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

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

Aqsa Noreen
Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan

Anjum Jabeen
Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan

Hasooba Hira
Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan

Arbab Ihsan
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

Elisa showing Cry1Ac protein uptake by pink bollworms in pyrethroid treated transgenic cotton

Zunnu Raen Akhtar, Zain Saeed, Aqsa Noreen, Anjum Jabeen, Hasooba Hira, Arbab Ihsan and Easha

Abstract
An experiment to detect the resistance development in pink boll worm against Bt cotton was conducted under laboratory and field condition. In laboratory pink boll worms were reared and whole life cycle was observed on Bt cotton as compared to control variety. While ELISA was conducted to observe the Bt protein presence in pink boll worms larvae. In field studies, population dynamics were observed in Bt cotton as compared to control to know, if Bt cotton expressing Cry 1Ac can control or reduce pink boll worm population. Results showed that life cycle parameters of pink boll worms on Bt cotton were similar to non-Bt cotton. In both of varieties, there was no significant difference in any life parameter when larvae were fed on Bt cotton as compared to control. ELISA results showed that Bt protein was present in pink boll worm in trace amounts. While Bt protein was present in higher concentration in bolls when collected from the fields. Field studies revealed that there was no significant difference in pink boll worm population dynamics in both varieties as compared to control. Laboratory results were in conjugation to field studies. These results show that the pink boll worm has developed the resistance against Bt cotton, so more research is required to observe the Bt protein role in pink boll worm.

Keywords: pink boll worm, resistance evaluation, Bt cotton, pyrethroids

1. Introduction
Cotton, *Gossypium hirsutum* the most important cash crop of Pakistan with natural fiber of great economic value as a raw material for cloth [1]. Cotton is also called "White Gold" has great value in the economy of Pakistan, it plays a key position in the economy of Pakistan and attributes 1.7% in the Agriculture and 1.5% in GDP of Pakistan due this in the world ranking production of cotton Pakistan have 4th position [2].

The quality and production of cotton are reduced due to the attack of lepidopterous insect pests, Pink bollworm *Pectinophora gossypiella* caused damage to different crops throughout the year but major pest of cotton and they caused heavy loss in cotton production. In the biology of pink bollworm, the larvae of pink bollworm fed on a natural diet like cotton bolls, flowers and squares while adult of pink bollworm fed on pollen and nectar. The optimum temperature on which their development rate is high at 27±1 ºC while their minimum development rate observed at 35±1 ºC. The percentage of survival rate of pink bollworm is 69.5% and heavy mortality occur in 1st instar larvae and minimum mortality rate occur in 4th instars larvae [3].

Bacillus thuringiensis (Bt) the soil born bacterium have resistance against different insect pests of cotton, in which present Cry1Ac toxin gram positive bacteria by which insects have susceptibility against Bt cotton. Transgenic cotton contains Cry1Ac toxin which provided protection against lepidopterons insects pests. The attack of insect pests on transgenic cotton are less than non-Bt cotton, while sowing of those Bt cotton which contain two or more than two distinct toxin that have more resistance than single toxin and non-Bt cotton against lepidopterous insects pests, Cry1Ac are not failure to control insects pests but it have less resistance against lepidopteran insect pests [4]. Genes are present in transgenic *Bacillus thuringiensis* for the creation of protoxin of the bacterium is used to control the pink bollworm, while due to the effective results of transgenic cotton the use of pesticides are decreased [5].

Insecticides are used to control many insect pests, pyrethorids are used to control bollworms of cotton and they show effective results against bollworms in cotton. Bollworms of cotton are susceptible against pyrethorids insecticides. It effect on the behavior of pink boolworm [6].

Different pesticides have different mode of action and damage, lambda-cyhalothrin have effective results against larvae of pink bollworm, while buprofezin create disturbance in the development process of pink bollworm. Lambda-cyhalothrin show more effective results against newly hatched larvae of pink bollworm than thiamethoxin and buprofezin. Some synergistic are used to enhance more toxicity in pesticides, PBO has an effective role to enhance the more toxicity in pesticides specifically with thiamethoxin[7]. Some pesticides are not showed good results against larvae of pink bollworm, while some pesticide showing effective results to control pink bollworm larvae. Triazon a pesticide which have a good results to control pink bollworm larvae while polythrin C and radiant are not showing effective results to control larvae of pink bollworm in both of them Bt and non-Bt cotton [8].

