

Elemental and Genetic Analyses of Cotton Crop



**Muhammad Nawaz Shareef University of Agriculture
Multan**

Table of Contents

Description	Page #
Executive Summary	4
Introduction	5
Material and Methods	7
Results	8
Discussions	18
References	20

List of Tables

Table #	Titles	Page #
1	Critical values (Normal ranges) of macro- and micro-elements in soil and leaf.	9
2	Correlation coefficients among the leaf elements, insect and disease infestation	13
3	Cotton leaf samples collected from 11 districts of South Punjab	15

List of Figures

Figure #	Titles	Page #
1	EC, pH, macro and micro-elements in soil samples collected from cotton fields in 11 districts of South Punjab	10
2	Macro and micro-elements in leaves of cotton growing in 11 districts of South Punjab	12

3	Pink Boll Worm (PBW), Army Worm (AW) and Whitefly (WF) infestation percentage of total collected leaf samples of eight most prevalent cotton varieties	14
4	Concentration of Cry1Ac protein in Pink Boll Worm non-infested (PBW-NI) and Pink Boll Worm infested (PBW-I) most prevalent cotton varieties	16
5	Concentration of Cry1Ac protein ($\mu\text{g/g}$) in Pink Boll Worm non-infested (PBW-NI) and Pink Boll Worm infested (PBW-I) most prevalent cotton varieties	17

List of Annexure

Annex #	Titles	Page #
I	Macro-elements in soil and plant samples collected from 11 districts of South Punjab	21
II	Micro-elements in soil and plant samples collected from 11 districts of South Punjab	27
III	Insect infestation of cotton crop in 11 districts of South Punjab	33
IV	Annexure IV: Bt protein expression of Cry1Ac in different cotton varieties	39

EXECUTIVE SUMMARY

Cotton contributes significantly in Pakistan's growth and development. Its cultivation faces complex stress phenomenon and becoming very tricky. Climate, soil health and seed system created present alarming situation in cotton. A field survey was conducted in 11 districts of cotton belt to collect soil and cotton leaf samples and insect infestation data to determine soil and plant elemental status, Bt endotoxin expression and relationship of plant nutrients with insect control. From each district, 20 samples and data collection points were randomly selected, however nearby adaptive research farms were preferred (five out of total 220 points). Standard procedures were followed for sampling and data collection and analysis. Nearly >70% soils of the cotton belt have EC (0.26-3.9 dS/m) and pH (7.14 - 8.50) that is not detrimental for cotton. Soil N was deficient in all soil samples. Soil P ranged from 7-13 ppm (normal) in Bahawalpur division, and Rajanpur and Layyah districts. Soils of Southern Punjab were rich in K and Cu in all districts while normal in Zn concentration in Bahawalpur, Rahim Yar Khan, Rajanpur, DG Khan, Muzaffar Garh and Layyah (>1 ppm). Soils were deficient in Fe in most parts of DG Khan division (<4.5 ppm), in Mn in Multan division (<1.0 ppm), and in B in Multan, Lodhran and some parts of Vehari (<0.5 ppm). Leaf N was low in some parts of Rahim Yar Khan and Bahawalpur (<1.40 ppm). Leaf P and Zn was normal in all samples. Cotton leaves were low in K in some parts of Bahawalpur, Rahim Yar Khan and Rajanpur (<1.49 ppm), in Cu in Bahawalnagar (<7 ppm), in Fe in Bahawalpur, Bahawalnagar and some parts of Multan (<149 ppm), in Mn in some parts of Layyah, DG Khan and Rajanpur (<20 ppm), in B in Bahawalpur division (<20 ppm). Contradictions in concentrations of macro and micro-elements in soil and cotton leaf samples were observed that could be originated where plant had up taken the required elements from soil thus making soil deficient and plant normal, or collected cotton leaf samples from soils/fields where nutrients were applied just before sampling thus making plant deficient and soil normal. In the present studies, plant elemental analysis depicted that leaf P and Mn had negative association with PBW infestation while leaf B had positive association with PBW infestation. Bt expression is dependent on optimum concentrations of plant nutrients. On the other hand, Bt resistance in boll worms is not only due to genetic mutations, plastic responses due to plant micro and macronutrients can produce resistant phenotypes. This also suggests that boll worms can also be managed through alternative means by maintaining micro and macronutrients in cotton plant that are unsuitable for boll worm survival and reproduction – an alternative to the Bt gene-centric view. Efficiency of cotton varieties to

uptake and utilize these elements also varies and has the potential to be exploited for improvement in their bollworm resistance. The present studies suggest further in-depth intensive surveying and breeding and biotechnological interventions to better understand the cotton boll worms susceptibility to Bt endotoxins and genetic improvement in cotton resistance against boll worms. Better crop husbandry techniques and varietal specific production packages may be developed to harvest better yields under these hostile situations. Precision agriculture practices may be promoted for the judicious use of farm inputs and improvement of cotton yield in Punjab.

INTRODUCTION

Cotton is major crop with highest share of 5% in agriculture value addition, 1% of GDP and it plays central role in rural economic development in poverty-stricken areas of cotton belt. In Pakistan, 15% of total cropped area is devoted to cotton that becomes ~3 million hectares. Cotton is mainly cultivated in Punjab and Sindh. The Punjab produces around 65% and Sindh contributes 35% of total cotton production (PCCC, 2016).

In the context of socio-economic growth in Pakistan, cotton has been and will remain as an iconic commodity for the governments. Over the past six decades, various governments took certain policy decisions and intervened for its sustained production at the farm and supply to industrial sector. Several factors are influencing cotton production including un-availability of proper quality inputs (seed, fertilizers, insecticides etc.), machinery, water, climate (rainfall, temperature, etc.) and market price. Among these, climate, soil health and seed system has created present alarming situation in cotton. Researchers attempt to provide solutions and cotton growers struggle to manage these factors and provide optimum environment for cotton production. Optimum environment including nutritional elements is not only essential for plant growth and development but also help to overcome biotic and abiotic stresses. Variation in environment affects plant response or phenotype through differences in gene expression and regulation. Bt cotton produces Cry protein toxic to bollworms but its expression depends on gene-by-environment ($G \times E$) interactions. The $G \times E$ interactions allow a single genotype (individual) to produce a range of phenotypes in different environments (Gibson, 2008).

The overriding assumption in Bt resistance monitoring is that genetic factors are primarily responsible for resistant phenotypes (Moar, 2008) but an alternative to this gene-centric view is that resistance is not solely dependent on resistant genotypes but ultimately on gene expression. In

case of Bt cotton for bollworm control, one Bt cotton variety could exhibit high resistance to bollworm (high susceptibility of insect to Bt) in one environment and low resistance to bollworm (low susceptibility of insect) in another – phenotypic plasticity (Deans et al., 2017). Macro and micro-elements status in plants controls chemical composition of plants (which serves as nutrition of boll worms) on one hand while expression of Bt endotoxin on the other. Ultimately nutrition affects boll worm (insect) susceptibility to Bt endotoxin (Deans et al., 2017).

Keeping in view the above situation, a survey was carried out to understand and determine:

- Soil and plant elemental status
- Bt endotoxin expression
- Relationship of plant nutrients with insect control

MATERIAL AND METHODS

Geography of sample collection site

Soil and cotton leaf samples and insect attack data were collected from 11 districts of Southern Punjab (cotton belt). From each district, 20 sampling and data collection points were randomly selected, however nearby adaptive research farms were preferred (five out of total 220 points).

Elemental analysis of soil samples

Soil and water testing laboratory of three divisions viz., Multan, Bahawalpur and DG Khan collected and analyzed soil samples for soil EC, pH, macro-elements (N, P, K) and microelements (Zn, Cu, Fe, Mn and B). Two soil samples from 0 to 6 and 6 to 12 inches depth were collected separately from each point having cotton crop.

Elemental analysis of cotton leaf samples

Soil and water testing laboratories of three divisions viz., Multan, Bahawalpur and DG Khan also collected and analyzed cotton leaf samples for macro-elements (N, P, K) and micro-elements (Zn, Cu, Fe, Mn and B). Fifty matured leaves were collected separately from cotton plants present at the soil sampling collection point.

Analysis of Bt gene expression

Cotton leaf samples were collected separately from same cotton plant used for soil and leaf elemental analysis at each sample point. Cotton leaves were collected from three positions i.e., bottom, middle and upper portion of plant. Leaf samples were collected in micro-tubes and immediately cryo-preserved in container containing liquid nitrogen for their logistics to Central Hi-Tech Laboratory at MNS-UAM. Concentration of Cry1Ac protein in leaves was determined using ELISA (Enzyme Linked Immuno Sorbent Assay).

Cotton insect and disease infestation

Infestation of cotton by insects like whitefly, Jassid, Thrips, cotton mealy bug, cotton dusky bug, pink boll worm and army boll worm, and disease like Fusarium wilt was observed and recorded. Standard threshold level of each insect and disease was considered for categorization of infested and non-infested cotton crop.

Statistical analyses

Correlations were estimated between soil, leaf elements and insect infestation and also between insect infestation and Bt protein (Cry1Ac) concentration.

RESULTS

Elemental Analysis of Soil Samples

Soil EC, pH, macro-elements (N, P, K) and micro-elements (Zn, Cu, Fe, Mn and B) were determined by Divisional Soil and Water Testing Labs at Multan, Bahawalpur and DG Khan and presented in Figure 1.

Soil Electric Conductivity (EC) and pH: Electric conductivity of soil samples collected from Layyah, Muzaffar Garh, Rajanpur, Multan, Bahawalpur, Bahawalnagar and Rahim Yar Khan ranged from 0-4 dS/m (Figure 1). The EC in the soil samples collected from districts of Vehari, Khanewal, DG Khan, Lodhran, border of Multan and Lodhran, and some parts of Bahawalpur ranged from 4.5-9.0 dS/m while EC of some samples from Multan, Vehari, Lodhran and DG Khan ranged from 9.0-46 dS/m. Soil pH of Bahawalpur and DG Khan Divisions ranged from 7.14-8.50 while that of Multan Division ranged from 8.50-9.70.

Nitrogen (N): The normal range of soil N is 0.043 – 0.064 % (Table 1). Soil N was deficient in all soil samples (Figure 1). It was extremely low ranging from 0.01-0.02 % in Multan division, Layyah, most of the parts of Rajanpur and DG Khan. In Bahawalpur Division and some parts of Layyah and DG Khan, soil N ranged from 0.02-0.03 %. In Muzaffar Garh and some parts of DG Khan, it ranged from 0.03-0.05 %.

Phosphorus (P): The normal range of soil P is 7 – 13 ppm (Table 1). Soil P was deficient in soil samples collected from district Vehari, Khanewal, Lodhran, Muzaffar Garh, most of the parts of DG Khan, Multan and some parts of Bahawalpur, Bahawalnagar and Rahim Yar Khan ranging from 2-7 ppm (Figure 1). It was normal in Bahawalnagar, Bahawalpur, Rahim Yar Khan, Rajanpur and Layyah districts ranging from 7-13 ppm. In some parts of district Bahawalnagar and Layyah, the phosphorus concentration was high ranging from 13-72 ppm.

Table 1: **Critical values (Normal ranges) of macro and micro-elements in soil and leaf.**
(Elemental values below these ranges are considered as deficient)

Sr No	Elements	Soil (ppm)*	Leaf (ppm)*
1	N	0.043-0.064	1.40-2.50
2	P	7-13	0.20-0.35
3	K	80-125	1.50-2.80
4	Zn	1	25
5	Cu	0.2	7
6	Fe	4.5	150
7	Mn	1	20
8	B	0.5	20

* values of all macro- and micro-elements are in ppm except N, which is in %.

Potassium (K): Soils of Southern Punjab were rich in potassium (80-125 ppm) except some parts of Layyah where it ranged from 16-80 ppm (Figure 1). In Bahawalpur, Rahim Yar Khan, Bahawalnagar, Muzaffar Garh and Layyah districts, its concentration ranged from 80-125 ppm.

Zinc (Zn): Zn concentration was sufficient in soil samples from Bahawalpur, Rahim Yar Khan, Rajanpur, DG Khan, Muzaffar Garh and Layyah ranging from 0.99-15.00 ppm (normal is 1 ppm, Figure 1). It was deficient in districts of Multan division and some parts of Bahawalnagar and Rajanpur districts ranging from 0.05-0.99 ppm.

