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RESISTANCE EVALUATION IN PINK BOLLWORM AGAINST TRANSGENIC COTTON UNDER LABORATORY AND FIELD CONDITIONS IN PAKISTAN

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ABSTRACT

Transgenic crops were developed to show resistance against major Lepidopteran pests attacking and causing loss to the crops. Transgenic cotton containing single gene of resistance against Lepidopteran pests is generally grown in Pakistan. With the time, insects started developing resistance against transgenic cotton. Our studies were conducted under laboratory and field conditions. In the laboratory studies, mortality tests were conducted while under field conditions population densities were observed. Results showed that higher survival of pink boll worms was found on transgenic cotton as well as on non transgenic cotton under laboratory conditions. Field studies were also in conjugation with laboratory studies where higher population densities were observed proving that resistance has been evolved in pink boll worm against transgenic cotton. Hence keeping the assumption it can be asserted that transgenic cotton containing single or pyramided Bt proteins expressed on different genes should be developed and commercialized in Pakistan to combat the resistance in Lepidopteran pests like pink boll worm against transgenic cotton.

Keywords: *Pectinophora gossypiella*, *Bacillus thuringiensis*, resistance, Bt cotton, population densities

INTRODUCTION

Transgenic crops expressing insecticidal proteins derived from *Bacillus thuringiensis* (mentioned here in after as *Bt*) have extensively been used to control insect pests of transgenic crops (Sanahuja*et al.,* 2011; James, 2015). These Bt are incorporated in important crops cotton, corn and soyabeans to avoid the heavy losses from insect pest attack. (Gill*et al*., 1992). These insecticidal proteins provide real tool for controlling lepidopteran pests, but harmless to non-target organisms, including humans and biota. (Mendelsohn *et al.,* 2003; Wu and Guo, 2005; Comas *et al.,* 2014). In 2015, the cultivated area of transgenic crops producing insecticidal proteins reached upto 84 million hectares in the world (James, 2015). Though transgenic crops provide considerable ecological and economic benefits (Wu *et al.,*2008; Tabashnik *et al.,* 2010; Lu *et al.,* 2012; Klumper *et al*., 2014), but resistance development in insect pests can hinder the benefits of Bt crops (Tabashnik *et al.,* 2013, Gassmann *et al*., 2014).

Cotton has been a very important cash crop of Pakistan. Textile industries need better quality of cotton to improve their production standards. Transgenic cotton is the first non-food crop providing a better control to lepidopteran pests (Shelton *et al*., 2002). Crystalline proteins (mentioned here in after as Cry) are called protoxin which are converted into activated toxins by insect midgut proteases and causing the death of the target species (Adang*et al*., 2014). About 40 amino acids remove from the amino terminus and 500 amino acid from the carboxyl terminal due to the activation, converting approximately 130 KDa protoxin into about 55-65 KDa activated toxins (Pardo-López*et al*., 2013). Bt cotton expresses Cry1Ac protein available from 1996 on the US market also available in Mexico, Colombia, Australia, China, India, Argentina and South Africa (James, 2006). Cry 1Ab and Cry 1Ac are considered as better tool to kill the bollworms in Bt cotton (Carrie`re *et al*., 2015).

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Pakistan is 4th largest cotton producing country of 2.30 million tonnes after China, India and United States in 2014-2015 (Pakistan central cotton committee 2014-2015). Cotton shows a major part in earning foreign exchange. The cotton crop production has 1.5 percent role in Gross Domestic Produce (GDP) and 7.1 percent in agriculture value addition (Anonymous 2014-2015). Pakistani farmers spend approximately $ 3 billion annually on different types of pesticides in which more than 80 percent spend on cotton, particularly cotton bollworms (Rao, 2007).

Pink bollworm, *Pectinophora gossypiella* (Saunders) (Gelichiidae: Lepidoptera) is one of the most damaging pest of cotton and causes heavy loss in terms of quantity and quality of cotton. The toxin that is articulated in Bt cotton is susceptible for *P. gossypiella* (Tabashnik *et al*., 2000). The larvae damage the floral outgrowths, bolls, seeds and flowers (Hassan *et al*., 2014). Recently, it has become a serious threat to transgenic and non-transgenic cotton in Sindh and southern Punjab (Ahmed, 2013).

