ORIGINAL ARTICLE

ISSN (Print): 2517-9675
ISSN (Online): 2518-2625

IMPACT OF MICRONUTRIENT ON THE RESISTANCE OF *HELICOVERPA ARMIGERA* TO SOME ADVANCE CRY1AC EXPRESSING COTTON VARIETIES

Saba Tahseen[1] • Muhammad Zeeshan Majeed[1] • Sohail Ahmed[2] • Qazi Waseem[3] • Idrees Ahmad Nasir[4] • Muhammad Asam Riaz[1*]

AUTHOR'S FFILIATION

Department of Entomology, University College of Agriculture, University of Sargodha, Sargodha, Pakistan.
[2]Department of Entomology, University of Agriculture, Faisalabad, Pakistan
[3]Nuclear Institute for Agriculture (NIA), Tandojam, Pakistan
[4]Seed Biotechnology Laboratory, Center of Excellence in Molecular Biology, 87-West Canal Bank Road, Thokar Niaz Baig, 53700 Lahore.
*Corresponding author's email: asam.riaz@uos.edu.pk

Keywords:
Helicoverpa armigera, Bt cotton, Bt resistance, micronutrient

ABSTRACT

Background *H. armigera* is a very notorious pest. It is widely present in all cotton growing areas globally.

Methodology Eleven Bt-cotton lines, viz., CIM-598, CIM-599, CIM-602, CIM-612, CIM-616, VH-305, MNH-886, FH-142, FH-LZ, CYTO-124, and CYTO-177 and one non-transgenic CIM-608 were sown for this study. Larvae were fed on Bt and non-Bt cotton with and without foliar application of $MgCl_2$ and data for Larval Survival Time (LST) and Percent Leaf Consumption (PLC) and Percent Larval Weight Change (PLWC) of bollworm larvae feeding on leaves was recorded. Infestation of bollworm and yield of cotton with and without foliar application of $MgCl_2$ was recorded. Enzyme Linked Immuno-Sorbent Assay (ELISA) was performed to determine the Cry1Ac concentration in the cotton leaves.

Results LST and PLC of bollworm larvae feeding on Bt varieties were significantly higher and PLWC was significantly lower than those feeding on non-Bt variety. Foliar application of magnesium chloride on the Bt varieties significantly changed the LST, PLC and PLWC of bollworm larvae while larvae feeding on non-Bt variety were unaffected. On the basis of Foliar Spray Response Index (FSRI), three Bt varieties; CIM-599, CIM-616 and VH-305 were found highly responsive to foliar application of $MgCl_2$. The results of ELISA revealed that Cry1Ac concentration in cotton leaves ranged from 30 to 40 ppm and foliar application of $MgCl_2$ significantly increased the concentration of Cry1Ac in CIM-599, CIM-602, CYTO-124, CYTO-177 and VH-305 by 20, 25, 19, 22 and 21% respectively and yield of CIM-612, CIM-599, CIM-602, CYTO-124, CYTO-177, FH-142, FH-LZ and VH-305 by 17, 21, 31, 23, 16, 23 and 33% respectively and significantly decreased the infestation of bollworm on Bt varieties CIM-599, CIM-602, CYTO-124, CYTO-177, FH-142, FH-LZ, MNH-886 and VH-305 by 33, 42, 33, 39, 26, 38, 34 and 57%, respectively. The decreased infestation of bollworm on CIM-599, CIM-602, CYTO-124, CYTO-177 and VH-305 was linked to the increased level of Cry1Ac concentration after foliar application of $MgCl_2$.

Conclusion Conclusively, all Bt varieties have got resistance to *H. armigera* larvae in Pakistan. The use of micro and macronutrients for better yield and production is recommended on transgenic cultivar to decrease the infestation o f *H. armigera.*

INTRODUCTION

Cotton is one of the most valuable crop of Pakistan. Pakistan is at number 4th for growing of cotton and number 3rd as an exporter of raw cotton globally. Overall production of cotton was 12.01 million bales (PES 2015-16). Many factors are involved in yield losses of cotton. Among these insect pests pose serious problem.

Cite As: Tahseen S, M Raza, M Fahad, MZ Majeed, M fzal, S Ahmed, Q Waseem, AM Farooq, IA Nasir, MA Riaz (2016) Impact of micronutrient on the resistance of *Helicoverpa armigera* to some advance Cry1Ac expressing cotton varieties. *J. Environ. Agric.*, 1(2): 111−119.

Cotton plant harbors 1326 insect species (Atwal 2005) and 93 insect and mites species have been reported in Pakistan (Yunus and Yousaf 1979). The annual losses caused by insect pests in a cotton crop are 2.9 million in Pakistan. Among all insect pest, bollworms caused a serious problems (Khan and Hameed 2005; Liu et al. 2006). *Helicoverpa armigera* is a very serious and destructive pest of cotton, *Gossypium spp.* and is considered to damage many crops like sorghum, cotton, oilseeds, maize, coarse grains, tomato, groundnut and vegetables in many areas of the world (Zaluckiet al. 1986; Butter and Singh 1996).