Copper (Cu): Concentration of Cu was sufficient in soils of Southern Punjab (Figure 1).

Iron (Fe): In most parts of DG Khan division soils have Fe deficiency i.e. 0.5-4.4 ppm (Table 1, Figure 1) except some parts of Rajanpur, Muzaffar Garh and Layyah districts were sufficient in iron concentration i.e. >4.5 ppm. Soil of Bahawalpur division was rich in iron i.e. 4.4-17.0 ppm. Multan division had normal range of soil Fe except some areas in Multan, Vehari, Lodhran and Khanewal districts.

Manganese (Mn): Deficiency of manganese was found only in Multan division 0.1-0.9 ppm (Figure 1). Soil Mn was found normal 0.9-19.0 ppm in Bahawalpur and DG Khan divisions.

Boron (B): The concentration of soil B was low in Multan, Lodhran and some parts of Vehari. While all other districts of Southern Punjab had normal range of boron in soil ranging from 0.40-3.80 ppm (Figure 1).

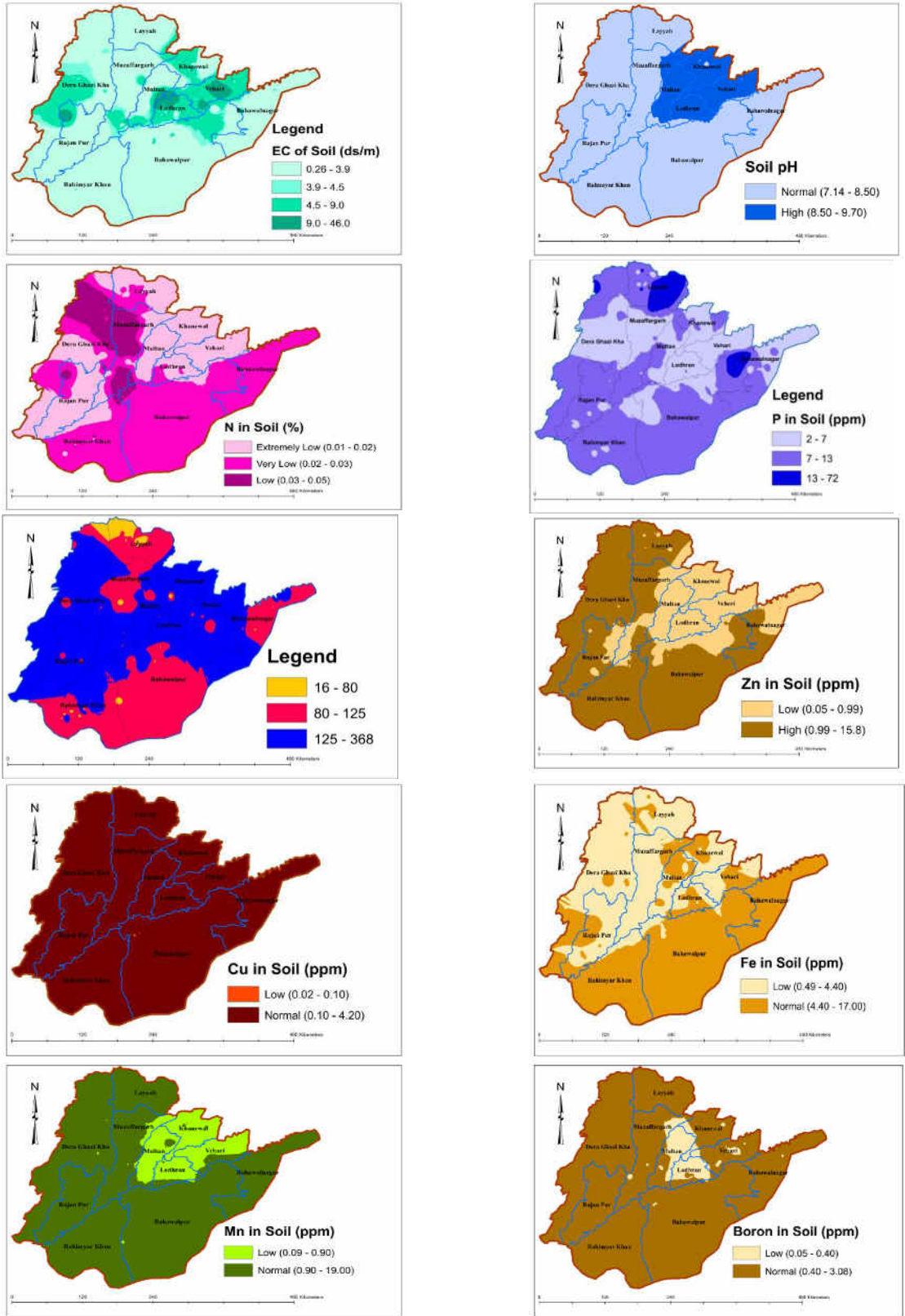


Figure 1: EC, pH, macro and micro-elements in soil samples collected from cotton fields in 11 districts of South Punjab

Elemental analysis of cotton leaf samples

Nitrogen (N): Nitrogen in leaves of cotton plant was found normal (1.40-2.50 %) except some parts of Rahim Yar Khan and Bahawalpur where it was low ranging from 0.52-1.39 % (Figure 2). Cotton leaves collected from DG Khan division were rich in N containing 2.50-5.70% N.

Phosphorus (P): Cotton growing in South Punjab had high leaf phosphorus ranging from 0.35-2.89 ppm, few spots in Multan and DG Khan division had 0.20-0.35 ppm (Figure 2). There was no deficiency of phosphorus in the leaf samples of cotton.

Potassium (K): In all districts potassium concentration in cotton leaves was normal from 1.49-2.8 ppm (Figure 2). Deficiency of potassium in cotton leaf was found only in some parts of Bahawalpur, Rahim Yar Khan and Rajanpur where it ranged from 0.85-1.49 ppm.

Zinc (Zn): Concentration of zinc was sufficient in all cotton leaf samples that ranged from 24-161.3 ppm except some from Multan district (Figure 2).

Copper (Cu): Copper deficiency in cotton leaf was found only in Bahawalnagar where it ranged from 1-6 ppm (Figure 2). It was normal in cotton leaf of all other districts where it ranged from 6-928 ppm.

Iron (Fe): Iron concentration in cotton leaf was low in Bahawalpur, Bahawalnagar and some parts of Multan where it ranged from 5-149 ppm (Figure 2). In all other districts iron concentration was normal ranged from 149-744.

Manganese (Mn): Cotton crop in Southern Punjab had normal range of manganese in leaves (19-156 ppm) except some parts of Layyah, DG Khan and Rajanpur where it was low ranging from (3-19 ppm, Figure 2).

Boron (B): Boron deficiency was found in Bahawalnagar, Rahim Yar Khan and some parts of Bahawalpur districts ranging from 5-19 ppm (Figure 2). In other two divisions boron concentration in cotton leaf was found high ranging from 20-95 ppm.

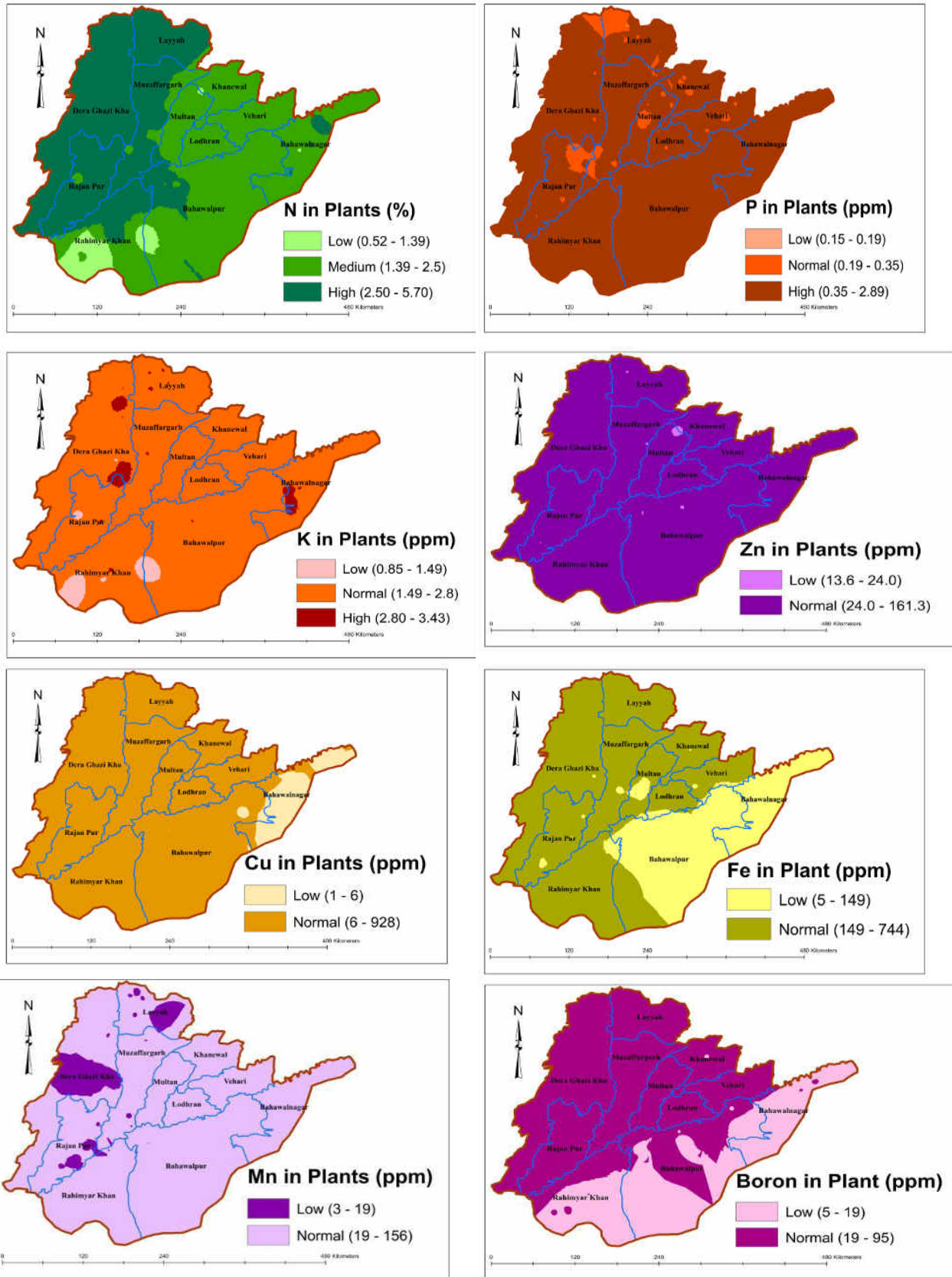


Figure 2: Macro and micro-elements in leaves of cotton growing in 11 districts of South Punjab

Correlation analysis between plant macro and micro-element with insect infestation

Correlation (Pearson coefficient, 'r') values were estimated between the leaf macro and micro-elements and insect infestation (Table 2). Leaf nitrogen was significantly positive correlated with dusky bug infestation. Significant positive correlation was also observed between leaf P and jassid and negative with PBW infestation. Leaf Mn was significantly negative correlated with PBW infestation while leaf boron was positively correlated with PBW infestation.

Table 2: Correlation coefficients (Pearson r) among the leaf elements, insect and disease infestation

Insect/Disease/Trait	Leaf elements							
	N	P	K	Zn	Cu	Fe	Mn	B
ANB	0.17	-0.08	-0.17	-0.69*	-0.13	0.24*	-0.26*	0.22*
White Fly	-0.01	-0.11	-0.04	-0.11	-0.17	-0.11	-0.03	0.05
Pink Boll Worm	0.19	-0.21*	0.11	0.05	-0.06	0.08	-0.23*	0.27*
Jassid	-0.06	0.20*	0.13	-0.01	-0.16	-0.31*	0.14	-0.15
Army Worm	-0.09	0.03	-0.03	0.01	-0.08	-0.11	0.12	-0.08
Thrips	-0.05	0.04	0.08	0.00	-0.10	-0.07	-0.02	-0.02
Mealy Bug	-0.04	-0.01	0.00	0.05	-0.05	0.04	0.04	0.01
Dusky Bug	0.21*	-0.09	0.10	0.04	-0.02	0.15	-0.18	0.25
Fusarium Wilt	0.15	-0.06	0.14	-0.12	-0.04	-0.08	-0.12	0.05

ANP, average number of bolls; *, significant at $P \leq 0.05$

Varietal response to insect infestation

In total 220 cotton leaf samples were collected from 11 districts of South Punjab (Table 3). Maximum leaf samples belonged to IUB-13 (61). Other dominating cotton varieties included FH-142 (29), IUB-15 (22), Z-33 (20), MNH-992 (14), BS-252 (11), Shahkar (10) and Lalazar (9). Eighty percent of the total collected leaf samples belonged to these eight cotton varieties. IUB-13 dominated in Multan and Bahawalpur divisions, FH-142 in DG Khan and Bahawalpur divisions while Z-33 in Bahawalpur and Rajanpur districts.