Resistance developed in *P. gossypiella* against the Cry1Ac under in-vitro condition is due to change in cadherin gene because Cry1 Ac resistance occurs due to mutation of 5 strains in-vivo culture of Pink bollworm in Arizona, United States (Carrie`re *et al*., 2006; Fabrick and Tabashnik 2012). Resistance against Cry1Ac was recognized by intake bioassay of pink bollworm on Cry1Ac and observed higher survival against Cry1Ac protein from Armali district in Gujrat (Dhurua, 2011).

The halo effect for *P. gossypiella* as global pest was first documented, which feeds exclusively on cotton in the United States and China (Wu, 2005). In the United States, planting of non-Bt cotton refuges was the primary strategy for delaying pink bollworm resistance to Bt cotton from 1996 to 2005 (Tabashnik *et al*., 2010). Monsanto also confirmed the resistance in pink bollworm against Bollgard I in four different districts of India including Bhavnagar, Armeli, Rajkot and Junagarh (Monsanto, 2010).

Refuges of non-Bt cotton areas are important factor that delay the resistance in *P. gossypiella* in Arizona State and recessive inherent resistant (Tabashnik *et al*., 2005; Tabashnik *et al*., 2012). Since 2006, pink boll worm eradication has been conducted by releasing the sterile moth in the field resulting in minimized attack of *P. gossypiella* (Tabashnik *et al*., 2010).

Pink bollworm resistance is due to four recessive cadherin alleles ($r_1 r_2 r_3$ and r_4) of Pgcad 1 which is responsible for resistance against Cry1Ac protein under laboratory condition in Arizona, United states. (Morin, 2003). The present study was conducted to evaluate the resistance development in pink bollworm (*Pectinophora gossypiella*) against commercially used transgenic and non-transgenic genotypes of cotton.

MATERIALS AND METHODS

Laboratory studies

Complete Randomized Design (CRD) was used in laboratory consisting of two treatments each with three replications. Population of pink bollworm was taken from the field and reared under the laboratory condition with relative humidity 60±5%, temperature 29±2°C and light dark photoperiods (16:8 h). *Pectinophora gossypiella* were placed in the rearing cages provided with non-Bt cotton bolls on daily basis. Adults were taken from the colony and were placed in separate rearing cages by providing them sugar solution soaked in cotton. After 5-6 days of mating female laid eggs and 1st instar larvae were hatched. This F1 generation was taken and used for bioassay studies.

Bioassay

For Bioassay**,** two treatments were performed to check the mortality on daily basis for consecutive 7 days. Different plant parts including bolls and squares of Bt and non-Bt cotton were fed with first instar neonate larvae of *Pectinophora gossypiella.*1st instar larvae from rearing cage were separated and were placed in petri-dishes wraped with parafilm. In 1st treatment, one larva was placed in each petri-dish feeding on Bt boll repeated three times as replications. Each replication was containing ten petri-dishes. Similarly, in 2nd treatments 1st instar larvae were provided with non-Bt boll as a food source. The bolls were changed at every 24 hours interval and data of mortality was recorded on daily basis till 7 days. Larvae which survived after 7 days exposure to Bt and non-Bt bolls. Total 3 bioassay studies were repeated without modification in procedure.

Field studies

Area dimensions and plot design

The field research was conducted at Entomological Research Area, University of Agriculture, Faisalabad, Pakistan. The experiment was laid out in Randomized Complete Block Design (RCBD) in the field with two treatments having three replication for each treatment. To avoid the effect of treatments on nearby insect population 5-m distance was left between plots (Men *et al*., 2003). Transgenic cotton FH-Lalazar (Ayub Agriculture Research Institute, Faisalabad, Pakistan) expressing Cry1Ac compared with the non-transgenic cotton NIAB-2008 (Nuclear Institute for Agriculture and Biotechnology, Faisalabad, Pakistan). The cotton was sown in last week of April with recommended seed rate. The experimental plot for each replicate was maintaining the row to row distance of 2.5 ft and plant to plant distance of 30 cm. Recommended agronomic practices were carried out throughout the growing season of the crop and no pesticide was sprayed in experimental area.

