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Pink bollworm showing genetic resistance against cry1Ac expressing transgenic cotton

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Abstract
Transgenic cotton expressing *cry1Ac* gene was tested for its resistance against it target pink bollworm. Objective of this experiment was to observe either Bt protein is expressed in Bt cotton or not. If it is expressed in Bt cotton then how pink bollworm can react to its Bt host. Experiment was consisted of laboratory as well as field studies. Laboratory studies showed that pink boll worm was not having effect from Cry1Ac expressing cotton. Though there were numerical differences in the life cycle of the larvae but there was no significant difference in Bt and non-Bt cotton varieties. Field results were also in conjugation with field studies in which it was found that no significant difference was observed in population dynamics of pink boll worms on Bt and non-Bt varieties. These results confirm that pink bollworm are either transporting Bt protein to excrete outside body successfully or they have developed system to down regulate the *cry1Ac* gene from Bt plant, or pink bollworms have up regulated resistant gene by unknown mechanism which is resulting in more resistant pink bollworms progeny in the fields of transgenic cotton. Further research should be carried out to reduce or eliminate resistance development in pink bollworms against Bt cotton.

Keywords: Pink bollworms, Bt cotton, resistance development, efficacy

Introduction
There are many cash crops in Pakistan. Cotton (*Gossypium hirsutum* L.) is one of the main cash crop of Pakistan after Sugarcane. It plays a vital role in the economy of Pakistan. It is used as a purpose of fiber and edible oil. It adds 1.5% in the GDP of Pakistan while 1.7% added in agriculture[1]. *Bacillus thuringiensis* (Bt) are gram positive bacteria that are present in the transgenic crops. These gram positive bacteria produce insecticide protein, these insecticidal proteins are used to reduce the infestation of bollworm larvae. It causes direct effect on the development of bollworm larvae [2]. The spray of crystalline proteins (Cry1Ac)on the cotton crop, it damage the larvae of Coleopterons, Dipterans and Lepidopterans [3]. In the insecticidal protein present Cry1Ac toxin that's provides economic and environmental benefits [4].
There are several factors that cause decline in cotton production but insect plays a vital role in the decline of quality and cotton production. In insects bollworms cause severe infestation in cotton crops. In a few previous year pink bollworm cause a heavy decline in the seed cotton production. It damages the seed cotton and flower, larvae of pink bollworm feed on the cotton bolls and flower while adult feed on the pollen and nectar [5].
The larval stage of bollworms are more susceptible to insecticidal protein than adult, because adult feed on the pollen and nectar while larvae are feed on the boll and they ingestion Cry1Ac toxin and died [6]. In the transgenic cotton are found Cry1Ac toxin that's are used to kill the larvae of bollworms, it observes that Bt cotton decrease the infestation of bollworms than non-Bt cotton. Bt cotton shows very effective results against pink bollworm and its failure to evaluate resistance in the pink bollworm against *Bacillus thuringiensis*[7]. The high concentration of Cry1Ac are shows effective results against larvae of pink bollworm, it decreases the infestation of pink bollworm in the field and increase the quality and quantity of cotton [8]. Bt cotton are used to suppress the development of pink bollworm, produced high yield of cotton and have no adverse effect on the non-target species[9]. The use of Bt cotton reduces the usage of pesticides and hence the risk posed by conventional insecticides, resulting in less damage to natural enemies and are found to be not affecting the environment and human health. It increases the quality, cotton production and reduce the infestation of insect pests [10].

In the Bt cotton contain Cry1Ac toxin that's bind the cadherin and damage the midgut, when insect feed on the transgenic crops these toxins are active in the stomach of the pink bollworm and cause starvation, due to starvation insects die [11]. In the larvae of bollworms cadherin are used as a receptors that bind the cadherin when Cry1Ac toxin are applied and hence it was observed that bollworms infestation was less [12]. Different insecticidal protein are used to control resistant pink bollworm and susceptible pink bollworm, Cry1Ac, Cry1Ab, Cry1Ba, Cry1Ca these different insecticidal proteins shows very effective results against *Pectinophora gossypiella* [13]. Cry Insecticidal proteins are present in the transgenic crop, after the ingestion of Cry toxin, these toxins are activated in the midgut of insect and it raptures the midgut of insect and insect died, it causes very limited effect on the non-target species [14]. Bt produced *Bacillus thuringiensis* that's are used to kill the lepidopterans insect pests and in 2002 it covers the 4.6 million hectares [15].