Highest frequency of Pink Boll Worm (PBW) infestation was observed in BS-252 and FH-142 (64 and 62%, respectively) (Figure 3). Shahkar, MNH-992, Z-33 and IUB-13 showed 40, 36, 35 and 28 % PBW infestation respectively. Army Worm (AW) infestation frequency was highest in Z-33 (30%). No AW infestation was observed in Shahkar. Whitefly (WF) infestation was highest in MNH-992 (79%). WF infestation frequency in other seven cotton varieties ranging 45 to 76%.

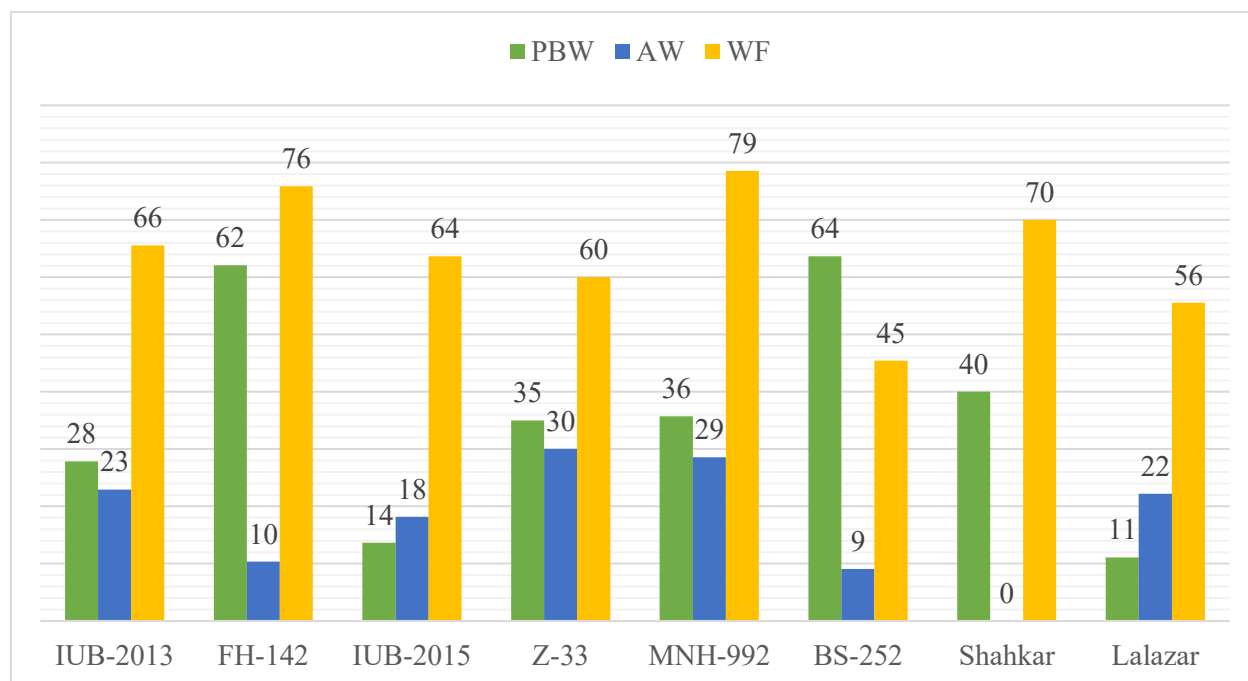


Figure 3: Pink Boll Worm (PBW), Army Worm (AW) and Whitefly (WF) infestation percentage (of total collected leaf samples) of eight most prevalent cotton varieties.

Table 3: Cotton leaf samples collected from 11 districts of South Punjab

Variety	Multan	Khanewal	Lodhran	Vehari	Bahawalnagar	Bahawalpur	Rahim Yar Khan	Rajapur	Layyah	DG Khan	Muzafargarh	Total
IUB-13	4	16	9	5	4	1	10	1	4	3	4	61
FH-142	1	3		3	4	1	1	2	7	5	2	29
IUB-15	6		6	6			1				3	22
Z-33			1			8	2	7		2		20
MNH-992	3				1	3		1		2	4	14
BS-252				2	1	1		4	3			11
Shahkar					2	1			1	2	4	10
Lalazar				1	2	2	1	2			1	9
MNH-1016	3		1									4
MNH-886					1				1			2
BT-146										3		3
Unknown				1		1	1					3
BT-114								2				2
BT-181	1			1								2
BT-602					1		1					2
Cyto-179					1						1	2
Deebal				1	1							2
Theliwala										2		2
B-70							1					1
BH-178									1			1
BS-3					1							1
BT-162					1							1
BT-173								1				1
BT-382											1	1
BT-555							1					1
BT-786										1		1
CIM-343	1											1
FH-786									1			1
FH-NOOR									1			1
KOT-1							1					1
P-09			1									1
S-12			1									1
S-299									1			1
S-32		1										1
Satluj			1									1
Sitara 009						1						1
Tarzan 1						1						1
Wheal Ag	1											1
Total	20	20	20	20	20	20	20	20	20	20	20	220

Genetic Analysis

Most prevalent cotton varieties were categorized into two groups i.e. infested and non-infested considering insect attack above and below standard threshold levels respectively. Cotton leaves were analyzed for the Bt expression to determine the concentration of Cry1Ac protein using ELISA technique. The concentration of Cry1Ac protein was less than critical value of toxin 1.90 $\mu\text{g/g}$ in all leaf samples collected from highly PBW non-infested (24 samples) and infested (11 samples) widely grown cotton varieties IUB-13, FH-142, IUB-15, BS-252, Lalazar, Shahkar and Z-33 (Figure 4). Only three leaf samples from Pink Boll Worm non-infested (PBW-NI) cotton variety IUB-13 had concentration of toxin between 1-1.90 $\mu\text{g/g}$. While concentration of toxin between 1-1.90 $\mu\text{g/g}$ was detected in one sample each from PBW-I cotton varieties viz., IUB-13, FH-142 and BS-252.

Variable Bt expression was detected in 10 tested samples of PBW-NI IUB-13 (0.08 to 1.34 $\mu\text{g/g}$) and in three tested samples of PBW-I IUB-13 (0.08 to 1.36 $\mu\text{g/g}$) (Figure 5). The concentration of toxin in PBW-NI cotton leaf samples from FH-142 was 0.13 $\mu\text{g/g}$ while that in PBW-I was 1.46 $\mu\text{g/g}$. PBW-NI BS-252 cotton leaf samples contained 0.53 $\mu\text{g/g}$ Bt toxin while PBW-I contained 1.32 $\mu\text{g/g}$. The concentration of Bt toxin was similar in PBW-NI and PBW-I leaf samples of Shahkar and Z-33.

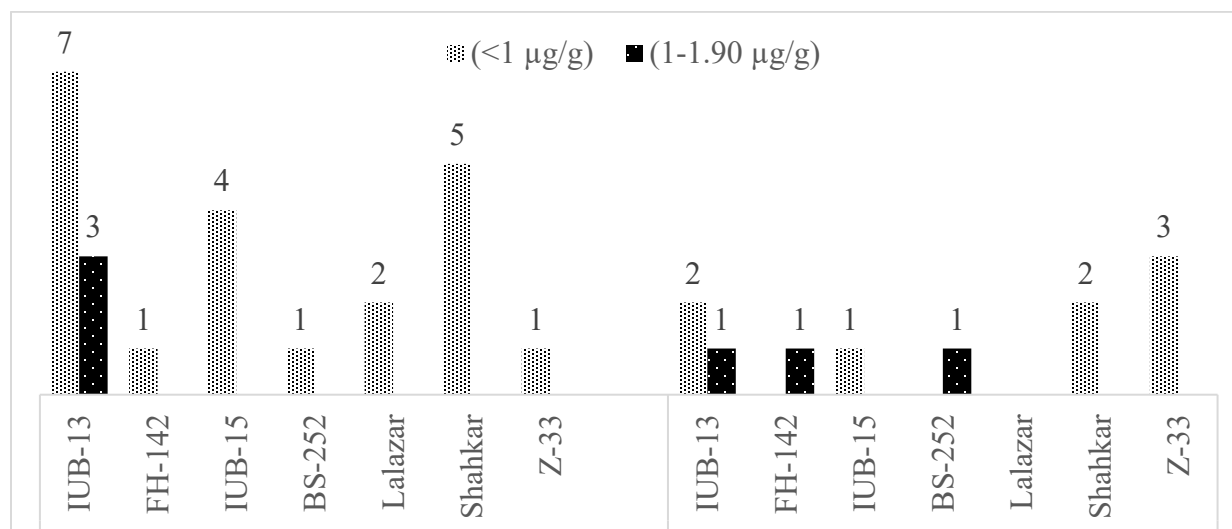


Figure 4: Concentration of Cry1Ac protein in Pink Boll Worm non-infested (PBW-NI) and Pink Boll Worm infested (PBW-I) most prevalent cotton varieties.

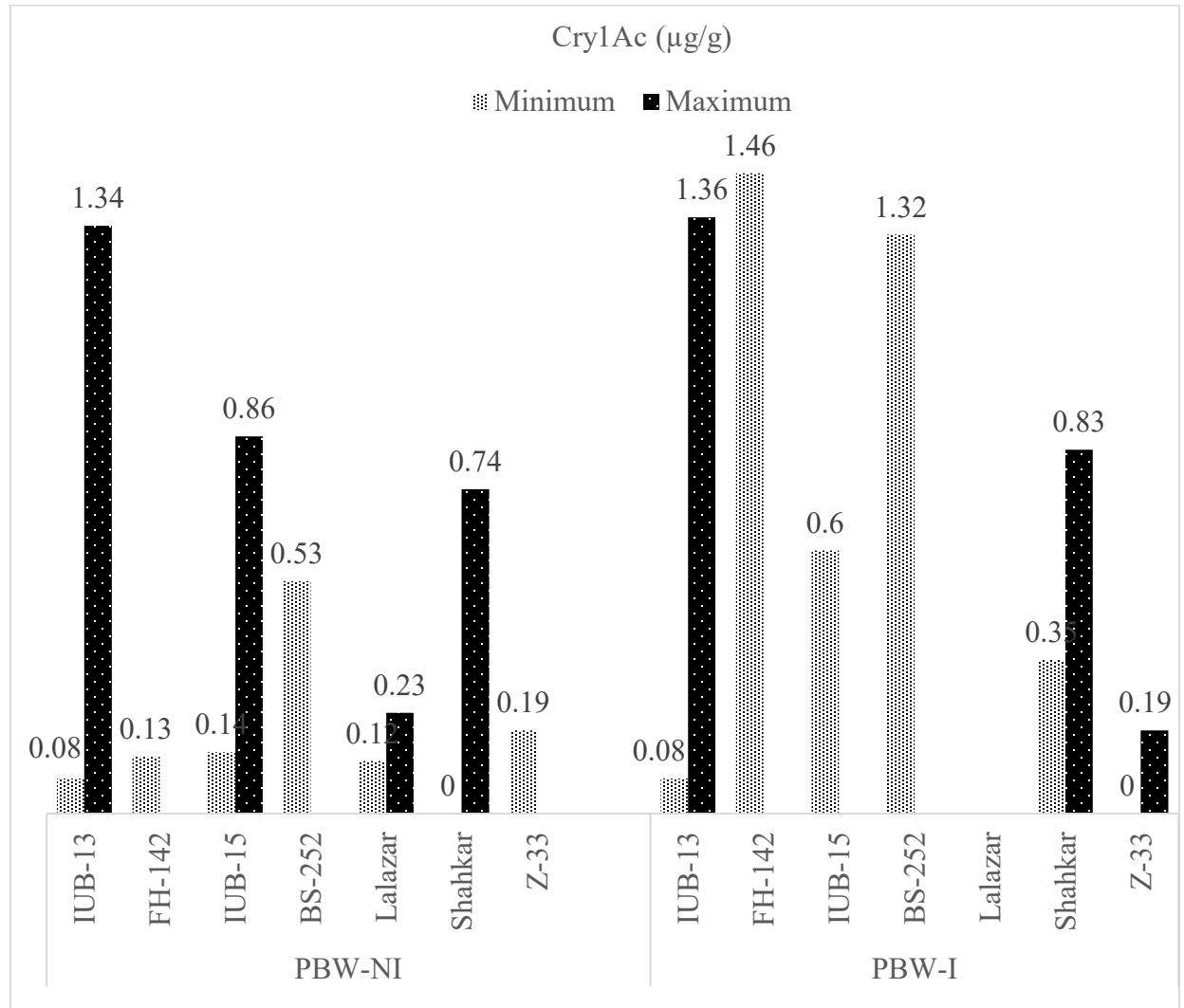


Figure 5: Concentration of Cry1Ac protein ($\mu\text{g/g}$) in Pink Boll Worm non-infested (PBW-NI) and Pink Boll Worm infested (PBW-I) most prevalent cotton varieties.