Small scale farmers apply the insecticides against this pest without complete information about insecticide and application methods (Bernard and David 2001). In Pakistan, farmers use much more pesticide in cotton for better production which ultimately causes resistance in the insect pests (Pray et al. 2002). The farmers spend about US 300$ for the pesticides to manage this pest every year, usually 80% pesticides are being applied on cotton, especially against bollworms (Rao 2007). All farmers use insecticide of various natures against bollworms (Arshad et al. 2009). American bollworm became extremely resistant against number of insecticides; pyrethroids, carbamate and organo-phosphate groups (GeMei et al. 1997; Tan and McCaffery 2007). Enhanced resistance to insecticides in *H. armigera* (Forrester et al. 1993; McCaffery 1998; Murray et al. 2005) is usually leading towards the interest in establishing alternate techniques for its management including the development of resistant varieties (Wilson et al. 1998; Khan et al. 2003).

Transgenic cotton is one of the alternatives to cope with bollworm. The Bt cotton is highly IPM compatible mainly because it can decrease use of insecticides and boosts up natural enemy populations (Fitt et al. 2000). Use of Bt cotton also adds other benefits like better control of targeted pests, low cost production, increase in yield and better biological management (Edge et al. 2001).

An emerging issue of Bt cotton is the appearance of increased tolerance of *Helicoverpa* spp. to Bt varieties. It has been stated that the frequency of resistance alleles has increased substantially in some field populations of *H. zea* in Australia, China, Spain and the United States (Tabashnik et al. 2008) and *H. armigera* in Pakistan (Alvi et al. 2012).

The larval growth and development might be disturbed due to difference in nutritive values of the host plants of *H. armigera* which nourish them (Liu et al. 2004; Ruan and Wu 2000). Particular immature stage development and body mass of pupae of bollworms are importantly afflicted with the host plants (Liu et al. 2004). The quality of host plant is strongly interrelated with the growth and development and population dynamics of pests (Bernays 1990; Ruan and Wu 2000; Liu et al. 2004). Promoting the growth of plant by using nutrient supplements could be another strategy to control the insects and hence, increase the farmers' yield. The optimum level of macro- and micronutrients are required for plant growth and yield improvement. These nutrients play a vital role in plant physiological and biochemical processes, therefore, improve plant health and yield (Ahmad et al. 2009; Pathak et al. 2012; Rab and Haq 2012). The foliar application of macronutrient, nitrogen in the form of urea produced higher delta endotoxin in transgenic cotton and subsequently higher seed cotton yield per hectare (Basavanneppa et al. 2015). Similarly, the foliar application of micronutrients particularly magnesium in combination with zinc increased the seed cotton yield with better net return and improved benefit cost ratio (Singh et al. 2015).

The present research was aimed at determining the resistance of *H. armigera* to Bt cotton and indirect impact of foliar application of micronutrient magnesium chloride on the resistance of *H. armigera.*

MATERIALS AND METHODS

Experimentation

Eleven Bt- and one non-Bt cotton varieties were sown during the last week of May 2014 in Research Area of University College of Agriculture, University of Sargodha, Sargodha. The transgenic cotton varieties; CIM-612, CIM-598, CIM-599, CIM-602, CIM-616, VH-305, MNH-886, FH-142, FH-LZ, CYTO-124, and CYTO-177 containing Cry1Ac toxin protein whereas non-transgenic cultivars CIM-608 were taken from Central Cotton Research Institute (CCRI), Multan. The above mentioned Bt and non-Bt varieties of cotton were sown according to the standard agronomic practices recommended by the Agriculture Department of Punjab Seeds were treated with imidacloprid (Confidor 70WSC @ 5g kg^{-1}) before sowing. Crop was sown under RCBD factorial and replicated thrice on the bed with 60 cm (bed to bed), 30 cm (RxR) and 20 cm (PxP) distance with a plot size of 3m x 1.8m. A pre-emergence weedicide Dual gold (S-metolachlor) was applied after sowing the crop.

Adults of *H. armigera* were collected from field, allowed to lay eggs, and reared in the Laboratory of Department of Entomology, University College of Agriculture, University of Sargodha, under controlled conditions (25±2°C and 65±5 % RH) in Petri dishes on artificial diet (Ahmed et al. 1998). Briefly, the artificial diet was prepared by mixing Part A (chickpea flour 100 g, methyl 1, 4 hydrobenzoate 1.6 g, ascorbic acid 1.6 g, yeast 16 g, streptomycin 1 g, distilled water 150 mL), Part B (Agar agar 3 g, Distilled water 150 mL) and Part C (Vitamin 1.6 mL and Corn oil 2 mL). Mixture of Part B and ingredients of Part C were added in the mixture of

Part A. After mixing the all mixtures, it was put into refrigerator for further usage. Synchronized population of larvae was used for further experiments.