Sampling of pink bollworm population densities under field condition

Data of Pink bollworm population was recorded from the flowers, squares and green bolls on weekly basis from last week of september to mid-november. 15 plants were selected from each replication in a treatment for recording the pest population. The mean population on each plant part i.e., flowers; squares and green bolls was recorded. Percent infestation was the indirect reflection of comparative resistance of transgenic and conventional cotton against Pink bollworm.

Statistical Analysis

The data regarding the *P. gossypiella* field population densities was subjected to the two way ANOVA with unequal variances. While mortality percentage in laboratory studies was subjected to the t-test with unequal variances. In all statistical analysis significant difference of $P<0.05$ was used.

RESULTS

The results showed moderate mortality of *P. gossypiella* in mortality tests (Fig. 1) in FH-Lalazar cotton variety as compared to non-Bt NIAB-2008 (*P*=0.37; df=12). Overall there was no significant difference in mortality of pink boll worm on Bt cotton as compared to non-Bt cotton. However numerical differences were found in mortality tests of pink boll worms on Bt cotton as compared to non-Bt cotton.

The results revealed moderate mortalities of *P. gossypiella* in mortality tests (Fig. 2) in FH-Lalazar cotton variety as compared to non-Bt NIAB-2008 (*P*=0.03; df=11). Overall significant difference was observed in mortality of pink bollworm on Bt cotton as compared to non-Bt cotton. However numerical differences were found in mortality tests of pink boll worms on Bt cotton as compared to non-Bt cotton.

The results exhibited moderate mortality of *P. gossypiella* in mortality tests (Fig. 3) in FH-Lalazar cotton variety as compared to non-Bt NIAB-2008 (*P*=0.35; df=12). Overall there was no significant difference was observed in mortality of pink boll worm on Bt cotton as compared to non-Bt cotton. However numerical differences were found in mortality of pink boll worms on Bt cotton as compared to non-Bt cotton.

The field experiments to compare the pink bollworm (*Pectinophora gossypiella*) larval population densities of flowers, squares and bolls on transgenic cotton as compared to non transgenic cotton variety (fig. 4), results reveal that some numerical differences in *P. gossypiella* population on transgenic and non-transgenic variety were found but overall there was no significant difference in mean population of *P. gossypiella* (df=1, F=0.42 and *P*=0.53).

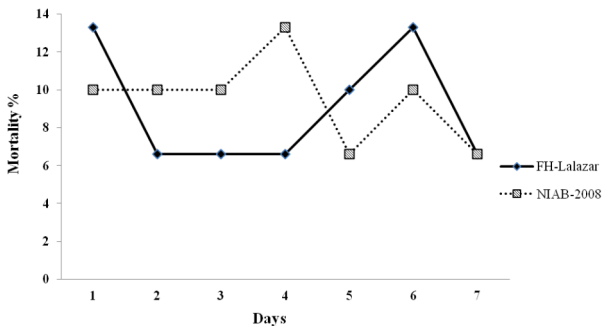

Fig. 1

1st mortality test of pink boll worm larvae on Bt and non-Bt cotton varieties.

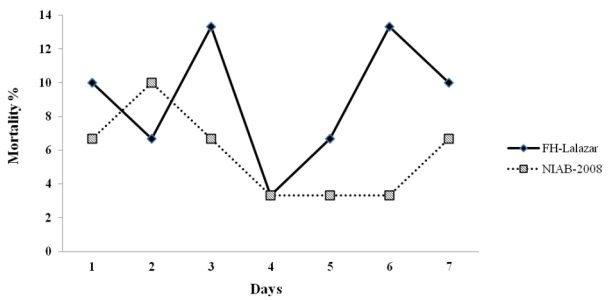

Fig. 2

2nd mortality test of pink boll worm larvae on Bt and non-Bt cotton varieties.