DISCUSSIONS

Variations in boll worm resistance (PBW and AW infestations) to Bt toxin have been observed in cotton varieties cultivated in South Punjab. This infestation was up to 64 % (Figure 3). Whereas production of Cry1Ac protein was below threshold level in all cotton varieties and also varied within each cotton variety resulting in infested and non-infested plants of a cotton variety (Figure 4 and 5). This variation could be due to:

- 1) phenotypic plasticity either in trait/genotype or boll worms (Gibson, 2008) and was likely to occur when boll worm populations experienced variable environments in different districts with contrasting fitness advantages (Ghalambor et al., 2007);
- 2) plant nutrient content variability that is often highly variable (Deans et al., 2016) and the nutritional balance of protein and digestible carbohydrates in plant resources has strong effects on insect performance, reproduction (Behmer, 2009), and detoxification ability (Behmer, 2002);
- 3) insects achieve an optimal balance in protein and carbohydrates through selective feeding on plant tissues to match their current nutritional needs (Behmer, 2009) and
- 4) variation in the concentration of plant defensive compounds within and between individual plants. These compounds can interact with plant nutrient content to affect insect herbivore performance (Behmer, 2002; Inbar, 2001).

Hence plant nutrients and environmental factors impacting susceptibility of boll worms to *Bt* toxins. In the present studies, plant elemental analysis depicted that leaf P and Mn have negative association with PBW infestation while leaf B has positive association with PBW infestation (Table 2). However, soil and plant elements varied across the 11 districts (Figure 1 and 2). Variable macro and micro-nutrients being part of insect total dietary concentration emerged as an important variable mediating the effects of Cry1Ac (Deans, et al., 2017). These elements also involved in Bt toxin production, for example, split treatment of N application (112/56 kg N ha⁻¹) exhibited 14% greater leaf Cry1Ac (Pettigrew and Adamczyk, 2006). In the present studies, nearly >70% soils have EC (0.26-3.9 dS/m) and pH (7.14 - 8.50) that is not detrimental for cotton (Figure 1). Contradictions in concentrations of macro and micro-elements in soil and cotton leaf samples were observed (Figure 1 and 2). These contradictions could be originated where plant had up taken the

required elements from soil thus making soil deficient and plant normal, or collected cotton leaf samples from soils/fields where nutrients were applied just before sampling thus making plant deficient and soil normal. Plant macronutrients affected Bt toxin production, that is varying within variety at various data collection points (Figure 5), and larvae performance. Larvae on the higher macronutrient p39:c29 diet took significantly longer to die (Deans, et al., 2017). Potential field implications of delayed mortality and an overall delay in development for larvae exposed to Cry1Ac suggests that larvae feeding on higher macronutrient tissues (Deans et al., 2016) (e.g., cotton seed) may produce greater crop damage. PBW infests cotton seed that is rich in protein and survives longer, consumes more diet and thus causes more damage.

Conclusively, gene-by-environment interactions have significant implications in the field to monitor and manage insect resistance. Bt expression is dependent on optimum concentrations of plant nutrients. On the other hand, Bt resistance in boll worms is not only due to genetic mutations, plastic responses due to plant micro and macronutrients can produce resistant phenotypes. This also suggests that boll worms can also be managed through alternative means by maintaining micro and macronutrients in cotton plant that are unsuitable for boll worm survival and reproduction – an alternative to the Bt gene-centric view. Cotton varieties efficiency to uptake and utilize these elements also varies and has the potential to be exploited for improvement in their bollworm resistance. The present studies suggest further in-depth intensive surveying and breeding and biotechnological interventions to better understand the cotton boll worms susceptibility to Bt endotoxins and genetic improvement in cotton resistance against boll worms.

Acknowledgements

The Divisional Soil and Water Testing Labs at Multan, Bahawalpur and DG Khan, Cotton Research Institute, Multan and Agriculture Biotechnology Research Institute, Faisalabad for their contribution in collection and analyses of soil and leaf samples. Their help is gratefully acknowledged.

REFERENCES

- Behmer, S. T. 2009. Insect herbivore nutrient regulation. *Ann. Rev. Entomol.* 54, 165–187.
- Behmer, S. T., Simpson, S. J. and Raubenheimer, D. 2002. Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. *Ecology* 83, 2489–2501.
- Deans, C. A., Sword, G. A. and Behmer, S. T. 2016. Nutrition as a neglected factor in insect herbivore susceptibility to Bt toxins. *Curr. Opin. Insect Sci.* 15, 97–103.
- Deans, C. A., Behmer, S. T., Fiene, J. and Sword, G. A. 2016. Spatio-temporal, genotypic, and environmental effects on plant soluble protein and digestible carbohydrate content: implication for insect herbivores with cotton as an exemplar. *J. Chem. Ecol.* 1–13.
- Deans, C. A., Behmer, S. T., Tessnow, A. E., Tamez-Guerra, P., Pusztai-Carey, M. and Sword, G. A. 2017. Nutrition affects insect susceptibility to Bt toxins. *Sci. Rep.* 7, 39705.
- Gibson, G. 2008. The environmental contribution to gene expression profiles. *Nat. Rev. Genet.* 9, 575–581.
- Ghalambor, C. K., McKay, J. K., Carroll, S. P. and Reznick, D. N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* 21, 394–407.
- Inbar, M., Doostdor, H. and Mayer, R. T. 2001. Suitability of stressed and vigorous plants to various insect herbivores. *Oikos* 94, 228–235.
- Moar, W. et al. 2008. Field-evolved resistance to Bt toxins. *Nature* 26, 1072–1074.
- PCCC. 2016. COTISTICS: Annual Cotton Statistical Bulletin. Directorate of Marketing & Economic Research, Pakistan Central Cotton Committee, Multan, Ministry of Textile Industry, Government of Pakistan. www.pccc.gov.pk
- Pettigrew, W. T. and Adamczyk, Jr. J. J. 2006. Nitrogen fertility and planting date effects on lint yield and Cry1Ac (Bt) endotoxin production. *Agron. J.* 98:691–697.

Annexure I: Macro-elements in soil and plant samples collected from 11 districts of South Punjab

Sr. No	(X)	(Y)	Plant			Soil		
			N %	P(ppm)	K(ppm)	N %	P(ppm)	K(ppm)
1	30.29	71.64	1.4	0.31	2.1	0.02	8.2	180
2	30.32	71.67	1.3	0.39	1.95	0.02	8.74	180
3	30.15	71.66	1.31	0.41	2.3	0.02	7.47	170
4	30.14	71.66	1.6	0.65	1.98	0.02	6.57	180
5	30.15	71.66	1.72	0.21	1.7	0.02	8.74	200
6	30.15	71.74	1.5	0.28	1.65	0.02	6.38	16
7	29.99	71.35	1.67	0.23	2.11	0.02	9.65	160
8	29.58	71.21	1.71	0.51	1.83	0.02	7.29	180
9	29.58	71.21	1.92	0.6	2.1	0.02	5.84	170
10	29.89	71.34	1.74	0.29	2.34	0.02	8.02	180
11	29.89	71.34	1.75	0.31	2.27	0.02	7.11	160
12	29.77	71.03	1.89	0.68	2.17	0.02	7.29	190
13	29.77	71.30	1.62	0.7	2.08	0.02	6.75	200
14	29.77	71.29	1.67	0.21	2.21	0.02	8.02	180
15	29.73	71.26	1.82	0.31	2.18	0.02	8.74	170
16	29.72	71.25	1.91	0.65	2.3	0.02	7.47	160
17	29.52	71.20	1.88	0.67	2.17	0.02	6.57	180
18	30.14	71.44	1.95	0.34	2.11	0.01	3.3	100
19	30.15	71.44	1.75	0.49	2.36	0.02	3.7	120
20	30.15	71.42	1.97	0.31	2.09	0.01	3.1	100
21	30.32	71.97	1.87	0.31	2.33	0.02	9.11	170
22	30.33	72.00	1.62	0.23	2.41	0.02	6.2	160
23	30.38	72.14	1.77	0.71	2.15	0.02	7.65	180
24	30.38	72.15	1.59	0.68	2.25	0.02	5.12	160
25	30.38	72.17	1.73	0.25	2.37	0.02	6.02	180
26	30.42	72.26	1.75	0.27	2.11	0.02	8.02	180
27	30.40	72.23	1.86	0.34	2.25	0.01	9.65	200
28	30.42	72.26	1.89	0.61	2.07	0.02	6.75	168
29	30.42	72.26	1.81	0.7	2.3	0.02	7.47	160
30	30.45	71.90	1.78	0.1	2.4	0.02	8.2	170
31	30.41	72.03	1.88	0.7	2.24	0.02	5.6	180
32	30.46	71.93	1.74	0.68	2.11	0.02	5.4	160
33	30.48	71.92	1.65	0.14	2.31	0.02	6.3	160
34	30.47	71.91	1.67	0.34	2.22	0.02	6.8	160
35	30.47	71.91	1.55	0.32	2.09	0.02	5.7	180
36	30.46	71.90	1.52	0.58	2.11	0.02	7.5	140
37	30.16	71.86	1.71	0.66	2.02	0.02	5.2	160
38	30.16	71.86	1.59	0.7	2.34	0.02	5.1	150

Sr. No	(X)	(Y)	Plant			Soil		
			N %	P(ppm)	K(ppm)	N %	P(ppm)	K(ppm)
39	30.13	71.25	1.78	0.47	2.26	0.02	7.3	160
40	30.13	71.85	1.67	0.51	2.35	0.01	5	120
41	29.57	70.74	1.76	0.29	2.27	0.02	7.3	220
42	29.58	71.77	1.81	0.34	2.51	0.02	7.6	240
43	29.59	71.77	1.87	0.29	2.38	0.02	7.1	200
44	29.59	71.78	1.56	0.71	2.21	0.02	6.9	220
45	29.61	71.86	1.96	0.76	2.27	0.03	6.7	220
46	29.62	71.87	1.78	0.67	2.17	0.02	5.5	220
47	29.61	71.84	1.84	0.28	2.18	0.03	5.2	180
48	29.60	71.66	1.66	0.29	2.09	0.02	4.9	200
49	29.60	71.66	1.51	0.31	2.14	0.02	5.6	180
50	29.61	71.61	1.64	0.78	2.13	0.02	5.1	180
51	29.57	71.54	1.67	0.7	2.31	0.03	5.3	210
52	29.58	71.52	1.95	0.29	2.11	0.03	5.7	180
53	29.60	71.50	1.68	0.34	2.24	0.03	5.9	220
54	29.68	71.59	1.73	0.25	2.17	0.03	5.1	180
55	29.71	71.59	1.75	0.76	2.36	0.03	5	180
56	29.74	71.98	1.81	0.7	2.07	0.02	5.2	200
57	29.74	71.57	1.93	0.34	2.1	0.02	5.1	180
58	29.76	71.56	1.84	0.75	2.21	0.02	4.9	120
59	29.77	71.56	1.81	0.31	2.3	0.02	5.3	180
60	29.81	71.57	1.76	0.34	2.26	0.02	4.6	120
61			1.8	0.61	2.15	0.02	6.1	140
62	29.92	72.03	1.84	0.58	2.06	0.01	4.8	180
63	29.86	72.08	1.93	0.74	2.23	0.02	5.8	120
64	29.84	72.07	1.59	0.34	2.35	0.01	4.7	120
65	29.77	72.19	1.6	0.74	2.33	0.02	6.3	120
66	29.77	72.18	1.66	0.7	2.29	0.02	6	160
67	29.82	72.22	1.71	0.21	2.21	0.02	5.5	120
68	29.98	72.49	1.77	0.34	2.31	0.02	5.3	180
69	29.98	72.49	1.72	0.23	2.37	0.02	4.9	140
70	30.01	72.49	1.9	0.27	2.29	0.02	5.3	160
71	30.11	72.54	1.84	0.78	2.28	0.02	5.5	180
72	30.12	72.54	1.69	0.65	2.27	0.01	4.6	160
73	30.12	72.54	1.72	0.78	2.38	0.01	4.7	120
74	30.15	72.62	1.63	0.21	2.18	0.02	5.7	120
75	30.16	72.62	1.74	0.25	2.21	0.02	5.6	160
76	30.17	72.62	1.75	0.29	2.14	0.03	6.5	200
77	30.13	72.63	1.59	0.34	2.17	0.02	5.8	210
78	30.13	72.63	1.84	0.7	2.07	0.02	4.9	120