Magnesium chloride (1%) was sprayed on sixty days old Bt and non-Bt varieties twice with an interval of one week. Leaves of sprayed and unsprayed Bt and non-Bt cotton plants were taken from field, washed, wiped to dry with tissue paper and cut into a leaf disc of four cm^2 and placed on wet filter paper in petri plates were fed to ten 2nd instar cotton bollworm larvae after 24 hours of $MgCl_2$ application. The experiment was laid out under Completely Randomized Design (CRD) factorial and repeated three times. The treatments were; a) leaves of non-Bt and Bt cotton, b) leaves of non-Bt and Bt cotton sprayed with 1% $MgCl_2$. The petri-plates with larvae were placed under controlled condition in laboratory. The developmental parameters such as weight of larvae, leaf consumption and survival time of cotton bollworm in each treatment were studied with an interval of 24 h till 96h. A disc of fresh leaves without larvae were placed to determine the losses due to evapotranspiration and the factor of evapo-transpiration was deducted from the leaf weight before calculating the leaf consumption. Fresh leaves were replaced with older leaves after every 24 hours till 96 hours. Leaves before and after 24 hours of larval release were weighed to determine the leaf consumption and leaves consumed was converted to percent leaves consumed (PLC). Similarly, larvae were also weighed before offering leaves and weighed again after an interval of 24 hours till 144 hours. The change in larval weight was converted to PLWC. The larval survival time (LST) was calculated by counting the number of dead and alive larvae after an interval of 24 hours till 144 hours.

Foliar spray response index (FSRI) was calculated by modifying the formula of Fisher and Maurer (1978) for insects.

$$FSRI = (1 - Wa \,/\, (Wb\,))/D$$

Where

Wa = PLWC with foliar spray

Wb = PLWC without foliar spray

$$D = \frac{Mean\ PLWC\ of\ all\ larvae\ with\ foliar\ spray}{Mean\ PLWC\ of\ all\ larvae\ without\ foliar\ spray}$$

To estimate the infestation of bollworm larvae on cotton varieties, the damage of bollworm larvae on BT and non-Bt cotton varieties was recorded on weekly basis. Insecticide was not used to control the insect-pest of cotton at any stage of crop. Five plants were randomly selected from each plot and each plant was examined for bollworm infestation. Number of infested bolls and total number of bolls per plant were counted to determine percentage infestation. The yield of seed cotton was estimated from each plot of cotton variety and yield was converted to tons per hectare.

Enzyme Linked Immunosorbent Assay (ELISA) was performed at Center of Excellence in Microbiology, University of Punjab, Lahore to detect the presence of *Cry*1Ac proteins in leafs of different transgenic varieties of cotton at different time interval. Three leaves of each variety were collected from the research field and stored at 4ºC before performing ELISA. The analysis was performed by using ENVIROLOGIX Qualiplate kit for *Cry*1Ac/*Cry*1Ab (500 River side Industrial Parkway, Portland, ME 04103-1486, Winooski, VT, USA) according to the manufacture's instruction. A brief procedure is that the samples for ELISA were prepared by adding ten mm^2 disc of cotton leaf to 250 µL of extraction buffer in 500 µL tubes. Leaf tissues were disrupted and mixed thoroughly. Fifty micro liters of extracted samples followed by 50 µL *Cry*1Ac/*Cry*1Ab enzyme conjugate was added to each well of strip previously coated with antibodies. Fifty micro liters of extraction buffer was added in blank. The contents were thoroughly mixed by moving the well plate on bench top in circular motion for 20-30 seconds. Then the wells were covered with tape and incubated for 1-2 hours at ambient temperature. After the incubation period, the contents of well were thoroughly shaken into a suitable container or sink. The wells were washed with wash buffer three times. Plates were placed on paper towel to remove the excessive water. One hundred micro liter of substrate was added to each well. The contents of well were mixed thoroughly and incubated for 15-30 minutes. 100 µL of stop solution (1.0 N HCl) was added to each of well and mixed thoroughly. Resultantly the contents of well-turned yellow due to the addition of stop solution. The plate was read by using Absorbance Microplate reader (ELx800, Biotek, Winooski, VT, USA) at 450nm after 30 minutes. The analysis software Gen5 Data was used for microplate reader data analysis. The standard curve of Cry1Ac was prepared by adding 4, 12 and 24 ng g^{-1} of toxin in the wells.