2. Material and Methods

2.1 Experimental Design

The trail was conducted at Postgraduate Laboratory, Department of Entomology, University of Agriculture Faisalabad. By using RCBD (Randomized Complete Block Design) consisting four treatment. In each treatment twenty five petri-dish were used. Pink bollworm larvae were collected from the field and reared under laboratory conditions temperature (35±1) and humidity (65±5) without exposure of pesticides. Cotton bolls were provided as a food. Larval population maintained on the cotton bolls in the laboratory. Four variety of cotton was used i) FH-Lalazar, ii) FH-142, iii) FH-146, iv)MNH-786. 1st three varieties was Bt while 4th one was non-Bt Cotton. In each twenty five petri-dishes of variety larval stage of pink bollworm were released in them and check the life cycle of pink bollworm. In fifth larval stage it goes to under pupation and after some time it become adult from pupation.

2.2 Statistical Analysis

t-Test with paired two samples for mean were applied for statistical analysis. In these analysis four treatments were used. This analysis was used to observe the population of insect pests in Bt and non-Bt cotton varieties. In each treatment twenty five petri-dishes were used. 25×3 petri-dishes were used for Bt cotton and only 25 petri-dishes was used of non-Bt.

3. Results

The results of life cycle of pink bollworm on Bt and non-Bt cotton in (Fig 1) showed that the Bt cotton played an effective role in decreasing the development of Pink bollworm while sprayed on the non-Bt cotton showed more disturbance in the development of pink bollworm. It showed that sprayed on the Bt cotton reduce the development of Pink bollworm. While overall results showed that there is a significant difference in the development of pink bollworm larvae on the FH-146 and MNH-786. As (df) = 24, (T) = 1.710882, (P) = 0.013985. Life cycle development test in (Fig 2) showed that the development of pink bollworm larvae slow on the Bt crops than non-Bt while sprayed on the non-Bt cotton reduce the development of pink bollworm. It showed that sprayed on Bt cotton also disturbed the development rate of pink bollworm larvae. While overall results showed that there is a significant difference in the development of pink bollworm larvae on the FH-142 and MNH-786. As (df) = 24, (T) = 1.710882, (P) = 0.000323. it is apparent in (Fig 3) that, the development of pink bollworm larvae was disturbed on the sprayed non-Bt cotton. It showed that sprayed on the Bt crop suppress the development rate of pink bollworm larvae. While overall results showed that there is a significant difference in the development of pink bollworm larvae on the FH-Lalazar and MNH-786. As (df) = 24, (T) = 1.710882, (P) = 0.012905. It is showed in (Fig 4) that, the high infestation was observed in non-Bt cotton while the infestation average in three Bt cotton was less than non-Bt cotton. These results showed that Bt cotton suppressed the development and infestation of bollworms than non-Bt cotton. While overall results showed that there is significant difference in the development/infestation of pink bollworm larvae on the FH-146, FH-142, FH-Lalazar and MNH-786. As (df) = 3, (F) = 13.35997, (P) = 4.24E-05.

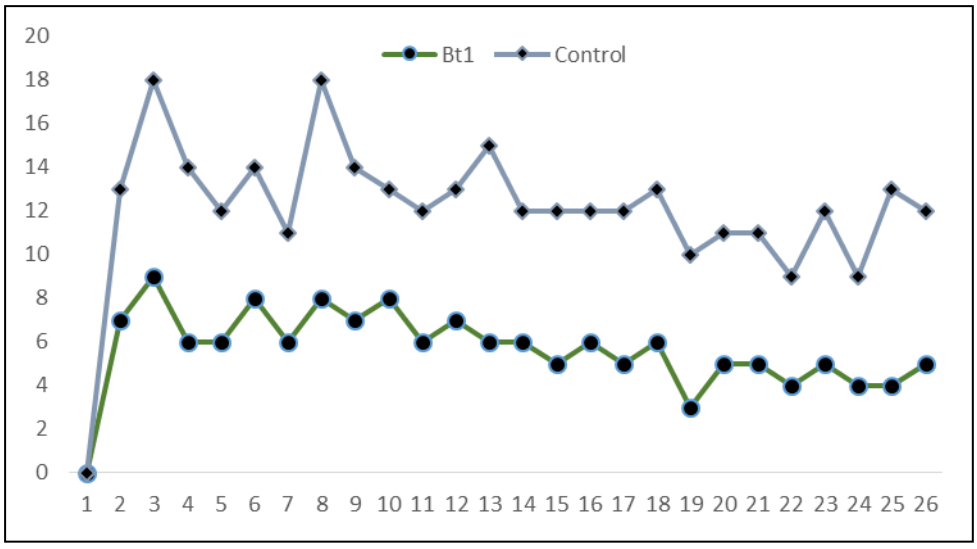

Fig 1: The life parameters of pink bollworms when reared on Bt 1 and non-Bt varieties.