Sr. No	(X)	(Y)	Plant			Soil		
			N %	P(ppm)	K(ppm)	N %	P(ppm)	K(ppm)
79	30.14	72.65	1.88	0.65	2.01	0.02	4.7	140
80	30.12	72.66	1.92	0.7	2.1	0.02	4.8	140
81	30.07	73.41	2.23	2.13	1.63	0.03	3.1	93
82	30.09	73.39	2.31	1.87	1.98	0.03	6.3	206
83	29.98	73.29	2.38	1.91	2.11	0.03	6	93
84	29.98	73.30	1.88	1.21	2.81	0.03	6	75
85	29.93	73.25	3.16	1.31	2.51	0.03	8.1	112
86	29.92	73.26	2.79	1.85	2.58	0.03	7.3	93
87	29.63	73.08	2.64	1.39	2.48	0.03	7.5	93
88	29.61	73.04	2.26	1.51	2.41	0.03	6.3	75
89	29.61	72.96	2.42	1.43	2.13	0.03	6.5	112
90	29.58	72.94	0.82	1.63	2.97	0.03	8.3	93
91	29.58	72.94	1.99	1.59	3.15	0.03	7.3	168
92	29.39	72.84	1.81	1.87	2.85	0.03	7.5	187
93	29.28	72.83	2.57	1.58	3.01	0.03	7.9	131
94	29.29	72.83	2.49	1.67	2.63	0.03	7.4	150
95	29.58	72.81	2.18	2.31	3.15	0.03	6.2	168
96	29.61	72.82	2.49	1.91	3.12	0.02	5.4	187
97	29.61	72.80	2.1	2.93	2.63	0.03	6.2	131
98	29.62	72.84	2.15	2.81	2.45	0.03	5.4	93
99	29.69	72.50	1.96	1.79	3.19	0.03	7.1	150
100	29.41	72.41	1.74	1.15	2.11	0.03	7.4	131
101	29.72	72.64	2.15	2.31	2.91	0.03	7.2	168
102	29.71	72.61	2.07	1.97	2.15	0.03	7.9	206
103	29.67	72.35	2.38	1.73	2.39	0.03	7.8	93
104	29.33	71.59	1.76	1.9	2.56	0.03	7.2	75
105	29.34	71.62	1.91	1.21	2.38	0.03	4.2	112
106	29.34	71.67	1.78	1.67	2.63	0.03	7.9	93
107	29.22	71.72	2.3	1.74	1.58	0.03	8.2	206
108	29.18	71.73	1.86	2.04	1.93	0.03	7.2	150
109	29.16	71.74	2.48	2.13	2.31	0.03	7.2	75
110	29.11	71.56	2.54	1.83	2.98	0.03	6.8	75
111	29.12	71.56	2.22	2.01	2.71	0.03	7.5	93
112	29.10	71.50	2.65	1.77	2.31	0.03	8.1	75
113	29.12	71.42	2.86	1.58	2.49	0.03	7.8	131
114	29.13	71.40	2.3	1.31	1.99	0.03	7.2	150
115	29.13	71.32	2.38	1.51	2.23	0.03	8.2	168
116	29.13	71.28	2.69	1.48	2.36	0.03	7.5	131
117	29.14	71.23	2.71	2.31	2.15	0.03	7.9	206
118	29.13	71.21	2.39	1.71	2.49	0.03	7.2	93
119	29.12	71.18	2.68	1.91	1.85	0.03	5.4	75

Sr. No	(X)	(Y)	Plant			Soil		
			N %	P(ppm)	K(ppm)	N %	P(ppm)	K(ppm)
120	29.10	71.14	2.89	2.38	1.93	0.03	2.1	112
121	28.23	70.23	1.13	0.62	1.97	0.03	5.2	115.5
122	28.31	70.23	0.97	0.73	1.52	0.04	9.5	32.8
123	28.23	70.25	0.93	0.57	1.85	0.03	5.6	74.1
124	28.33	70.23	1.15	0.97	1.63	0.02	8.2	115.5
125	28.23	70.35	0.95	1.15	2.15	0.03	9.4	74.1
126	28.23	70.35	0.91	1.03	1.89	0.03	8	74.1
127	28.30	70.40	0.76	0.89	1.18	0.03	3.6	198.2
128	28.33	70.43	1.13	1.39	1.34	0.03	4.5	115.5
129	28.34	70.42	0.91	1.09	1.49	0.03	9.5	32.8
130	28.38	70.41	1.15	0.74	2.15	0.03	8.4	280.9
131	28.39	70.42	1.21	0.59	1.91	0.02	9.2	74.1
132	28.30	70.40	3.12	1.95	1.76	0.03	8.4	115.5
133	28.45	70.51	5.91	2.38	3.47	0.02	9.4	198.2
134	28.47	70.51	3.85	1.71	2.39	0.04	10.2	115.5
135	28.47	70.95	0.52	0.67	0.85	0.03	8.5	74.1
136	28.28	70.14	0.69	0.45	1.11	0.03	9.6	115.5
137	28.26	70.13	1.57	0.93	1.39	0.03	7.8	32.8
138	28.19	70.08	1.29	0.95	1.15	0.02	11	74.1
139	29.19	70.10	1.38	1.3	1.03	0.03	9	115.5
140	28.22	70.15	2.31	1.15	1.15	0.03	8.4	280.9
141	28.93	70.24	3.67	0.29	2.36	0.02	8.05	158.8
142	28.88	70.16	4.26	0.46	2.24	0.02	6.75	164.9
143	28.80	70.02	4.56	0.35	1.47	0.02	7.95	149.5
144	28.78	69.99	4.78	0.47	1.86	0.02	8.4	191.5
145	28.72	69.95	4.22	0.55	2.69	0.02	5.65	124.4
146	28.72	69.38	2.86	0.34	2.56	0.02	7.05	127.1
147	29.06	70.33	3.67	0.36	2.78	0.02	7.25	155.5
148	29.06	70.33	3.88	0.49	3.16	0.02	6.75	153.1
149	29.07	70.30	4.16	0.69	2.48	0.02	7.8	146.3
150	29.06	70.30	4.85	0.42	2.65	0.02	8.7	157.7
151	29.04	70.34	4.88	0.38	2.49	0.01	3.9	149.9
152	29.09	70.34	4.69	0.27	1.26	0.02	6.65	114.4
153	29.10	70.37	3.56	0.38	3.26	0.02	8.15	106.1
154	29.41	70.52	3.77	0.48	2.47	0.02	9.2	127.5
155	29.42	70.50	3.46	0.27	2.68	0.02	5.9	155.5
156	29.42	70.48	3.59	0.21	2.69	0.02	7.45	145.5
157	29.44	70.46	3.58	0.36	2.57	0.02	8.3	139.7
158	29.50	70.41	4.49	0.28	2.36	0.02	8.4	144.5
159	29.51	70.41	4.26	0.26	2.78	0.02	8.2	133.3
160	29.69	70.59	4.15	0.42	3.06	0.02	8.1	188.5

Sr. No	(X)	(Y)	Plant			Soil		
			N %	P(ppm)	K(ppm)	N %	P(ppm)	K(ppm)
161	30.96	70.24	4.25	0.43	2.16	0.04	16	120
162	30.95	71.03	3.18	0.43	3.14	0.02	17	92
163	30.99	71.10	2.85	0.28	2.46	0.03	9	92
164	31.02	71.08	2.77	0.47	1.58	0.04	3	92
165	31.02	71.07	2.69	0.26	1.67	0.03	4	92
166	31.03	71.06	3.44	0.48	2.16	0.03	11	60
167	31.02	71.04	3.16	0.35	2.34	0.01	7	92
168	31.01	70.99	2.66	0.47	2.77	0.03	5	92
169	31.16	71.01	3.45	0.18	3.06	0.02	7	92
170	31.22	70.98	2.89	0.24	2.65	0.01	6	60
171	31.24	70.99	4.26	0.26	1.46	0.02	4	60
172	31.25	71.03	3.86	0.28	1.68	0.01	19	40
173	31.17	71.14	4.56	0.14	1.68	0.01	14	60
174	31.19	71.17	2.78	0.56	3.14	0.02	5	60
175	31.17	71.23	4.26	0.46	2.64	0.02	4	120
176	31.01	71.25	4.47	0.37	3.09	0.04	17	92
177	31.01	71.27	3.69	0.29	2.56	0.02	16	60
178	30.99	71.28	3.85	0.36	1.95	0.01	13	28
179	30.96	71.30	4.16	0.46	1.87	0.03	36	120
180	30.93	71.42	2.65	0.35	2.26	0.02	14	92
181	30.03	70.16	3.68	0.47	2.62	0.02	4.3	97
182	30.00	70.73	4.25	0.46	2.74	0.04	7	183
183	29.96	70.57	4.46	0.53	1.26	0.03	6.1	143
184	29.96	70.41	3.26	0.66	1.42	0.01	4.5	95
185	29.96	70.68	4.68	0.24	1.89	0.04	5	109
186	29.96	70.68	3.85	0.29	2.41	0.03	4.1	110
187	29.96	70.41	3.26	0.35	2.63	0.05	4.1	153
188	29.96	70.41	2.85	0.44	2.78	0.01	4	305
189	29.91	70.66	3.47	0.47	3.26	0.02	5.2	199
190	29.90	70.65	4.56	0.59	3.45	0.02	6	103
191	30.52	70.70	4.58	0.35	2.46	0.03	7.21	143
192	30.56	70.68	4.27	0.26	1.85	0.04	7.1	165
193	30.55	70.67	4.63	0.39	3.19	0.04	7.5	181
194	30.63	70.68	4.86	0.47	2.84	0.04	5.9	155
195	30.63	70.68	4.75	0.69	2.75	0.04	7	173
196	30.63	70.67	3.26	0.36	2.63	0.03	6	129
197	30.63	70.67	3.69	0.45	2.63	0.04	7.3	184
198	30.66	70.67	3.85	0.45	2.86	0.03	6.9	191
199	29.67	70.66	2.69	0.42	3.16	0.04	5.9	155
200	30.68	70.64	3.75	0.66	3.27	0.04	6	180
201	29.40	70.96	3.69	0.26	1.62	0.04	7.3	134

Sr. No	(X)	(Y)	Plant			Soil		
			N %	P(ppm)	K(ppm)	N %	P(ppm)	K(ppm)
202	29.37	70.98	4.75	0.26	2.57	0.04	8.1	291
203	29.52	70.96	2.96	0.35	2.69	0.04	8.9	154
204	29.54	70.10	4.58	0.58	2.14	0.04	10.5	368
205	29.45	70.99	4.78	0.47	1.65	0.04	8.3	348
206	29.51	70.95	3.65	0.56	1.86	0.04	7.9	198
207	29.54	71.02	3.85	0.69	2.04	0.05	6.8	89
208	29.36	71.00	3.44	0.54	2.36	0.04	6.9	89
209	29.51	70.95	3.69	0.23	3.26	0.06	7.3	134
210	29.54	71.01	4.75	0.27	3.16	0.04	6.9	178
211	30.05	70.98	5.02	0.36	2.88	0.04	6.9	65
212	30.00	71.11	3.58	0.57	1.69	0.05	6.7	95
213	30.00	71.11	4.53	0.28	2.46	0.06	7.5	99
214	30.00	71.11	3.66	0.36	1.85	0.04	6.5	96
215	30.00	71.11	3.26	0.46	1.63	0.04	6.3	72
216	30.27	70.87	2.58	0.25	2.58	0.05	4.3	44
217	30.27	70.87	3.68	0.58	2.45	0.04	7.4	75
218	30.27	70.87	4.46	0.49	2.63	0.05	6.8	89
219	30.27	70.87	4.25	0.58	2.68	0.06	6.7	109
220	30.27	70.87	5.26	0.36	2.75	0.04	7.4	198