Statistical analysis

The values of LST and toxin concentration were subjected to CRD factorial analysis. The means were compared by Tukey's HSD test. Analysis of covariance was performed on PLC, PLWC and percentage infestation by taking time interval as covariance. Data of yield was analyzed under factorial RCBD and means were compared by Tukey's HSD test. The analysis was performed by using R-software.

RESULTS

Larval survival time, percent larval weight change and leave consumption of H. armigera larvae

Mean comparison of larval survival time (LST) on Bt and non-Bt varieties without foliar application of magnesium chloride showed that the non-Bt varieties

CIM-608 was non-significantly different from Bt varieties CIM-598 and FH-LZ. Bt varieties CIM-612, CIM-599, CIM-616, CYTO-124 and VH-305 were having higher survival time than non-Bt variety. Foliar application of magnesium chloride significantly changed the larval survival time on all varieties except CIM-612 and non-Bt variety CIM-608. The PLC of non-Bt variety was significantly different from all Bt varieties while non-significantly different from CIM-612, FH-LZ. The foliar application of magnesium chloride significantly changed the leaf consumption of seven Bt varieties; CIM-598, CIM-599, CIM-616, CYTO-124, CYTO-177, MNH-886 and VH-305. There was a significantly increase in weight of larvae feeding on Bt varieties CIM-612 and CIM-616 while larval weight decreased significantly when fed on other varieties as compared to non-Bt variety. Foliar application of magnesium chloride significantly changed the larval weight when fed on CIM-612, CIM-598, CIM-599, CIM-616, CYTO-177 and VH-305. Foliar spray response index of all Bt and non-Bt plants indicated that only three Bt varieties CIM-599, CIM-616 and VH-305 responded well and positively to the foliar application while the rest responded either less or negatively (Table 1).

Biplot analysis

A genotype into environment (GGE) Biplot is constructed by plotting the first PC1 (Principal component) scores of insect growth parameters and genotype against the scores of second PC2. The "which-won-where" of GGE is an effective tool and consists of irregular polygon and a set of lines drawn from the biplot origin. The three insect parameters have been divided into five sectors with different winning cultivars. According to the biplot shown in Figure 1, the polygon has five corners with five corner genotypes VH-305, FH-LZ, CIM-612, CIM-616 and FH-142 that were the most responsive ones. The first sector represents PLWC with genotype CIM-616 and CIM-612 as the most favorable. The second sector represents PLC with genotype CIM-599, CIM-598, MNH-886, CYTO-177 and FH-142 G1 as the most favorable. The third sector have VH-305, CIM-602 and CYTO-124 as most favorable genotype. The fourth sector represents LST with FH-LZ and CIM-608 as most favorable genotype. The angle between the parameters describes the correlation coefficient. In other words, the cosine angle describes the correlation coefficient between the related plant parameters.

Principal component analysis was for LST, PLC, PLWC and FSRI of twelve Bt and non-Bt varieties of cotton with and without foliar application of cotton with and without foliar application of magnesium chloride. The data presented in Figure, revealed that Bt and non-Bt genotype were located very close to each other. However, Figure revealed that foliar application of magnesium chloride changed the life parameters and response of transgenic varieties was changed.

The four insect parameters have been divided into four sectors with different winning cultivars. According to the biplot shown in Figure 2, the polygon has four corners with four corner genotypes CIM-612, CIM-616, CIM-599 and FH-142 that were the most responsive ones. The first sector represents FS.PLWC the most favorable. The second sector represents FS.PLC with genotypes FH-142, CIM-598, MNH-886 and CIM-602 as the most favorable. The third sector represents FSRI

Table 1 Mean comparison of Larval Survival Time (LST), Percent Larval Weight Change (PLWC) and Leaf Consumption (PLC) by *H. armigera* larvae feeding on cotton varieties with and without foliar application of $MgCl_2$