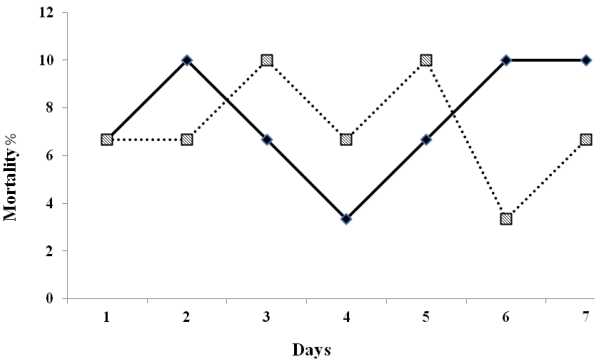

Fig. 3

3rd mortality test of pink boll worm larvae on Bt and non-Bt cotton varieties.

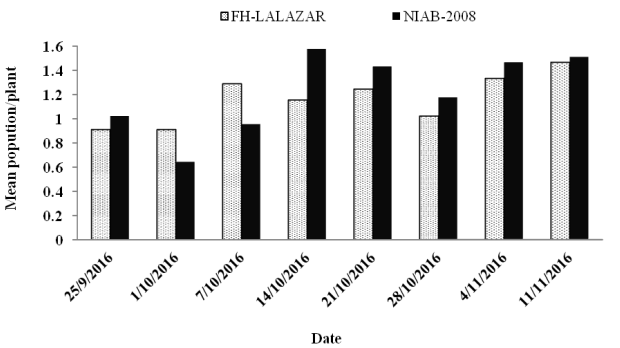

Fig. 4

Population densities of pink boll worms on Bt and non-Bt cotton varieties under field conditions.

DISCUSSION

Transgenic crops were found as good substitute instead of insecticidal spray in fields with more production and profitability to the farmers (Choudhary *etal*., 2014).Transgenic crops are the best source of getting higher economical benefits as compared to conventional crops and also have less effect on the environment (Wu *et al.,*2008). Bt crops were more successful for controlling the Lepidopteran pests, but with passage of time they have developed resistance against Bt crops (Tabahsnik *et al*., 2012). transgenic cotton is the first harmless non-food crop providing genuine tool for controlling bollworms (Wu and Guo, 2005). In Pakistan resistance was developed against Bt cotton in Lepidopteran pests at field level (Arshad *et al*., 2015).

In Pakistan studies to determine the allele were conducted at limited level espceically in pink boll worms, previous studies were conducted for Lepidopteran pests in Bt cotton which have similar phenomena of resistance development as of pink boll worms. Our laboratory studies were in agreement with Tabashnik *et al*.,2005 in which resistance was found at laboratory level, hence in our studies resistance was found during mortality tests which showed moderate survival on Bt cotton as compared to control.

Pink bollworm resistance in India was evolved against single Bt toxin Cry 1Ac and it was first time reported in 2008

(Carrie`re*et al*., 2016). In 2002 reported that *Helicoverpa zea*got field evolved resistance against single gene Bt Cotton in USA(Carrie`re*et al*., 2016). In our studies resistance was also found in pink boll worms at field level where more population densities were found in Bt and non-Bt cotton fields. Our results showed that pink boll worms showed moderate resistance against Bt cotton might be with recessive allele which were in agreement with (Tabashnik *et al*., 2000). Different strategies for controlling resistance development in Lepidopteran pests were used including high dose, refuge strategy, push pull technique and CRISPRCas, but only few techniques proved to be successful to eliminate resistance in Lepidopteran pests. In Arizona, USA resistance was reduced upto the level and pink boll worms were controlled with passage of time (Tabashnik *et al*., 2012). So it can be asserted that Bt cotton containing double or multiple pyramided toxins can help to delay the resistance development in Lepidopteran pests in Pakistan.

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