Annexure II: Micro-elements (ppm) in soil and plant samples collected from 11 districts of South Punjab

Sr. No	Plant					Soil				
	Zn	Cu	Fe	Mn	B	Zn	Cu	Fe	Mn	B
1	19.8	10	155	53	29	0.56	0.49	4.51	0.73	0.21
2	20	15	154	54	31	0.73	0.41	3.91	0.75	0.39
3	20.21	18	155	58	35	0.56	0.43	3.71	0.81	0.28
4	23	20	194	52	32	0.71	0.47	4.69	0.91	0.28
5	35	16	189	48.9	35	0.69	0.37	3.26	1.17	0.37
6	37	19	176	52	31	0.72	0.28	7.31	0.95	0.23
7	41	15	156	58	40	0.61	0.43	3.5	0.75	0.33
8	29	22	152	56	33	0.68	0.26	9.28	0.77	0.39
9	40	18	177	53	32	0.58	0.37	4.11	0.79	0.48
10	43	16	178	49	37	0.61	0.73	6.79	0.57	0.45
11	46	14	110	54	34	0.49	0.27	3.43	0.73	0.41
12	48	12	108	57	34	0.41	0.51	3.1	0.89	0.38
13	52	18	100	53	32	0.42	0.31	3.99	0.87	0.47
14	48.5	20	110	62	31	0.59	0.51	3.83	0.47	0.44
15	52	18	102	54	30	0.52	0.31	2.87	0.63	0.27
16	39	15	180	52	32	0.54	0.23	4.42	0.65	0.48
17	32	17	178	57	31	0.58	0.29	0.49	0.77	0.43
18	38	12	172	51	42	0.29	0.43	3.55	0.67	0.32
19	40	16	169	55	44	0.37	0.05	6.38	0.71	0.39
20	42	18	164	51	46	0.42	0.33	8.23	0.85	0.28
21	35	18	165	49	33	0.36	0.18	6.65	0.87	0.44
22	39	13	145	52	35	0.47	0.18	4.68	0.48	0.48
23	38	14	155	51	36	0.44	0.45	0.58	0.89	0.39
24	29	13	167	56	34	0.51	0.33	4.24	0.75	0.43
25	32	12	155	54	32	0.55	0.61	3.55	0.53	0.39
26	33.5	18	165	55	35	0.45	1.11	3.99	0.71	0.3
27	32	16	178	57	32	0.57	0.71	3.56	0.99	0.49
28	37	17	188	53	36	0.41	0.73	4.2	0.78	0.41
29	28	18	198	53	37	0.49	0.41	4.11	0.91	0.45
30	29	20	155	52	42	0.45	0.31	2.16	1.21	0.48
31	28.6	15	159	54	6	0.4	0.27	3.21	0.81	0.41
32	32	16	160	52	34	0.41	0.22	4.99	1.01	0.42
33	34	18	198	48	36	0.74	0.43	3.55	0.67	0.43
34	44	14	167	44	38	0.37	0.05	6.38	0.71	0.49
35	34	18	188	52	36	0.42	0.33	8.23	0.85	0.45
36	36	16	156	51	42	0.29	0.51	3.87	0.57	0.46
37	39	15	174	49	34	0.48	0.26	2.72	0.2	0.48
38	28.7	12	155	45	38	0.15	0.49	4.51	0.73	0.42

Sr. No	Plant					Soil				
	Zn	Cu	Fe	Mn	B	Zn	Cu	Fe	Mn	B
39	22.5	14	168	52	42	0.13	0.41	3.91	0.75	0.48
40	29	18	210	51	43	0.3	0.43	3.71	0.89	0.4
41	44	20	169	55	39	0.19	0.37	3.26	1.17	0.27
42	49	21	172	56	36	0.12	0.28	7.31	0.95	0.37
43	54	18	158	52	38	0.02	0.26	9.28	0.77	0.43
44	44	18	162	51	42	0.01	0.37	4.11	0.79	0.43
45	39	14	164	53	33	0.61	0.43	3.5	0.75	0.27
46	46	15	166	51	36	0.61	0.73	6.79	0.57	0.33
47	45	14	172	53	38	0.49	0.27	3.43	0.73	0.39
48	48	18	176	55	36	0.41	0.51	3.1	0.89	0.48
49	42.8	15	168	52	34	0.42	0.31	3.99	0.87	0.42
50	44	18	172	48	38	0.59	0.51	3.83	0.47	0.23
51	38	17	156	52	42	0.52	0.31	2.87	0.63	0.33
52	37	14	168	51	38	0.54	0.23	4.42	0.65	0.23
53	44	19	172	52	36	0.58	0.29	0.49	0.77	0.33
54	48	13	162	50	34	0.36	0.18	6.65	0.87	0.34
55	35	10.9	166	55	32	0.47	0.18	4.68	0.83	0.29
56	38	12	154	54	35	0.44	0.45	0.58	0.89	0.27
57	52	18	158	56	36	0.51	0.33	4.24	0.75	0.33
58	48	17	172	54	38	0.55	0.61	3.55	0.53	0.39
59	53	14	168	54	44	0.45	1.11	3.99	0.71	0.23
60	32	13	172	55	38	0.57	0.71	3.58	0.99	0.35
61	32	15	188	55	46	0.29	0.51	3.87	0.57	0.46
62	30.5	18	168	54	36	0.48	0.26	2.72	0.2	0.48
63	33	15	199	51	34	0.15	0.49	4.51	0.73	0.42
64	34	14	103	53	35	0.13	0.41	3.91	0.75	0.48
65	32	18	156	55	36	0.3	0.43	3.71	0.81	0.4
66	36	16	178	53	38	0.21	0.47	4.69	0.91	0.36
67	44	15	186	49	35	0.19	0.37	3.26	0.95	0.37
68	43	17	149	50	34	0.12	0.28	7.31	0.95	0.26
69	48	18	152	52	36	0.72	0.26	9.28	0.77	0.39
70	46	20	156	53	42	0.67	0.37	4.11	0.79	0.36
71	43	12	183	55	34	0.61	0.43	3.5	0.75	0.4
72	46	15	180	56	33	0.61	0.73	6.79	0.57	0.44
73	57	16	179	54	35	0.49	0.27	3.43	0.73	0.41
74	45	16	177	56	36	0.41	0.51	3.1	0.89	0.45
75	44	14	172	52	35	0.42	0.31	3.99	0.87	0.49
76	42	16	167	52	38	0.59	0.51	3.83	0.47	0.42
77	45	18	168	51	41	0.15	0.49	4.51	0.73	0.36
78	48	12	163	50	42	0.13	0.41	3.91	0.75	0.26
79	50	14	158	53	38	0.3	0.43	3.71	0.81	0.55

Sr. No	Plant					Soil				
	Zn	Cu	Fe	Mn	B	Zn	Cu	Fe	Mn	B
80	41	18	166	54	36	0.21	0.47	4.69	0.91	0.48
81	54	1	21	125	22.77	0.64	0.42	6.98	3.92	0.85
82	62	5	5	85	15.18	0.68	2.18	6.62	3.28	1.66
83	162	27	95	131	11.39	0.8	2	7.96	2.88	1.87
84	116	2	61	100	10.44	0.5	1.3	8.98	6.82	0.33
85	164	3	100	137	22.77	1.18	0.92	10.4	4.44	0.33
86	71	1	13	140	17.08	0.46	0.5	6.82	3.48	0.33
87	33	3	17	82	12.33	0.72	0.72	7.76	4.5	0.85
88	35	3	17	99	14.23	0.68	0.3	9.22	3.74	0.54
89	46	1	21	95	15.18	0.88	0.52	10.28	4.9	1.03
90	47	1	24	82	12.33	0.64	0.32	10.42	4.86	1.00
91	65	1	32	125	19.92	0.8	0.04	10.88	5.64	0.76
92	42	2	19	78	6.64	2.02	1.12	17.02	7.34	0.57
93	48	2	27	106	11.39	1.2	1.08	9.68	4.64	1.45
94	57	1	31	97	21.82	0.86	0.52	11.92	2.8	1.72
95	38	1	14	89	17.08	1.3	0.74	8.94	4.06	0.45
96	46	1	10	100	16.13	1.06	0.24	9.3	2.44	2.30
97	36	3	28	83	18.03	2.96	1.84	16.16	2.76	1.54
98	46	3	25	107	11.39	1.34	1.32	11.52	3.14	0.57
99	55	1	32	97	12.33	4.7	0.76	7.34	2.5	1.99
100	30	1	11	125	12.22	0.7	0.14	4.56	1.06	1.84
101	176	17	169	59	16.13	1.44	0.26	5.54	4.34	1.60
102	44	66	48	92	21.83	2.02	2.48	15.96	7.1	0.90
103	40	30	48	79	17.08	1.06	0.36	6.04	3.02	1.80
104	40	52	43	34	19.92	0.22	1.32	14.82	4.96	1.00
105	43	38	41	46	21.82	0.2	0.96	12.6	4.6	2.74
106	60	27	53	36	18.03	0.92	0.64	9.72	4.12	3.09
107	17	50	29	71	16.13	2.04	0.92	6.36	3.06	1.55
108	26	21	24	66	17.08	1.76	0.84	7.08	4.18	1.95
109	38	14	33	120	15.18	1.62	1.08	6.58	5.26	2.29
110	46	36	37	84	22.77	1.6	1.32	8.48	4.92	2.64
111	40	43	28	93	19.92	1.16	0.68	6.02	3.72	1.70
112	34	48	31	141	23.72	1.14	0.68	4.62	2.24	2.19
113	37	37	30	128	20.87	1.08	0.92	5.78	3.18	1.60
114	25	2	12	118	19.92	0.94	0.22	4.38	2.84	1.75
115	37	43	29	110	19.92	1.08	4.2	4.9	2.14	2.79
116	38	14	19	117	21.82	0.94	0.42	0.5	4.1	2.39
117	35	9	35	91	14.23	0.8	0.12	4.54	2.86	0.55
118	10	70	9	3	15.18	0.82	0.02	5.1	4.6	1.40
119	38	20	27	111	16.13	1	0.16	7.88	2.2	0.05
120	36	16	20	132	15.18	1.04	0.04	5.58	4.8	0.30