Varieties	Larval survival time (hours)		Percent leaf consumption		Percent larval weight change		
	-MgCl$_2$	**+MgCl$_2$**	**-MgCl$_2$**	**+MgCl$_2$**	**-MgCl$_2$**	**+MgCl$_2$**	**FSRI of plants**
CIM-608	64.7±5.3 D	60±4.3C	31.8±4.5E	37.9±1.8D	26.7±4.9 C	23±2.1B	0.53
CIM-612	120.6±4.2a	122±4.4a	29.8±3.2e	31.6±4.9d	**170±2.1b**	**349±2.8a**	-8.70
CIM-598	**72.8±2.3cd**	**21.5± 3.5e**	**70.5±3.1b**	**52±2.1b**	**-29.1±3.9c**	**-38.4±2.9e**	-3.00
CIM-599	**80.2±3.5c**	**24.4±3.7e**	**63.9±2.9c**	**45.9±3.8c**	**8±2.1d**	**-7.2±3.1c**	**17.86**
CIM-602	**48.3±2.8 e**	**22.6±1.9e**	50.2±3.9d	43.9±3.4c	-43.1±3.1 e	-41.7±3.8e	-0.32
CIM-616	**80.7±3.2c**	**26.8±2.9e**	**57.5±1.5d**	**11.9±3.2e**	**182.3±2.5a**	**-25.5±3.8d**	**10.69**
CYTO-124	**82.6±3.4c**	**50.3±1.6d**	**54.8±3.5d**	**32.9±2.1d**	-48.8±3.2e	-45.3±2.2e	-0.66
CYTO-177	**32.5±2.5f**	**48.3±4.6d**	**67.5± 4.1b**	**51.1±3.8b**	**-41.3±3.9e**	**-22.9±3.5d**	-4.18
FH-142	**48.8±2.6 e**	**20.5±2.9e**	80.2±3.2a	86.4±2.9a	-56.9±2.5 f	-63.8±4.2g	1.13
FH-LZ	**74.6±5.6cd**	**82.6±3.5b**	36.6±2.8e	37.5±2.1d	24.1±2.8c	21.5±2.9b	0.90
MNH-886	**40.3±6.5e**	**27.4±4.1e**	**81.9±2.4a**	**57.3±3.5b**	-62.8±2.9g	-64.9±3.7g	0.19
VH-305	**90.6±6.2b**	**24.4±3.9e**	**20.6±2.1f**	**43.6±4.1c**	**-38.1±3.1e**	**-55.2±3.1f**	**4.20**

*Similar alphabets in columns show the non-significant difference between the varieties and different alphabets show the significant difference between varieties (Tukeys HSD test). Bold numbers indicate the significant difference between the treatments (T-test). Negative values indicate the percent decrease in the weight of larvae/leaf consumption and positive values indicate the percent increase in the larval weight/leaf consumption. FSRI (foliar spray response index) was calculated by modifying Fischer and Maurer (1978) formula. Higher values of FSRI indicate higher response of variety towards magnesium chloride. Positive value indicated the increased weight and negative values indicated the decreased weight. CIM-608: Non-Bt

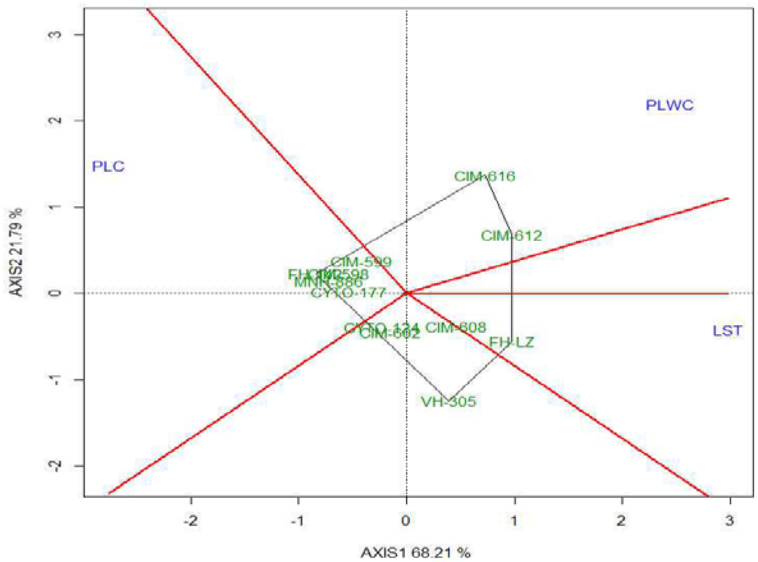

Figure 1 Principal component analysis of Larval Survival Time (LST), Percent Leaves Consumption (PLC) and Percent Larval Weight Change (PLWC) of twelve Bt and non-Bt cotton varieties without foliar application of $MgCl_2$

