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EVALUATION OF TRANSGENIC COTTON CULTIVARS CONTAINING CRY TOXINS FROM *BACILLUS THURINGIENSIS* AGAINST THRIPS

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is a significant fiber crop that is contributing 7 to 10% in the GDP of Pakistan. In the production of cotton, Pakistan is ranked at 4th position among the major cotton growing countries. Various insect pests, especially sucking and chewing pests, attack cotton crop. Among the sucking pests, thrips (*Thrips tabaci* Lindeman) causes the most significant damage to cotton at seedling stage. Due to tremendous usage of insecticides, these pests have developed resistance against insecticides. Therefore, cultivation of resistant cotton varieties is the best tactic that can minimize the attack of thrips on this crop. In the present study, five Bt cotton varieties with different cry toxins were evaluated for their resistance/susceptible response to thrips. The cotton cultivars selected for this study were including four Bt cotton cultivars (two single and two double toxins) and one non-Bt cotton cultivar. The experiment was carried out under climatic conditions of Faisalabad, Pakistan. There was not any pronounced difference in thrips population on different Bt cotton cultivars either they were single or dual toxin gene cotton cultivars. MNH-186 and FH-141 showed decreasing trend in population of thrips with the passage of time as compared to other Bt cotton cultivars. The little variation in thrips population among the Bt cotton cultivars showed that all Bt cotton varieties had good control effect on the thrips population. Thrips population was insignificant with respect to leaf position i.e. upper, middle and lower leaves. Correlation study of thrips population with different environmental factors exhibited r values of -0.2213, 0.49837 and 0.41735 for temperature, humidity and rain fall, respectively.

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INTRODUCTION

Agriculture is the backbone for progress of industrialization. In Pakistan, 5000 industries have been established, of which 60% are agro-based. Cotton plays an important role in agriculture economy of the country. Sucking and chewing insect pests damage cotton crop up to 4.6% and 19.21%, respectively, while 14% damage was recorded by mealybug on cotton crop (Anonymous, 2005; Khan et al., 2013). About 162 species are known to cause yield losses in cotton crop. In Pakistan, cotton insect pests are generally controlled through broad spectrum insecticides. The plenty of microplitis, campoletis and trichommatids was found in small quantity in Bt cotton as compared to non-Bt cotton. Cultivation of Bt cotton has been found good to control the population of cotton pests (Wu and Guo, 2004).

Thrips, *Thrips tabaci* (Thripidae: Thysanoptera) attack cotton at early vegetative stage because of the presence of good quality of nutrients in the tissues for these insects at this growth stage. In cotton, decline in insecticides application particularly at early growth stage has led to reappearance of aphids and thrips (Men et al., 2005). Cultivation of transgenic cotton is an excellent approach for the control of insects' population nowadays (Gore et al., 2001). The effects of Bt cotton toxins is more pronounced in newly emerging leaves and less in fruiting bodies because of the fact that toxicity is reduced with plant age (Wan et al., 2005). Cotton is viewed as backbone of Pakistan's economy since it is a main source of overseas exchange and play important role in the financial growth of the country. In the world, there are more than 1326 insect species which cause damage on cotton (Atwal, 2002).

Bacillus thuringiensis (Bt) is a gram-positive bacterium known due to its entomopathogenic activity. During the sporulation, it produces an insecticidal protein. These proteins comprise of one or more proteins (Cyt and cry-toxins) known as alpha-endotoxins. Cry proteins show lethal properties and cause the death of targeted insects without any harmful effects on vertebrates and plants. Bt is used as an excellent alternate method to control pests and diseases (Bravo et al., 2005; Gulzar et al., 2020). Cultivation of Bt cotton on a large area is changing the pest situation because Bt cotton is an excellent source for controlling the chewing pests and reduction of pesticides cost. In this study, five Bt cotton varieties having different cry toxins were assessed for their response to thrips attack.

MATERIALS AND METHODS

A field experiment was conducted at University of Agriculture, Faisalabad during 2018 to record the population densities of thrips on five cotton varieties with different levels of cry toxins. The cotton varieties included two single Bt toxin cultivars (FH-142 and MNH-186), two dual toxin gene cultivars (CAM-33 and CA-12), and one non-Bt cultivar (FH-141). Each cultivar was sown in the field using a RCBD design with three replicates. The cotton genotypes were sown during the 2nd week of May 2018. The recommended agronomic practices for cotton cultivation were applied. The research area was kept unsprayed throughout the season; however, necessary and recommended agronomic practices were performed when required. The climatic factors such as temperature, relative humidity, rainfall were also recorded from experimentation site.