Sr. No	Plant					Soil				
	Zn	Cu	Fe	Mn	B	Zn	Cu	Fe	Mn	B
121	57	85	557	80	26.48	7.32	1.22	8.1	5.94	1.52
122	31	17	418	79	18.33	7.5	1.14	5.56	0.08	1.79
123	103	403	551	73	21.38	5.88	0.7	7.06	2.52	1.16
124	104	928	464	156	9.16	8.34	1.44	6.86	19.02	1.22
125	46	34	627	55	17.31	8.24	0.28	9.06	1.64	0.71
126	63	693	524	55	19.35	8.38	0.32	5.3	1.92	0.63
127	39	150	416	71	6.11	7.66	0.78	8.64	5.5	0.48
128	50	63	367	49	16.29	11.28	0.04	5.08	0.14	1.16
129	46	17	431	55	11.2	7.08	0.92	6.46	4.54	1.49
130	46	160	372	133	16.29	16.72	0.96	8.86	5.6	2.14
131	50	55	585	73	20.37	5.96	0.44	9.32	2.88	2.83
132	37	70	426	72	18.33	4.8	0.02	10.86	3.26	1.31
133	28	43	542	77	21.38	6.48	0.84	9.08	4	2.35
134	25	37	623	104	7.13	6.82	0.26	8.86	1.02	1.90
135	64	16	533	125	5.09	8.94	0.24	9.3	0.84	2.32
136	44	8	655	114	17.31	8.88	0.56	9.72	3.24	1.73
137	45	10	685	72	15.27	6.24	1.68	8.64	6	2.65
138	29	9	744	82	22.4	8	1.52	13.72	6.74	2.89
139	28	11	725	76	19.35	8.24	1.22	10.84	4.2	2.32
140	24	11	348	76	8.15	7.08	1.44	12.86	7.76	3.07
141	36	16	266	15	46	1.32	0.18	4.51	1.46	0.811
142	48	24	274	16	82	0.6	0.43	3.71	2.62	0.748
143	27	15	158	12	74	1.38	0.36	3.26	2.34	0.571
144	26	28	98	35	59	1.36	0.26	6.14	1.54	0.787
145	69	57	86	24	68	1.22	0.43	3.5	2.5	0.795
146	23	69	356	27	48	1.04	0.32	2.87	2.26	0.571
147	26	63	244	17	49	1.16	0.29	2.47	1.54	0.621
148	45	35	247	15	36	0.94	0.18	4.68	1.66	0.631
149	49	26	263	26	68	1.02	0.38	4.24	1.5	0.652
150	68	35	278	34	84	0.9	1.12	3.99	1.42	0.739
151	57	24	269	19	43	0.82	0.53	4.2	1.56	0.832
152	26	57	178	12	46	0.9	0.3	2.16	2.42	0.742
153	38	15	97	17	57	0.82	0.22	4.94	3.02	0.571
154	42	26	106	16	52	0.58	0.51	3.87	1.14	0.785
155	66	63	149	20	56	0.3	0.49	4.51	1.46	0.675
156	29	65	216	22	58	0.6	0.42	3.71	1.62	0.744
157	35	24	317	24	62	0.38	0.37	3.26	2.34	0.808
158	37	26	265	27	42	1.44	0.26	2.58	1.54	0.739
159	26	42	274	29	75	1.22	0.43	3.5	1.5	0.804
160	35	16	266	24	46	0.98	0.27	3.43	1.46	0.571
161	35	24	156	26	65	2.68	0.76	3.89	1.98	0.691

Sr. No	Plant					Soil				
	Zn	Cu	Fe	Mn	B	Zn	Cu	Fe	Mn	B
162	26	16	265	15	58	0.94	0.38	3.25	1.06	0.711
163	59	35	285	19	59	1.22	0.34	3.96	2.42	0.563
164	68	26	274	36	67	1.64	0.42	8.9	1.4	0.764
165	26	24	258	26	81	1.6	0.49	4.58	2.24	0.681
166	35	45	269	24	83	1.54	0.44	4.67	4.3	0.812
167	48	26	285	19	95	0.89	0.35	3.72	2.04	0.774
168	74	48	169	26	87	0.99	0.42	4.26	1.42	0.784
169	15	85	187	27	48	0.86	0.28	3.97	2.38	0.571
170	26	26	268	12	56	1	0.37	4.08	1.42	0.844
171	59	14	342	29	75	1.08	1.22	3.66	3.04	0.719
172	44	56	256	11	48	1.34	0.92	4.76	1.54	0.544
173	58	28	286	16	59	1.92	0.35	4.32	2.7	0.842
174	29	35	269	22	62	0.94	1.07	4.12	1.34	0.776
175	38	24	168	24	65	0.78	0.52	2.58	1.46	0.683
176	62	48	196	19	67	0.94	0.3	3.36	2.9	0.791
177	58	57	248	27	82	1.24	0.16	2.73	1.14	0.843
178	67	26	316	6	92	1.58	0.32	2.58	1.94	0.689
179	36	35	345	18	74	1.32	0.35	4.84	2.94	0.719
180	42	26	285	13	68	0.89	0.25	3.25	1.62	0.649
181	36	25	256	9	48	1.7	0.28	3.97	1.38	0.839
182	42	26	384	8	46	1.86	0.27	4.08	1.42	0.731
183	46	35	352	11	53	0.88	0.88	3.24	0.7	0.681
184	26	24	26	12	57	1.66	1.22	3.65	2.34	0.563
185	35	14	86	14	49	1.36	0.42	4.76	1.54	0.791
186	25	25	66	10	74	1.86	1.35	4.5	2.7	0.785
187	45	26	326	12	75	1.94	0.44	3.88	0.98	0.572
188	26	35	258	14	68	1.98	1.07	5.24	2.34	0.775
189	36	45	257	16	63	1.6	0.74	5.7	1.86	0.819
190	65	46	246	32	65	1.56	0.56	3.55	2.9	0.775
191	26	26	268	24	85	1.09	1.42	3.14	0.66	0.839
192	35	35	344	26	81	1.8	0.34	2.69	2.94	0.8
193	24	24	327	28	43	1.07	0.6	3.99	3.08	1.019
194	44	15	316	27	64	0.98	0.78	2.99	2.18	0.771
195	48	22	326	26	76	1.48	0.34	5.3	1.58	0.571
196	46	23	398	16	83	0.75	0.26	4.12	2.86	0.782
197	49	36	247	13	95	1.52	1.02	3.93	1.14	0.681
198	27	26	246	17	74	1.8	0.32	5.46	1.46	0.739
199	36	24	268	19	55	1.39	0.42	4.13	1.04	0.723
200	49	27	344	20	49	1.2	0.45	4.3	1.67	0.781
201	26	36	265	26	56	1.48	0.37	3.14	1.15	0.719
202	47	26	286	16	81	1.37	0.14	3.05	2.44	0.755

Sr. No	Plant					Soil				
	Zn	Cu	Fe	Mn	B	Zn	Cu	Fe	Mn	B
203	45	35	274	13	75	1.1	0.38	1.9	2.46	0.731
204	46	24	275	25	45	0.94	0.16	3.16	1.34	0.531
205	26	45	306	24	56	1.16	0.14	1.37	1.34	0.571
206	68	28	328	16	86	1.3	0.59	4.58	1.84	0.582
207	59	46	384	35	53	1.05	0.4	2.66	2.3	0.796
208	54	25	269	24	54	1.14	0.24	2.85	2.9	0.687
209	41	26	178	18	42	1.01	0.63	3.98	2.04	0.631
210	42	35	65	16	49	0.92	0.69	1.2	2.06	0.811
211	35	35	246	26	58	1.93	0.34	1.34	1.63	0.781
212	25	25	275	23	54	0.34	0.81	4.25	1.62	0.803
213	36	47	365	27	75	2.2	0.2	3.21	1.02	0.661
214	51	26	248	9	69	3	0.63	3.68	2.48	0.843
215	58	24	326	28	75	1.25	0.52	4.18	1.07	0.784
216	57	15	366	31	42	1.26	0.28	1.36	0.94	0.763
217	62	35	275	24	46	2.54	0.37	4.22	0.99	0.811
218	54	26	269	19	53	0.99	0.32	3.2	1.43	0.717
219	53	35	241	17	52	1.36	0.96	1.62	1.39	0.599
220	48	64	246	16	59	3.21	0.34	4.85	2.25	0.775

Annexure III: Insect infestation of cotton crop in 11 districts of South Punjab. X and Y are coordinates.

Sr. No	Y	X	W.F	PBW	Jassid	A.W	Thrips	M.B	D.B	F.W
1	30.29	71.64	1	0	1	0	0	0	0	0
2	30.32	71.67	0	0	1	0	0	0	0	0
3	30.15	71.66	1	0	1	0	0	0	0	0
4	30.14	71.66	1	0	1	0	0	0	0	0
5	30.15	71.66	1	0	0	0	1	0	0	0
6	30.15	71.74	0	0	1	0	0	0	0	0
7	29.99	71.35	0	0	1	0	1	0	0	0
8	29.58	71.21	0	0	1	0	0	0	0	0
9	29.58	71.21	1	0	1	0	1	1	0	0
10	29.89	71.34	1	0	1	1	1	1	0	0
11	29.89	71.34	1	0	0	1	0	1	0	0
12	29.77	71.03	0	1	1	0	0	1	0	0
13	29.77	71.30	1	0	1	1	0	0	0	0
14	29.77	71.29	1	1	1	0	0	0	0	0
15	29.73	71.26	0	0	0	0	1	1	0	0
16	29.72	71.25	1	0	1	0	0	0	0	0
17	29.52	71.20	0	0	0	0	0	0	0	0
18	30.14	71.44	1	1	0	1	1	0	0	0
19	30.15	71.44	1	1	1	0	1	0	0	0
20	30.15	71.42	0	1	1	0	1	0	0	0
21	30.32	71.97	0	1	1	0	1	0	0	0
22	30.33	72.00	1	1	1	1	1	0	0	0
23	30.38	72.14	1	0	0	0	0	0	0	0
24	30.38	72.15	0	0	0	0	0	0	0	0
25	30.38	72.17	0	0	0	0	0	0	0	0
26	30.42	72.26	1	0	0	0	0	1	0	0
27	30.40	72.23	1	0	0	0	0	0	0	0
28	30.42	72.26	1	0	0	0	0	0	0	0
29	30.42	72.26	1	0	0	0	0	0	0	0
30	30.45	71.90	0	0	0	0	0	0	0	0
31	30.41	72.03	1	0	0	0	0	0	0	0
32	30.46	71.93	1	0	0	0	0	0	0	0
33	30.48	71.92	1	1	1	1	1	0	0	0
34	30.47	71.91	0	1	1	1	1	0	0	0
35	30.47	71.91	1	1	1	1	1	0	0	0
36	30.46	71.90	1	1	0	0	0	0	0	0
37	30.16	71.86	1	0	0	0	0	0	0	1
38	30.16	71.86	1	0	0	0	0	0	0	1

Sr. No	Y	X	W.F	PBW	Jassid	A.W	Thrips	M.B	D.B	F.W
39	30.13	71.25	0	0	1	1	1	0	0	1
40	30.13	71.85	1	0	0	0	0	0	0	0
41	29.57	70.74	1	1	1	1	1	0	0	0
42	29.58	71.77	1	1	0	0	0	0	0	0
43	29.59	71.77	1	0	0	0	0	0	0	0
44	29.59	71.78	1	1	0	0	0	0	0	0
45	29.61	71.86	0	0	1	1	0	0	0	0
46	29.62	71.87	1	1	1	1	0	0	0	0
47	29.61	71.84	1	0	1	1	1	0	0	0
48	29.60	71.66	1	1	1	1	1	0	0	0
49	29.60	71.66	1	0	1	0	1	0	0	0
50	29.61	71.61	1	0	1	0	1	0	0	0
51	29.57	71.54	1	0	1	1	1	0	0	0
52	29.58	71.52	1	0	1	1	1	0	0	0
53	29.60	71.50	1	0	1	1	1	0	0	0
54	29.68	71.59	1	0	1	0	1	0	0	0
55	29.71	71.59	1	0	1	0	1	0	0	0
56	29.74	71.98	1	0	0	0	0	0	0	0
57	29.74	71.57	1	0	0	0	0	0	0	0
58	29.76	71.56	1	0	0	0	0	1	0	0
59	29.77	71.56	1	0	1	0	1	1	0	0
60	29.81	71.57	1	0	0	0	0	0	0	0
61			1	0	1	1	1	1	0	0
62	29.92	72.03	0	0	1	0	0	0	0	0
63	29.86	72.08	0	1	1	0	0	0	0	0
64	29.84	72.07	0	1	0	0	0	0	0	0
65	29.77	72.19	1	0	0	1	1	1	0	0
66	29.77	72.18	1	0	1	0	1	0	0	0
67	29.82	72.22	1	0	1	0	0	0	0	0
68	29.98	72.49	1	1	0	0	0	0	0	0
69	29.98	72.49	1	0	1	0	0	1	0	0
70	30.01	72.49	1	1	0	0	0	0	0	0
71	30.11	72.54	0	0	1	0	0	0	0	0
72	30.12	72.54	1	0	0	0	0	0	0	0
73	30.12	72.54	0	0	0	0	0	0	0	0
74	30.15	72.62	1	0	1	0	1	0	0	0
75	30.16	72.62	1	0	0	0	0	0	0	0
76	30.17	72.62	1	0	0	0	0	0	0	0
77	30.13	72.63	1	0	1	0	0	0	0	0
78	30.13	72.63	1	1	1	0	0	0	0	0
79	30.14	72.65	1	1	1	0	0	0	0	0
80	30.12	72.66	0	0	1	0	0	0	0	0