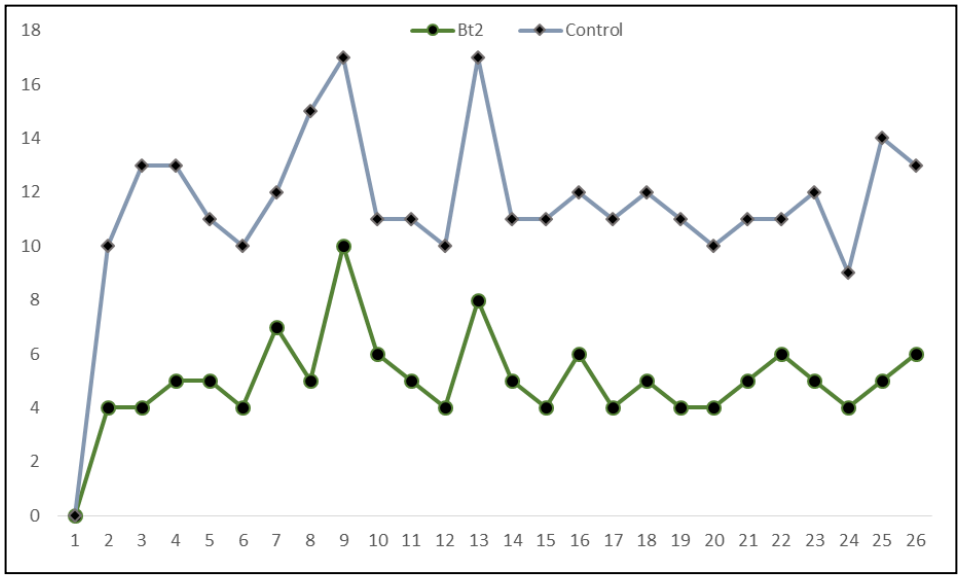

Fig 2: The life parameters of pink bollworms when reared on Bt 2 and non-Bt varieties.

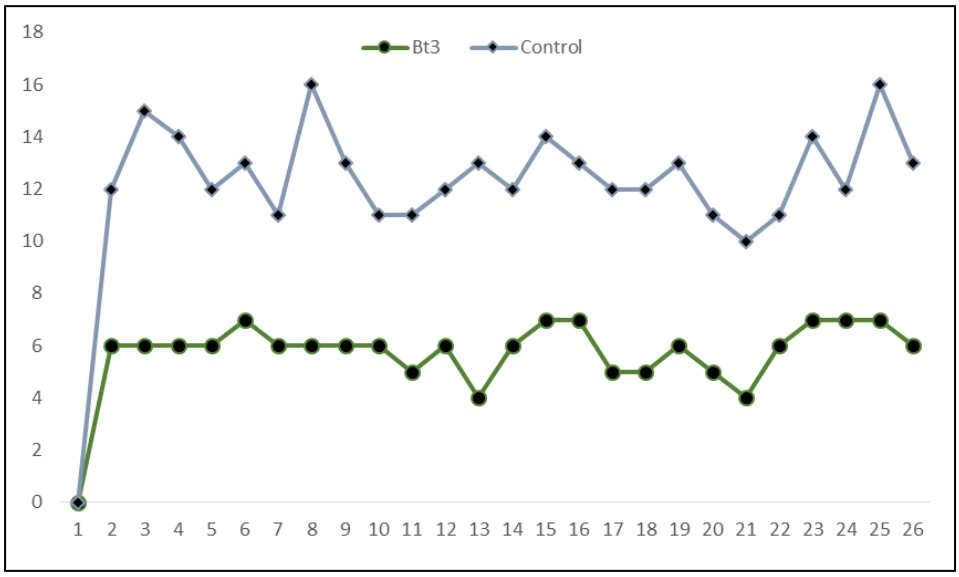

Fig 3: The life parameters of pink bollworms when reared on Bt 3 and non-Bt varieties.

