Pak. J. Agri. Sci., Vol. 51(3), 725-731; 2014
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

THE ROLE OF MORPHOLOGICAL AND CHEMICAL PLANT TRAITS IMPARTING RESISTANCE IN *BT* COTTON GENOTYPES AGAINST THRIPS, *Thrips tabaci* (Lind.)

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A study was carried out in the experimental area of Ayub Agricultural Research Institute Faisalabad during (2012) to determine the role of morphological and biochemical parameters of *Bt* cotton genotypes influencing resistance against thrips *Thrips tabaci* (Lind). Six advance lines and one *Bt* cotton approved variety IR-NIBGE-5, SITARA 10-M, SITARA 11-M, PB-38, SITARA-009, IR-NIBGE-4 and BT-121 respectively were planted in Randomized Complete Block Design with 3 replications. Varieties differ significantly in response to thrips population on per leaf basis. SITARA 10-M showed maximum population of thrips (5.01/leaf) and did show significant difference with all other genotypes. Various morphological and biochemical traits also differ significantly among seven genotypes of cotton. Gossypol glands on Leaf lamina and phosphorus contents of leaves showed negative and highly significant correlation -0.447 and -0.490 respectively with the thrips population while hair density on veins and lamina and hair length on lamina showed positive and significant correlation 0.806, 0.574 and 0.45 respectively with the thrips population. All others parameters showed non-significant correlation with the thrips population. The R^2 values, computed together for multiple regression analysis for thrips population indicated that hair density on vein was the most important factor which contributed maximum i.e. 58.2% in pest population fluctuation of the pest. Gossypol gland on lamina was the second important factor showing impact of 20%, Hair density on lamina and hair length on lamina showed 6.90% and 0.30 % impact in per unit change in pest population. Phosphorus contents showed 24% impact in per unit change in the pest density. Principle component analysis of morphological and chemical factors revealed that gossypol gland on lamina showed contrasting behavior having maximum variance value 95.159%. Hair density on vein also showed contrasting behavior with variance values of 3.741%. Hair density on lamina, hair length of lamina and Phosphorus content was not an important factor due to minimum variance value of 0.804%, 0.234% and 0.064%. Morphological and biochemical take part in resistance against pest which can be used as important tool in integrated pest management model.
Keywords: Cotton, morphology, resistance, thrips, insecticide

INTRODUCTION

Thrips infestation has repeatedly caused severe damage to young cotton plants (Arif *et al.*, 2005). A study in Pakistan revealed 37.6% loss in yield of seed cotton by combined attack of Thrips 14.6 per leaf (Saleem *et al.*, 2013) It has been observed that Thrips tabaci (Lind.) is the specie which caused serious destruction to cotton crop in Pakistan (Baloch and Soomro, 1980). According to Williams (2006) during 2005 the greatest losses from thrips occurred in Virginia and Oklahoma with 3.66 and 3%, respectively. Ottens et al. (2004) reported that in Georgia severe infestations can cause yield reduction as high as 50-60% if not controlled. Transgenic Bt cotton genotypes can effectively control specific lepidopteran species (Cotton bollworm Helicoverpa armigera and pink bollworm Pectinophora gossypiella (Arshad and Suhail, 2010) but they are not effective in controlling sucking insect pests (Hofs *et al.*, 2004). Hence Bt genotypes demands regular use of pesticides for effective management of thrips (Hilder and Boulter, 1999). The reduced use of pesticides in Bt cotton increases the population sucking insect pests (Men *et al.*, 2005) so sucking insect pests majorly thrips, jassid and white fly have become a major significant part of Bt cotton insect pest community (Wu *et al.*, 2002). As another aim of Bt cotton is to reduce pesticide usage for environmental safety, there is a need for the establishment of environmental friendly, safer, long lasting and more reliable control measures for the control of sucking insect pests particularly thrips (Ashfaq *et al.*, 2010). Plant resistance is an important factor of controlling agricultural pests. The resistance may be due to physico-morphic and biochemical characters of plant (Raza *et al.*, 2000). Sucking of cell sap can reduce photosynthesis activity (Khan *et al*., 2010).