Pink bollworm developed resistance against conventional pesticides due to highly application of conventional pesticides in the field, *Bacillus thuringiensis* (Bt) are used to control pink bollworm that have resistance against pesticide and reduced the used of conventional pesticide in the field. After some time pink bollworm larvae also developed resistance against single gene Cry1Ac transgenic cotton but susceptible against double gene Bt cotton which are a combination of Cry1Ac and Cry2Ab toxin [9].

2. Materials and Methods

Bt cotton planting

Bt cotton seeds were sown using normal agronomic conditions as of farmers under field conditions. All experiments were conducted in the vicinity of Faisalabad. The farmers fields were used to randomly collect the bolls. Whole of the crop was kept unsprayed to observe severity or incidence of pink boll worms under field conditions. No crop rotation or refuge was used in these experiments.

Study was conducted in entomological research area, Department of Entomology, University of Agriculture Faisalabad from may to december 2017, Randomize Complete Design (CRD) containing four varieties and three replications was used.

Laboratory experiments

Laboratory experiments of observing pink boll worms life cycle was conducted in petri dishes, in which cuttings of Bt and non-Bt cotton bolls were used and one larvae for each petri-dish was released.

Pink boll worms rearing on Bt plants

Whole of the laboratory rearing experiment was conducted at the post graduate laboratory, University of Agriculture, Faisalabad. Colonies were reared on a natural diet i.e. conventional cotton variety.

Pink boll worms rearing for spray application

Fifteen petri dishes were used in each replication. The larvae of pink bollworm were collected from bolls of cotton from the field and reared under the laboratory conditions temperature $(26\pm2)^0$C and relative humidity (60±5%) without the exposure of insecticides. A larva of pink bollworm population was maintained on cotton bolls. Larvae of pink bollworms feed on bolls of cotton while adult feed on sugary material and bolls of cotton.

Insecticide preparation and Applications

Cypermethrin @ 0.1%, 0.2% and 0.3% on three different cotton varieties was tested against the larval population of pink bollworm (*Pectinophora gossypiella*) under the laboratory conditions. The standard solution of tested pesticides was ready and different necessary concentrations were formed.

Mortality Test of Pink bollworm (*Pectinophora gossypiella*)

Three mortality test were conducted by using the cypermethrin with three different concentrations viz. 0.1%, 0.2%, 0.3%. In each petri dish one larvae of pink bollworm were released.

3. Results

Laboratory studies

Bt protein detection

From table 1, it is clear that Bt protein was found in Bt variety while no Bt protein was detected in control variety. There were significant differences in values in terms of Bt and non-Bt variety. However, concentration of Bt protein uptake by pink bollworm was slightly lower than leaves.

Life cycle and reproductive parameters

From table 2, it is clear that no significant difference was observed in life cycle parameters of pink boll worm while reared on Bt and non-Bt varieties (df= 23, P= 0.612). All stages were found to have some numerical differences but without any significant difference. Total number of eggs laid were found significantly different in Bt and non-Bt variety (df= 23, P= 0.00571).

Mortality test results using pesticide

From Fig. 1, it is clear that there were significant differences in the pesticide applied as compared to control. The mortality was less on standard or recommended concentration to prove that standard dose can be applied under field conditions successfully.

Population Dynamics of pink bollworms

Fig. 2, Population dynamics of pink bollworms showed that there was no significant difference in Bt and non-Bt variety pink boll worms population dynamics (df= 29, P= 0.3316)

Table 1: Concentration of Bt protein in field collected and laboratory reared samples of pink boll worms

Variety /control	Concentration of Bt in leaf µg/m1	Concentration of Bt in boll µg/ml	Concentration of Bt in field collected 3rd instar larvae µg/ml	Concentration of Bt in laboratory collected 3rd instar larvae µg/ml
Variety 1	0.75±0.02	1.25±0.1	0.25±0.008	0.69±0.01
Control	N.D	N.D	N.D	N.D

N.D= Not detected

Table 2: Laboratory reared parameters of female pink boll worms under laboratory conditions

