enzyme plays a significant role in the development of resistance against different insecticides. Among Lepidopteran insects, *Spodoptera litura* developed the resistance against pyrethroids due to the detoxification activity of enzymes which was confirmed by using RNA interference technique [21].

Some genes are over expressed to produce the P450 enzyme which is responsible for the detoxification of the deltamethrin in *Helicoverpa armigera*. Detoxification of the chemicals through cytochrome P450 enzyme is the main reason for development of resistance in *Helicoverpa armigera* against pyrethroids [22]. Similarly, in china resistance in *Helicoverpa armigera* was developed due to the detoxification of pyrethroids with the help of different enzymes, mono oxygenase and glutathione-*S*-transferase [23].

Helicoverpa armigera was found to be resistant to spinosad (IGR) in China. Activity of the metabolic enzymes increases to overcome the toxicity when *Helicoverpa armigera* exposed to spinosad either alone or combination with other insecticides [24]. In contrast to this, spinosad was most effective in controlling pink bollworm population in the field in Egypt. Maximum reduction of pink boll worm population was observed when alone used as compared to combination with other insecticides that was the indication of having no resistance.

Different insecticides were used to control pink bollworm population causing serious damage in the cotton crop in Egypt. Pyrethroids were more effective in controlling pink bollworm population in field as compared to the other insecticides. The use of synergistic agent increases the toxicity of the insecticides [25]. Our result was in contradicted to this as pyrethroids were less effective in controlling pink bollworm larval population as compared to the organophosphate.

Spodoptera litura was also found to have developed resistance against triazophos, carbamates and pyrethroids in the field. In field observation *Spodoptera litura* was highly resistant to carbamates as compared to triazophos and bifenthrin. Whereas resistance to triazophos was more as compared to bifenthrin. Lot of research has been conducted on new novel insecticides resistance evaluation against pests in cotton.

Different enzymes were found involved in resistance development in Lepidopteran pests in the World. Such as, in American boll worms, developed resistance was found against pyrethroids, where esterase was known to be involved in resistance development in central Africa [26]. Similarly inheritance and stability of American boll worm against pyrethroids was observed in central Africa [27].

Different insecticides were used for the inhibition of the enzymes which are involved in the development of resistance in different Lepidopterans pest. Among this Lepidopterans pest *Spodoptera litura* shows resistance to organophosphate and no cross resistance was observed when combined with pyrethroids [28]. In agreement to this work pink bollworm also developed resistance against organophosphate and pyrethroids due to the involvement of enzyme activity mainly acetylecholinsterase enzyme. No cross resistance was observed in these insecticides [29]. But the present study was contradicted to this work as organophosphate was effective in control of *Pectinophora gossypiella* as compared to pyrethroids. Pink bollworm was found resistant to pyrethroids than organophosphate.

Due to the resistance development in pink bollworm in the world, different types of insecticides were developed to control that serious pest. New modern insecticides chlorantraniliprole was better for the control of pink bollworm and less toxic to the natural enemies [30]. In our study pyrethroids was less effective in controlling to pink bollworm population.

Exposure to various pesticides may develop resistance in *Helicoverpa armigera*; emmamectin benzoate and lufenuron were found more toxic to the *H. armigera* [31]. For pink boll worm, work is still less in the World especially Pakistan. So in our research resistance was found to be developed however its biochemical and physiological reason is unknown till date.

5. Conclusion

As a conclusion it can be asserted that pink boll worm in Pakistan has developed the resistance against insecticides and this resistance development should be further analyzed to observe the concerns and new techniques should be developed to overcome this emerging resistance in pink boll worms.

6. Acknowledgement

We are grateful to Mr. Haseeb Jan for his valuable comments and suggestions.

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