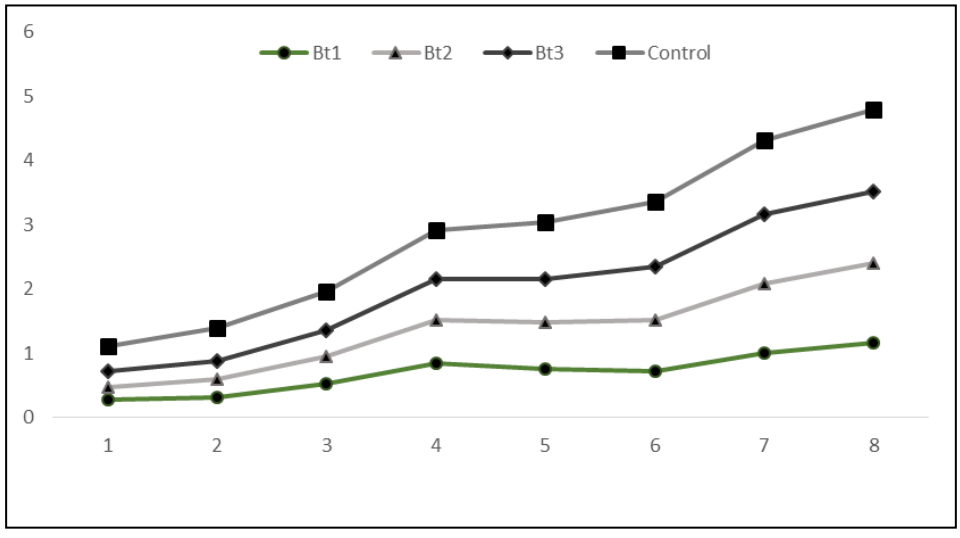

Fig 4: Population dynamics of pink bollworms when observed on Bt and non-Bt varieties under field conditions.

4. Discussion

According to [16] *Bacillus thuringiensis* toxin showed very powerful results against bollworms, it reduces the low infestation and suppressed the development rate of lepidopterous insect pests, so our results were also in agreement because its observed that sprayed non-Bt cotton disturbed the life cycle of pink bollworms while the high concentration of insecticidal protein is also upset the life cycle

duration of the pink bollworm larvae. [17] described that the high concentration of Cry toxin showed low infestation/high mortality than low concentration of Cry toxin, so our results were in also agreement because sprayed cotton effect the development rate of the bollworm larvae while low concentration of insecticidal protein showed cause less effect on the life cycle of pink bollworm. According to [18] *Bacillus thuringiensis* produced Cry1Ac toxin that's are used to control pink bollworm but due to high use of Bt pink bollworm gain resistance against Bt cotton ndBt showed less effective in the control of pink bollworm, so our results were also in agreement because Sprayed non-Bt cotton showed very effective results in the disturbing of life cycle of pink bollworm while Cry1Ac insecticidal protein showed less effective. It was observed that insecticidal proteins in transgenic crop are used to control insect pests and diseases [19], it is used to suppress the population of the bollworms and reduce the attack of bollworms on Bt crops, so our results were also in agreement because sprayed non-Bt cotton affect the development rate of the bollworms while high concentration of insecticidal protein or sprayed Bt cotton were also showed effective results against the development of bollworms. According to [20] the Cry1 toxin are showed effective results against bollworms, it reduce the infestation of bollworms and interfere in the life cycle of pink bollworm, so our results were also in agreement because the life cycle was disturbed on the sprayed non-Bt cotton and high concentration of insecticidal protein also suppressed the development rate of bollworm larvae. [21] observed that the sprayed of insecticidal protein on the cotton field showed effective results against bollworms, it reduce the infestation of bollworms or high mortality, its high concentration directly effect on the development of bollworms larvae, so our results were also in agreement because sprayed non-Bt cotton cause suppression of the development of bollworms while high concentration of insecticidal proteins that's are present in the transgenic cotton showed also very powerful results against development of bollworms. According to [22] the insecticidal protein produced by gram positive bacteria caused effects on bollworm larvae, it reduces the population of bollworm and directly affect life cycle of bollworms, so our results were also in agreement because the sprayed non-Bt cotton showed low infestation and disturbed the life cycle of pink bollworm while high concentration of Cry also showed that disturbed the development rate of pink bollworm.

5. Conclusion

It seems like Bt cotton if expressing Cry1Ac against Lepidopteran pests, then pests like pink boll worms have developed resistance or they up regulate the gene for resistance in terms and/or upregulate detoxification mechanism inside the body. Further research should carry out to observe this phenomena clearly.

6. Acknowledgement

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