Variety/control	Total larval duration	Pre-oviposition duration	Days of fecundity	Total number of eggs
Variety 1	14.5±0.01	4.25±0.02	12.75±0.03	47.25±0.01
Control	14.0±0.06	4.55±0.04	13.03±0.01	50.21±0.4

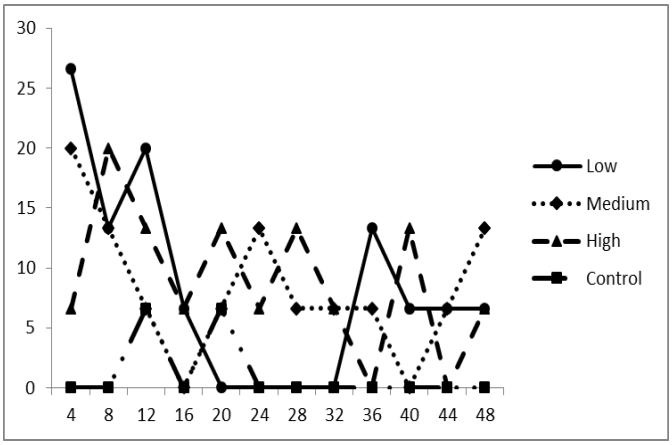

Fig 1: Mortality test variety FH-142 as compared to control at low, medium and high concentrations of pyrethroid (cypermethrin)

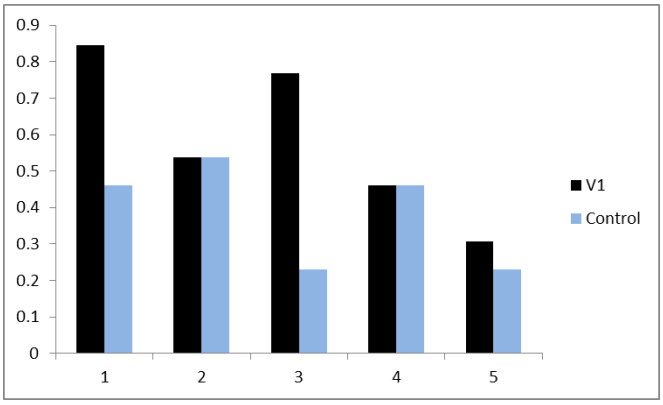

Fig 2: Field population dynamics results of year 2016 in FH- 142 as compared to control variety

4. Discussion

Bt cotton was found more effective to control pink bollworm as compared to conventional cotton in Yangtze area [10], in which pink bollworm resistance was observed under sprayed field condition, so our results were in agreement because pink bollworm infestation was found lower/high mortality was observed when cypermethrin was sprayed in Bt cotton. Our results were also in agreement to another studies in which higher mortality was observed when Bt cotton was sprayed with pesticide [11], so we also asserted that Bt cotton would not result in higher infestation in fields sprayed with pesticides. Pink bollworm has susceptibility to Bt cotton and the ratio of development of pink bollworm was slow on Bt cotton [12], our results were also in agreement to above study as higher mortality was observed when Bt cotton was treated with pesticide. Bt cotton has resistance against pink bollworm [13], our results to the study were slightly different as pesticides in present research caused higher mortality of pink bollworm. The low concentration of Bt protein has partial resistance against pink bollworm while high concentration of Bt protein has full resistance against pink bollworm [14], our results were also in agreement to above studies that high concentration of pesticides sprayed on Bt cotton caused high mortality. Bt cotton provided high yield production and effective control against pink boll worm, in which bollworms has less damaged to cotton [15], so our results were in agreement because the less infestation of pink bollworm was observed on sprayed Bt cotton. The high concentration of Bt cotton showed resistance against pink bollworm [16], our results were also in agreement to above study as the high mortality of pink bollworm in pesticide sprayed Bt cotton was observed. Results indicated that larvae of pink bollworm both newly hatched and mature were having susceptibility to Bt cotton, it prohibited the pupation of pink bollworm and decreased the weight of pupal when increased the concentration [17], so our results were also in agreement that high concentration of pesticide in Bt cotton caused high mortality of pink bollworm.

5. Conclusion

As a conclusion, it can be stated that ELISA showed uptake of Bt protein upto second trophic level in Bt cotton cropping system. But less mortality in Bt cotton also proved that pink bollworms were having resistant strains.