The data regarding thrips population were recorded weekly (starting one week after germination) for 7 weeks, early in the morning. For collecting data, arbitrarily 3 plants from each variety were carefully chosen from every plot for thrips population. A single leaf was selected from upper portion as first vegetal, one leave from the central part of the 2nd vegetal and 2 leaves from the lower portion of the 3rd vegetal were selected for collection of thrips from each selected plant.

Collected data regarding population of thrips were statistically analyzed by analysis of variance followed by mean separation by Tukey's HSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Data regarding population of thrips on different Bt cotton varieties at different time intervals after sowing is presented in Figure 1. There were significant pairwise differences among the population means of thrips on different cotton cultivars at all the tested growing stages. The maximum population abundance of thrips (3.30) was on CA-12 and FH-142 followed by FH-141 (3.10) and MNH-186 (2.8). CAM-33 showed the minimum abundance of thrips i.e. 2.50 (Figure 1A). After 2-week growth, the maximum population abundance of thrips was on CA-12 and FH-142 i.e. 3.73 followed by CAM-33 and then MNH-186 having 2.93 and 2.73 thrips, respectively. FH-141 showed the lowest population of thrips that was 2.66 (Figure 1B). After week 3, the highest thrips population was recorded on CA-12 (6.70) followed by MNH-186 (5.6), CAM-33 (5.36) and FH-142 (4.20). On the other hand, FH-141 exhibited the lowest thrips population (2.50) (Figure 1C). After 4-week growth, the greatest thrips population was recorded on MNH-186 and FH-141 (6.00 each) followed by 5.76 and 5.00 in FH-142 and CA-12, respectively. CAM-33 showed the lowest population of thrips that was 4.83 (Figure 1D). After 5-week growth, the highest thrips population was on CA-12 followed by FH-142 i.e. 5.16 and 4.26, respectively. Varieties FH-141 and CAM-33 exhibited moderate thrips population of 3.86 and 3.20 respectively. MNH-

186 had the lowest population of thrips i.e. 3.86 per plant (Figure 1E). After 6-week growth, CA-12 had the maximum thrips population (6.13) followed by 5.86 thrips on FH-142. FH-141 and MNH-186 had moderate populations of thrips i.e. 4.86 and 4.46 respectively while the lowest thrips population (4.33) was recorded on CAM-33 (Figure 1F). After 7-week growth, FH-142 showed the highest thrips population (8.03) followed by CA-12 (7.86), FH-141 (6.46), CAM-33 (6.33) and MNH-186 (6.20) (Figure 1G).