Sr. No	Y	X	W.F	PBW	Jassid	A.W	Thrips	M.B	D.B	F.W
81	30.07	73.41	0	0	1	1	1	0	0	0
82	30.09	73.39	1	0	0	0	0	0	0	0
83	29.98	73.29	0	0	1	0	0	0	0	0
84	29.98	73.30	0	1	1	1	0	0	0	0
85	29.93	73.25	1	1	1	0	0	0	0	0
86	29.92	73.26	1	0	1	0	0	0	0	0
87	29.63	73.08	1	0	0	0	0	0	0	0
88	29.61	73.04	1	0	1	0	0	1	0	1
89	29.61	72.96	1	0	1	0	0	0	0	1
90	29.58	72.94	1	1	1	0	0	0	0	1
91	29.58	72.94	1	0	1	1	1	0	0	0
92	29.39	72.84	0	0	1	0	1	0	0	0
93	29.28	72.83	0	0	1	0	1	0	0	0
94	29.29	72.83	0	0	1	1	1	1	0	0
95	29.58	72.81	1	0	1	0	1	0	0	0
96	29.61	72.82	1	0	0	0	0	0	0	0
97	29.61	72.80	0	0	1	0	1	1	0	0
98	29.62	72.84	0	0	1	0	0	0	0	0
99	29.69	72.50	1	0	1	0	0	0	0	0
100	29.41	72.41	1	0	1	1	0	0	0	0
101	29.72	72.64	1	0	1	0	1	0	0	0
102	29.71	72.61	1	0	1	0	0	1	0	0
103	29.67	72.35	1	0	1	0	0	0	0	0
104	29.33	71.59	0	0	1	0	0	0	0	0
105	29.34	71.62	0	1	1	0	1	0	0	0
106	29.34	71.67	1	1	0	1	0	0	0	0
107	29.22	71.72	1	1	1	0	1	0	0	0
108	29.18	71.73	1	1	1	0	1	0	0	0
109	29.16	71.74	1	1	1	0	0	0	0	0
110	29.11	71.56	0	1	1	1	1	0	0	0
111	29.12	71.56	0	0	1	0	1	0	0	0
112	29.10	71.50	1	0	0	0	0	1	0	0
113	29.12	71.42	1	0	1	0	0	0	0	0
114	29.13	71.40	1	0	1	0	0	0	0	0
115	29.13	71.32	1	0	1	0	0	0	0	0
116	29.13	71.28	0	1	1	1	0	0	0	0
117	29.14	71.23	1	0	1	1	1	0	0	0
118	29.13	71.21	0	0	1	0	0	0	0	0
119	29.12	71.18	1	0	1	1	1	0	0	0
120	29.10	71.14	1	0	1	0	0	0	0	0
121	28.23	70.23	0	0	0	1	0	0	0	0
122	28.31	70.23	0	0	0	0	0	0	0	0

Sr. No	Y	X	W.F	PBW	Jassid	A.W	Thrips	M.B	D.B	F.W
123	28.23	70.25	0	0	0	0	0	0	0	0
124	28.33	70.23	0	0	0	0	0	0	0	0
125	28.23	70.35	0	1	0	0	0	1	0	0
126	28.23	70.35	0	0	0	0	0	0	0	0
127	28.30	70.40	1	0	0	0	1	0	0	0
128	28.33	70.43	0	0	0	0	0	0	0	0
129	28.34	70.42	1	0	0	0	0	0	0	0
130	28.38	70.41	1	0	0	0	0	0	0	0
131	28.39	70.42	0	1	1	0	0	0	0	0
132	28.30	70.40	0	0	0	0	0	0	0	0
133	28.45	70.51	0	0	0	0	0	0	0	0
134	28.47	70.51	0	0	0	0	0	0	0	0
135	28.47	70.95	1	1	0	0	0	0	0	0
136	28.28	70.14	1	1	0	0	0	1	0	0
137	28.26	70.13	1	0	0	0	1	1	0	0
138	28.19	70.08	1	0	1	0	0	0	0	0
139	29.19	70.10	1	1	1	1	1	0	0	0
140	28.22	70.15	1	0	0	1	0	0	0	0
141	28.93	70.24	1	0	1	0	0	0	0	1
142	28.88	70.16	0	0	1	0	0	0	0	0
143	28.80	70.02	1	0	0	0	0	0	0	1
144	28.78	69.99	1	0	0	0	0	0	0	1
145	28.72	69.95	1	0	1	0	0	0	0	0
146	28.72	69.38	1	1	1	0	0	0	0	1
147	29.06	70.33	1	1	1	0	1	0	0	1
148	29.06	70.33	0	1	0	0	0	0	0	0
149	29.07	70.30	1	0	0	0	0	0	0	0
150	29.06	70.30	0	1	0	1	1	1	0	0
151	29.04	70.34	1	1	1	0	1	0	0	1
152	29.09	70.34	1	1	1	0	0	0	0	0
153	29.10	70.37	0	1	0	0	0	0	0	1
154	29.41	70.52	1	1	1	0	0	0	0	1
155	29.42	70.50	1	0	1	0	0	1	0	0
156	29.42	70.48	1	1	1	0	0	0	0	0
157	29.44	70.46	0	0	1	0	0	0	0	0
158	29.50	70.41	0	0	1	0	0	0	0	0
159	29.51	70.41	1	1	1	0	0	1	0	0
160	29.69	70.59	1	1	1	0	0	1	0	0
161	30.96	70.24	1	1	1	1	0	0	0	0
162	30.95	71.03	0	1	1	0	0	0	0	0
163	30.99	71.10	0	0	0	1	1	0	0	0
164	31.02	71.08	0	0	0	0	0	0	0	0

Sr. No	Y	X	W.F	PBW	Jassid	A.W	Thrips	M.B	D.B	F.W
165	31.02	71.07	1	1	0	0	0	0	0	0
166	31.03	71.06	1	1	1	0	1	0	0	0
167	31.02	71.04	0	1	1	0	1	0	0	0
168	31.01	70.99	1	1	0	0	0	1	0	0
169	31.16	71.01	0	1	0	0	0	0	0	0
170	31.22	70.98	1	0	0	0	0	0	0	0
171	31.24	70.99	0	1	0	0	0	1	1	0
172	31.25	71.03	1	1	0	0	0	0	0	0
173	31.17	71.14	0	1	0	0	0	0	0	0
174	31.19	71.17	1	1	0	0	0	0	0	0
175	31.17	71.23	0	1	0	0	0	0	0	0
176	31.01	71.25	1	0	0	0	0	0	0	0
177	31.01	71.27	0	1	0	0	0	0	0	0
178	30.99	71.28	0	1	0	0	0	0	0	0
179	30.96	71.30	1	1	0	0	0	0	0	0
180	30.93	71.42	1	1	0	0	0	1	0	0
181	30.03	70.16	1	0	1	0	1	0	0	0
182	30.00	70.73	1	0	1	0	1	0	0	0
183	29.96	70.57	1	0	1	0	1	0	0	0
184	29.96	70.41	1	0	1	0	1	0	0	0
185	29.96	70.68	1	0	1	0	1	0	0	0
186	29.96	70.68	1	1	0	0	0	0	0	0
187	29.96	70.41	1	1	1	0	1	0	0	0
188	29.96	70.41	1	0	0	0	0	0	0	0
189	29.91	70.66	1	0	1	0	0	0	0	1
190	29.90	70.65	1	0	1	0	1	0	0	0
191	30.52	70.70	1	0	1	0	1	0	0	0
192	30.56	70.68	1	0	0	0	0	0	0	0
193	30.55	70.67	1	0	1	0	1	0	0	0
194	30.63	70.68	1	1	0	0	0	0	0	0
195	30.63	70.68	0	1	1	0	1	0	1	0
196	30.63	70.67	1	0	0	0	0	0	1	0
197	30.63	70.67	1	0	1	0	1	0	1	0
198	30.66	70.67	0	1	1	0	0	0	1	0
199	29.67	70.66	1	0	1	0	1	0	0	0
200	30.68	70.64	1	0	1	0	1	0	1	0
201	29.40	70.96	1	0	1	0	0	0	0	0
202	29.37	70.98	1	1	0	1	0	0	0	0
203	29.52	70.96	1	0	1	0	0	0	0	0
204	29.54	70.10	1	0	1	0	0	1	1	0
205	29.45	70.99	0	0	0	1	0	0	0	0
206	29.51	70.95	1	1	0	0	0	0	0	0

Sr. No	Y	X	W.F	PBW	Jassid	A.W	Thrips	M.B	D.B	F.W
207	29.54	71.02	0	0	0	0	0	0	0	0
208	29.36	71.00	1	0	0	0	0	0	0	0
209	29.51	70.95	0	1	0	0	0	0	0	1
210	29.54	71.01	1	1	0	1	0	0	0	0
211	30.05	70.98	0	1	0	1	0	0	0	1
212	30.00	71.11	1	0	0	0	0	0	0	0
213	30.00	71.11	1	0	0	0	0	0	0	0
214	30.00	71.11	1	0	0	1	0	0	0	0
215	30.00	71.11	0	1	0	0	0	0	0	0
216	30.27	70.87	0	1	0	0	1	1	1	0
217	30.27	70.87	0	1	0	0	0	0	0	0
218	30.27	70.87	1	1	1	0	1	1	0	0
219	30.27	70.87	1	1	0	0	1	0	0	0
220	30.27	70.87	1	1	0	0	0	0	0	0

** W.F= White Fly, P.B.W= Pink Boll Worm, A.W= Army Worm, M.B= Mealy Bug,
D.B=Dusky Bug, F.W=Fusarium Wilt

Annexure IV: Bt protein expression of Cry1Ac in different cotton varieties

Coordinate (X)	Coordinates (Y)	Tehsil	Variety	Cry1Ac ($\mu\text{g/g}$)
29.60076	71.66372	Lodhran	Z-33	0.17
28.2292	70.246	RYK	Z-33	0.19
29.06148	70.30428	Rajun Pur	Z-33	0.19
29.40652	70.52386	jam pur	Z-33	0
29.61069	73.03545	Haroon abad	Shahkar	0
29.60679	72.96171	Haroon abad	Shahkar	0.21
31.16871	71.22659	Karor	Shahkar	0.83
30.55943	70.6805	Taunsa	Shahkar	0.05
30.55344	70.67413	Taunsa	Shahkar	0.19
30.00108	71.10705	Muzafargarh	Shahkar	0.74
30.26687	70.86542	Kot Adu	Shahkar	0.35
29.28421	72.826147	Fort Abbas	Lalazar	0.12
28.77723	69.98522	Rojhan	Lalazar	0.23
30.31574	71.66743	Multan	IUB-2015	0.86
30.14695	71.73853	Multan	IUB-2015	0.43
29.61261	71.85587	kahrorpakka	IUB-2015	0.14
30.00382	71.1127	Muzafargarh	IUB-2015	0.29
30.2734	70.86935	Kot Adu	IUB-2015	0.6
29.88847	71.34013	Shujaabad	IUB-2013	1.43
30.32546	71.99847	Khanewal	IUB-2013	1.36
30.38004	72.15472	Khanewal	IUB-2013	0.64
30.38332	72.16967	Khanewal	IUB-2013	1.34
30.44729	71.89658	Mian Channo	IUB-2013	1.22
30.47928	71.92271	Kabirwala	IUB-2013	0.96
29.57204	70.74367	kahrorpakka	IUB-2013	0.08
28.3292	70.227	RYK	IUB-2013	0.14
28.2996	70.4039	Liaqt Pur	IUB-2013	0.11
28.4476	70.5089	Liaqt Pur	IUB-2013	0.11
29.6072	71.83761	kahrorpakka	IUB-2013	0.008
29.58739	71.76853	kahrorpakka	IUB-2013	0.11
28.4722	70.5135	Liaqt Pur	IUB-2013	0.12
30.46861	71.9117	Kabirwala	FH-142	1.46
29.54486	71.02104	Jatoi	FH-142	0.13
29.06248	70.33233	Rajun Pur	BS-52	1.32
31.01911	71.08188	Layyah	BS-52	0.53
30.14451	71.66337	Multan	BS-1016	0.98
29.5813	71.2111	Multan	BS-1016	1.3
29.61629	71.86823	kahrorpakka	S-12	0.07
29.58322	71.76832	kahrorpakka	S-12	0.11
29.58904	71.77727	kahrorpakka	Satluj	0.06