Syed *et al*., (1996) investigated the relative resistance of twenty cotton varieties and observed the highest and the lowest thrips population was observed on Super Okra and Riode Okra, respectively. Raza *et al*. (2000) tested 10 genotypes of cotton viz., HR-107NH, HR-17H, HR-101, HR-102, HR-103, HR-Vol, FH-900, MNH-552, CIM-443 and FH-643 for resistance and reported that genotype HR-103 (1.61) was found susceptible, whereas, HR-107NH was resistant to thrips. Hairy varieties were reported to be susceptible to thrips population (Baloch *et al.,* 1982). Ali *et al.* (1995) reported that less number of hair on leaf midrib and leaf lamina were found to play a part in contributing resistance to thrips. Raza (2000) reported negative correlation between hair density on leaf lamina and thrips population but positive correlation between gossypol glands and thrips population.

Ali (1999) observed that cotton cultivars having nitrogen, potassium, zinc and copper were positively correlated with thrips infestation while these are negatively correlated with jassid infestation. Protein contents and total minerals are also important for determining resistance against insect attacking cotton, similarly high quantities of total minerals, proteins, zinc, iron, copper, magnesium and lower levels of calcium, phosphorus, potassium and manganese were found to contribute towards resistance against jassid and susceptibility to thrips (Ahmad *et al.*, 1989).

Keeping in view, the existing situation of outbreaks of piercing sucking insects on Bt cotton, there is a direct need to develop an effective and sound pest-management program, that is well suited to the ecological requirements. The present study was undertaken to find out the exact nature of relationship between pest population and morpho-chemical traits of different cotton genotypes in relation to resistance towards thrips infestation with an ultimate aim of helping the plant breeders to incorporate specific elements in the breeding material that proves to be effective against thrips infestation.

MATERIALS AND METHODS

Experiment was carried out in the experimental research area of Ayub Agriculture Research Institute (AARI), Faisalabad during 2012 crop season. The role of morphological and biochemical factors of *Bt*-cotton cultivars in developing resistance against thrips was determined. Recommended and on-season seven *Bt*-cotton genotypes (one approved and six advance lines) IR-NIBGE-5, SITARA 10-M, SITARA 11-M, PB-38, SITARA-009, IR-NIBGE-4 and BT-121 were planted in the Randomized Complete Block Design (RCBD) with three replications to each *Bt*-cotton genotype. The plot size was 3.83m x 8.82m with plant to plant and row to row distance was maintained as 23 cm and 76cm respectively. Data on *Thrips tabaci* population was recorded at seven days intervals from each plot by selecting 15 leaves from fifteen plants (Ali, 1999) per variety in each replication in such a way that first leaf from upper portion of first plant, second leaf from the middle portion of the second plant and third leaf from lower portion of third plant and so on (Khan, 2013).

Different morphological and bio chemical plant traits were studied at crop maturity. Chemical analysis include various macro and micro nutrients in the plant leaves viz., Nitrogen was determine by Kjeldhal apparatus (Winkleman *et al.*, 1986), Phosphorus was determined by calorimetrically vanadomolyhydro-phospharic acid color method (Jackson, 1958), Potassium by flame photometer (Sparks, 1996), Proteins, Iron as well as Manganese contents were determined by using AOAC method (1995) and chlorophyll contents of leaves was measured by using chlorophyll meter model SPAD-502Plus made in Japan (Khan, 2013) and correlated with thrips population.