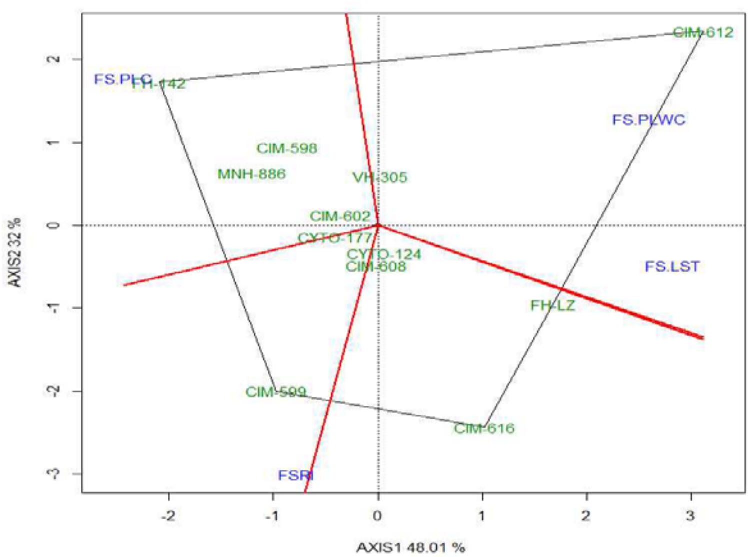

Figure 2 Principal Component Analysis of Larval Survival Time (LST), Percent Leaves Consumption (PLC), Percent Larval Weight Change (PLWC) and Foliar Spray Response Index (FSRI) of twelve Bt and non-Bt cotton varieties after foliar spray (FS) of $MgCl_2$

with genotypes CIM-599 and CYTO-177 as most favorable genotype. The fourth sector has CIM-616, FH-LZ, CYTO-124 and CIM-608 as most favorable genotype.

Concentration of Bt toxin Cry1Ac, larval infestation and yield of Bt and non-Bt cotton varieties

All Bt varieties have almost the same concentration of toxin except CIM-616, FH-142, FH-LZ and MNH-886 have significantly higher concentration of Bt toxin. All varieties have more than 30ppm of Cry1Ac toxin. Magnesium chloride application significantly changed the concentration of five Bt varieties; CIM-599, CIM-602, CYTO-124, CYTO-177 and VH-305 (Figure 3). The infestation of bollworm on Bt varieties was not significantly different from non-Bt variety while three Bt varieties CIM-612, FH-142 and FH-LZ were having even significantly higher level of bollworm infestation.

The application of $MgCl_2$ significantly changed the level of bollworm infestation on all Bt and non-Bt varieties except CIM-612, CIM-598 and CIM-616 (Figure 4). The average yield of two Bt varieties CIM-612 and FH-142 was higher as compared to non-Bt variety while all other varieties were having comparatively lower yield. Foliar application of magnesium chloride significantly changed the yield of Bt varieties except CIM-598, CIM-616 and MNH-886 (Figure 5).

DISCUSSION

As the bollworm is getting resistance to Bt cotton world over (Tabashnik et al 2008). In this context, the present research is aimed at investigating the resistance of *H. armigera* to Bt cotton in Pakistan and the impact of micronutrient on the concentration of Bt toxin. Our results shows that larvae fed on Bt and non- Bt varieties showed significant difference of Larval Survival Time, Leaf Consumption and Larval Weight Change. The survival time of larvae on the most Bt varieties was even more than that of non-Bt variety, one Bt variety CYTO-177 showed minimum value of LST, similarly, the leaves of Bt varieties consumed by bollworm larvae was significantly higher than non-Bt cotton. Larvae consumed very small quantity of VH-305 leaves. However, the weight of larvae decreased significantly when fed on Bt varieties. Out of the used Bt varieties, only four showed the increased weight of bollworm larvae. One Bt variety MNH-886 significantly decreased the weight of larvae. Foliar Spray Response Index (FSRI) of plant suggested the three Bt varieties CIM-599, CIM-616 and VH-305 as the most responding varieties to foliar application. Larval weight decrease or increase seems good parameters to explain tolerance response as compared to Larval Survival Time (LST) and Percent Leaf Consumption (PLC). Because larvae feeding different Bt and non-Bt varieties with similar LST and PLC may have difference in larval weight. We found that the foliar application of magnesium chloride on the Bt varieties significantly changed the survival time, leaf consumption and weight of bollworm larvae while larvae were not affected by non-Bt variety (Table 1). The response of all Bt varieties was not same to foliar application of magnesium chloride. The results of ELISA revealed that the most Bt varieties have the same level of Cry1Ac concentration in leaves. Interestingly, foliar application of magnesium chloride significantly increased the concentration of Cry1Ac in CIM-599, CIM-602, CYTO-124, CYTO-177 and VH-305 by 20.6, 24.9, 19.3, 22.4 and 21.1% respectively.

Change. The survival time of larvae on the most Bt varieties was even more than that of non-Bt variety, one Bt variety CYTO-177 showed minimum value of LST, similarly, the leaves of Bt varieties consumed by bollworm larvae was significantly($p \leq 0.05$) higher than non-Bt cotton. Larvae consumed very small quantity of VH-305 leaves. However, the weight of larvae decreased