6. Acknowledgement

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7. References

1. Aiken CS. The Cotton Plantation South. Transportation Information Service of Germany, Gesamtverb and der DeutschenVersicherungswirtschafte. V. (GDV), Berlin, http://www./tis_e/baumwoll/baumwoll.htm, 2002-2006.
2. Economic Survey of Pakistan, 2015-16.
3. Shah MA, Memon N, Shaikh MA, Mal B. Biology of pink bollworm *Pectinophora gossypiella* (Lepidoptera: Galechiidae) on different temperatures under controlled conditions. Sindh University Research Journal.(Sci. Ser.). 2013; 45(2):321-324.
4. Tabashnik BE, Wu K, Wu Y. Early detection of field-evolved resistance to Bt cotton in China: Cotton bollworm and Pink bollworm. J Invert. Path. 2012; 110:301-306.
5. Gutierrez AP, Ponsard S. Physiologically based demographics of Bt-cotton Pest interactions I. Pink Bollworm resistance, refuge and risk. 2006; 191:346-359.
6. Ramalho FS, Gonzaga JV. Methodology of the application of pyrethroids against cotton boll weevil and pink bollworm. Tropical Pest Management. 1991; 37(4):324-328.
7. Kazafy Al, Sabry H. Effect of some pesticides with different taget sites on the pink bollworm, *Pectinophora gossypiella*. Archives of phytho and Plant protection. 2013; 46(8):942-951.
8. Rajput IA, Syed TS, Gilal AA, Ahmed AM, Khoso FN, Abro GH *et al*. Effect of Different Synthetic Pesticides Against Pink Bollworm *Pectinophora gossypiella* (Saund.) on Bt. and non-Bt. Cotton Crop. Journal of Basic and Applied Science. 2017; 13:454-458.
9. Tabashnik BE, Dennehy TJ, Sims MA, Larkin K, Head GP, Moar WJ *et al*. Control of Resistant Pink Bollworm (*Pectinophoragossypiella*) by Transgenic Cotton That Produces *Bacillus thuringiensis* Toxin Cry2Ab. Applied and environmental Microbiology. 2002; 68(8):3790-3794.
10. Wan P, Wu KM, Huang M, Wu J. Seasonal pattern of infestation by pink bollworm *Pectinophora gossypiella* (Saunders) in field plots of Bt transgenic cotton in the Yangtze River valley of China. Crop Protection. 2003; 23:463-467.
11. Wan P, Huang Y, Wu HH, Minsong H, Shengbo C, Tabashnik BE *et al*. Increased Frequency of Pink Bollworm Resistance to Bt Toxin Cry1Ac in China. Plos One. 2012; 7(1-9):e29975.
12. Liu YB, Tanashnik BE, Timothy J, Patin AL, Sims MA, Meyer SK *et al*. Effects of Bt Cotton and Cry1Ac Toxin

on Survival and development of Pink Bollworm (Lepidoptera: Gelechiidae). Journal of Economic Entomology. 2001; 94:5.
13. Tabashnik BE, Wu KM, Wu Y. Early detection of field-evolved resistance to Bt cotton in China: Cotton bollworm and pink bollworm. Journal of Invertebrate Pathology. 2012; 110:301-306.
14. Liu YB, Tabashnik BE, Meyer SK, Carriere Y, Bartlett AC. Genetics of Pink Bolllworm Resistance to *Bacillus thuringiensis* Toxin Cry1Ac. Journal of Economic Entomology. 2001; 94:248-252
15. Dhillon MK, Pampapathy G, Wadaskar RM, Sharma HC. Impact of Bt transgenic cottons and insecticides on target and non-target insects pests, natural enemies and seed cotton yield in India. Journal of Agriculture Science. 2012; 82:248-54.
16. Wang L, Wan P, Cong S, Wang J, Huang M, Tabashnik BE *et al*. Adult Exposure to Bt Toxin Cry1Ac Reduces Life Span and Reproduction of Resistant and Susceptible Pink Bollworm (Lepidoptera: Gelechiidae). Journal of Economic Entomology. 2016; 109:1357-1363.
17. Lgnoffo CM, Graham HM. Laboratory and field cage test with *Bacillus thuringiensis* against pink-bollworm larvae. 1967; 9:390-394.