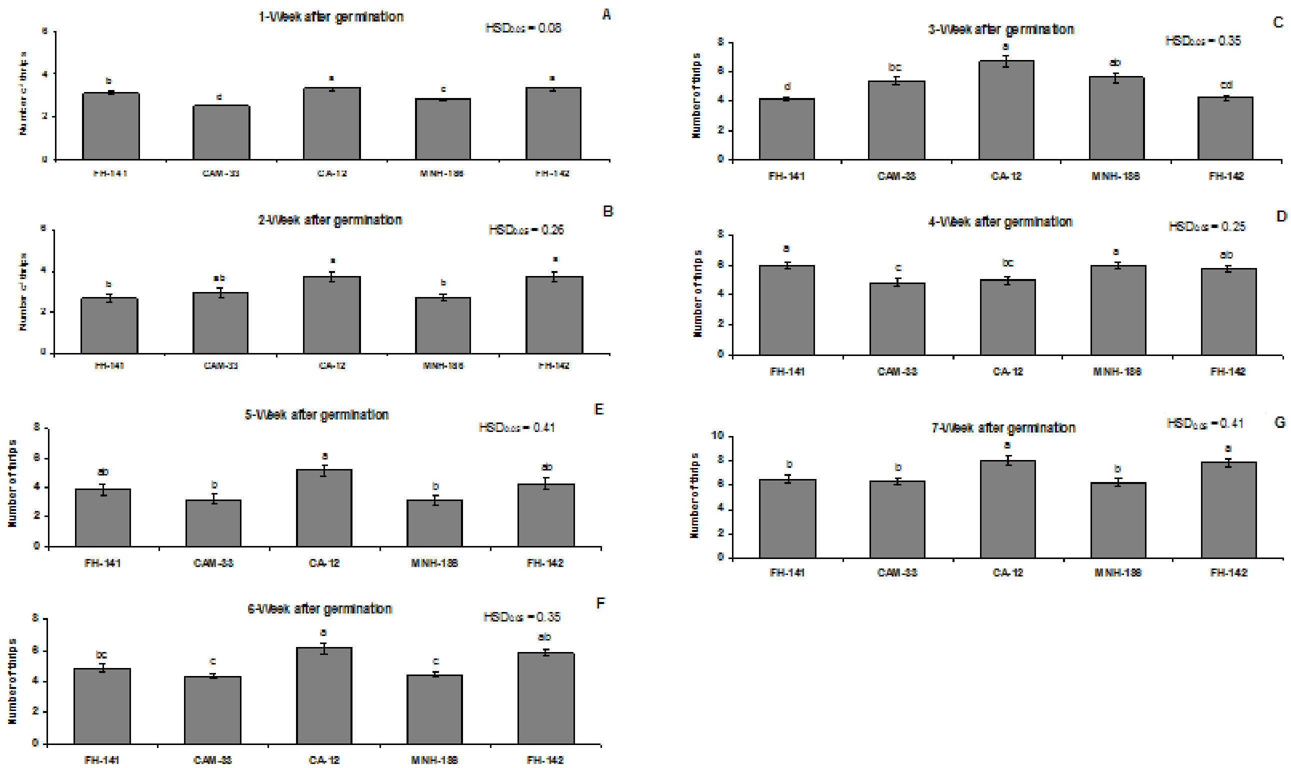

Figure 1: Thrips population at different growth stages of transgenic cotton cultivars containing Cry toxins from *Bacillus thurigiensis*. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD test.

The findings of this research work are in lines with the results of various previous studies. The studies of Bakhsh et al. (2009) were similar to our study where the advance cultivars of Bt cotton were cultivated to study the level of protection they gave to insect's damage and also the agronomy and morphology related characteristics of these varieties. There was 70-100% control of insect damage on different cotton varieties. In Bt cotton varieties, the effect of cry toxin was significant. Likewise, Abro et al. (2004) studied the infestation level of insects on both Bt and non-Bt varieties under field conditions. The level of infestation of thrips population was different on Bt than on non Bt varieties of cotton at different growth stages. By contrast, Akram et al. (2013) reported a different behavior of whitefly and thrips populations on 8 Bt and 5 non-Bt varieties. They found that the population of these sucking pests was more on the Bt as compared to non-Bt varieties. Storer et al. (2001) compared the life cycle of army worm on Bt and non-Bt corn varieties. The toxin present in the ear of corn crop had negative effect on growth of army worm at larval stage. Larval death was more on Bt than on non-Bt varieties where only 15-40% larvae survived on Bt plants. Bt corn also had 80% less feeding of adults. Bt maize TC1507 produced Cry1F protein to give protection to the plants against feeding by many lepidopteran pests (Storer et al., 2010).

Table 1 shows the coefficient of correlation 'r' values of thrips population against meteorological factors to explain the linear relationship. For temperature, r value was -0.2213, which showed negative correlation between temperature and thrips population. It means for every single degree increase in temperature there will be a decrease of 0.2213 populations of thrips. For humidity, r value was 0.49837, which indicated positive correlation between humidity and thrips population showing that for every single percent increase in humidity, there will be an increase of 0.49837 in thrips population. For rainfall, r value was 0.41735 that represents positive relationship between rainfall and thrips population indicating that for every single mm increase in rainfall, an increase of 0.41735 in thrips population will occur.

Table 1: Correlation between thrips population and metrological factors.

Metrological factors	r values
Temperature	-0.2213
Humidity	0.49837
Rainfall	0.41735

CONCLUSION

In this research work, population growth of thrips was studied on different Bt cotton cultivars. Growth of thrips was not affected on different Bt cotton cultivars either they were single or dual toxin gene cotton cultivars. MNH-186 and FH-141 showed decrease growth of thrips with the passage of time than other Bt cotton cultivars. However, the population growth difference among the Bt cotton cultivars was little showing that all Bt cotton cultivars had good control potential of thrips population with the passage of time without the spray of insecticides.

AUTHORS' CONTRIBUTION

SM and MA designed the studies, did experimental work and collected data, SM and IHK contributed in paper writing, AJ prepared graphs and carried out statistical analysis, NA edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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