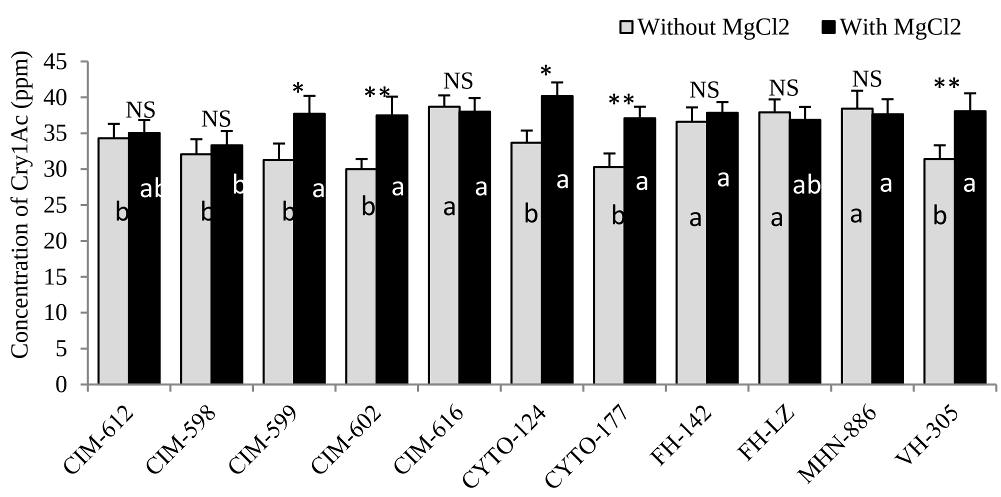

Figure 3 Mean comparison of Bt toxin Cry1Ac Concentration in leaves of Bt cotton with and without the foliar application of $MgCl_2$. Similar alphabets on bars show the non-significant difference between the varieties and different alphabets show the significant difference between varieties (Tukeys HSD test). Black font indicates the mean comparison among the varieties without foliar application of magnesium chloride while white font shows the mean comparison among the varieties with foliar application of magnesium chloride. Means of toxin concentration of each Bt variety with and without foliar application were compared by T-test and significance level is shown above the bars. ($P< 0.05$ *, $P< 0.01$ **, $P< 0.001$ ***, NS non-significant)

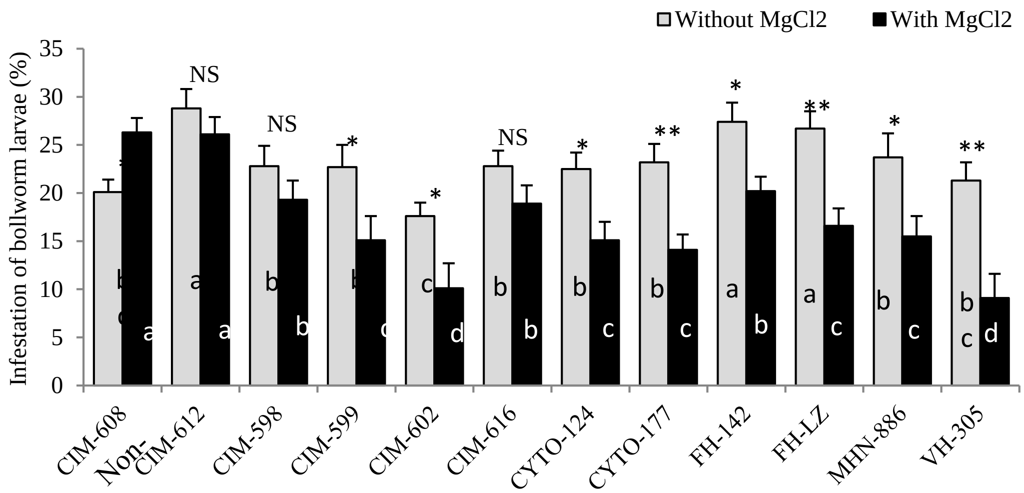

Figure 4 Percent infestation of *H. armigera* larvae on Bt and non-Bt cotton varieties with and without the foliar application of $MgCl_2$. Similar alphabets on bars show the non-significant difference between the varieties and different alphabets show the significant difference between varieties (Tukeys HSD test). Black font indicates the mean comparison among the varieties without foliar application of magnesium chloride while white font shows the mean comparison among the varieties with foliar application of magnesium chloride. Means infestation on each Bt variety with and without foliar application was compared by T-test and significance level is shown above the bars. (P< 0.05 *, P< 0.01 **, P< 0.001 ***, NS=non-significant)

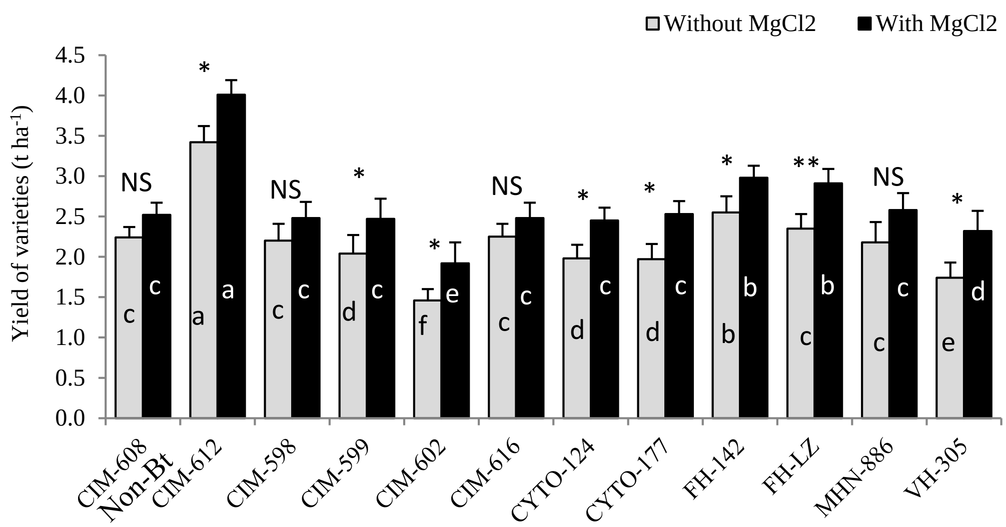

Figure 5 Mean comparison of yield of Bt and non-Bt cotton varieties with and without the foliar application of $MgCl_2$. Similar alphabets on bars show the non-significant difference between the varieties and different alphabets show the significant difference between varieties (Tukeys HSD test). Black font indicates the mean comparison among the varieties without foliar application of magnesium chloride while white font shows the mean comparison among the varieties with foliar application of magnesium chloride. Means yield of each Bt and non-Bt variety with and without foliar application was compared by T-test and significance level is shown above the bars. (P< 0.05 *, P< 0.01 **, P< 0.001 ***, NS=non-significant)

significantly when fed on Bt varieties. Out of the used Bt varieties, only four showed the increased weight of bollworm larvae. One Bt variety MNH-886 significantly decreased the weight of larvae. Foliar Spray Response

Index (FSRI) of plant suggested the three Bt varieties CIM-599, CIM-616 and VH-305 as the most responding varieties to foliar application. Larval weight decrease or increase seems good parameters to explain tolerance response as compared to Larval Survival Time (LST) and Percent Leaf Consumption (PLC). Because larvae feeding different Bt and non-Bt varieties with similar LST and PLC may have difference in larval weight. We found that the foliar application of magnesium chloride on the Bt varieties significantly changed the survival time, leaf consumption and weight of bollworm larvae while larvae were not affected by non-Bt variety (Table 1). The response of all Bt varieties was not same to foliar application of magnesium chloride. The results of ELISA revealed that the most Bt varieties have the same level of Cry1Ac concentration in leaves. Interestingly, foliar application of $MgCl_2$ significantly increased the concentration of Cry1Ac in CIM-599, CIM-602, CYTO-124, CYTO-177 and VH-305 by 20.6, 24.9, 19.3, 22.4 and 21.1% respectively.

The data of percent infestation suggest that bollworms have got resistance to Bt varieties as all varieties have the infestation similar to non-Bt variety. The infestation level decreased significantly in Bt varieties CIM-599, CIM-602, CYTO-124, CYTO-177, FH-142, FH-LZ, MNH-886 and VH-305 by 33.4, 42.6, 32.8, 39.2, 26.2, 37.8, 34.6 and 57.2 respectively. It is concluded that the decreased infestation of bollworm on CIM-599, CIM-602, CYTO-124, CYTO-177 and VH-305 is due to the increased level of Cry1Ac concentration after foliar application of magnesium chloride. The increased infestation of bollworm on non-Bt variety after foliar application could be due to good plant growth which acts as feeding stimulant to bollworm. As magnesium is the central part of chlorophyll, so the increased larval attack, leaf consumption and larval weight gain could be explained by increased chlorophyll contents of leaves and subsequently greenish look of cotton leaves. The seed cotton yield of all Bt varieties was not significantly higher than non-Bt variety, however, the foliar application of magnesium chloride significantly increased the yield of CIM-612, CIM-599, CIM-602, CYTO-124, CYTO-177, FH-142, FH-LZ and VH-305 by 17, 21, 31, 23, 16, 23 and 33% respectively. The response of Bt-varieties seems selective. As nutrient supplement promote plant growth and yield by improving the plant physiological and biochemical processes (Ahmad et al. 2009; Putra et al. 2012; Rab and Haq 2012). The application of macronutrient and micronutrients has been reported to increase the yield of transgenic cotton by increasing the expression of delta endotoxin in transgenic cotton (Basavanneppa et al. 2015).

CONCLUSION

Conclusively, all Bt varieties have got resistance to *H. armigera* larvae in Pakistan. The use of biological control methods along with cultivation of non-Bt varieties and application of micro and macronutrient for better yield and production is recommended.

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