Sample prepared for the nutrient determination were digested first. First drying and ashing was done by taking accurately weight 1g sample dried and ground into glazed, high from porcelain crucible. Ash 2 hours at 500°C and let it cool. Wet ash with 10 drops water and carefully add 3-4 ml HNO_3. Evaporate, excess HNO_3 on hot plate set at 100-120°C. Return crucible, dissolve ash in 10ml HCl and transfer quantity to 50ml volume flask. After that Wet ashing was done by taking accurately weight 1g sample, dried and ground into 150ml Pyrex beaker. Add 10ml HNO_3 and let soak thoroughly. Add 3ml 60% $HClO_4$ and heat on hot plate, slowly at first, until frothing ceases. Heat until HNO_3 is almost evaporated. If charring occurs then cool it and add 10ml HNO_3 and continue heating. Heat it to white fumes of $HClO_4$. Cool it and add 10ml HCl and transfer quantity to 50 ml. volumetric flask.

Three plants were selected, at random, from each plot, and one leaf from the top, middle and bottom parts of each, taken to study the trichome-density, number of gossypol-glands, length of hair and thickness of the leaf-lamina on its midrib as well as on its veins counted from the lower side of the leaves, under a binocular microscope from three different places of each leaf. The length of midrib and veins, thus, chosen was 1 cm; whereas, the area of leaf-lamina was 1 cm^2. For this purpose, an iron-dye of 1 cm^2 was used. The cross-section of each leaf was cut with the help of a fine razor and the thickness of leaf-lamina, determined from 3 different sites of each leaf, with the help of an ocular micrometer, under a CARL ZEISS binocular microscope. Leaf area was measured by using leaf area meter model L-3100. The data were also analyzed statistically by using Mstst-C and Statistica 8.1 to find the significance of the results within the genotypes and means were compared by LSD test at 5% probability. Simple correlation was worked out between population density of thrips, morphological characters and biochemical traits of the plant. The combined effect of the factors viz., number of gossypol glands, hair density, length of hair and thickness of leaf lamina, leaf area, nitrogen contents, phosphorus contents, potassium contents, zinc contents, iron contents, copper contents, protein contents, chlorophyll contents and manganese contents of the leaves on the population of thrips was measured by developing stepwise multiple linear regression models.

RESULTS

The results (20, (F=82.06; df = 6; P= 0.00)) revealed that the genotypes differ significantly in their response to thrips population per leaf. SITARA 10-M showed maximum population of thrips (5.03/leaf) and did show significant difference with those of IR-NIBGE-5, SITARA 11-M, PB-38, SITARA-009, IR-NIBGE-4 and BT-121. The minimum population of thrips was recorded to be 1.76 per leaf on PB-38 and showed significant difference with those of recorded on all other varieties. So it can be concluded that SITARA 10-M is a susceptible variety and as for showing minimum population of thrips PB-38 can be classified as resistant variety among all other genotype under study. The various morphological plant traits viz., hair density, length of hair

Table 1. Mean comparison of various morphological plant traits of Bt cotton

Genotypes	Thrips pop. per leaf	Trichome density			Trichome length mµ			Gossypol Glands			Thick. of leaf lamina mµ /cm²	Leaf area /cm²
		Lamina /cm²	Vein /cm	Midrib /cm	Lamina /cm²	Vein /cm	Midrib/cm	Lamina /cm²	Vein /cm	Midrib /cm		
IR-NIBGE-4	1.87F	169.87BC	157.50CD	194.80AB	2.247AB	2.91A	2.75A	39.97B	6.97C	25.56BC	0.88D	121.67A
IR-NIBGE-5	2.00E	128.07D	148.63D	195.67AB	1.29C	2.94A	2.81A	28.59D	4.65D	24.28CD	1.04D	124.01A
SITARA 10-M	5.03A	194.87A	183.77AB	205.17A	2.42AB	2.83A	2.92A	26.84D	3.37E	20.75D	1.23C	105.03C
SITARA 11-M	2.95C	148.37CD	171.43BC	186.27AB	2.76AB	1.87B	2.59A	34.68C	6.00C	35.33A	1.35C	98.88D
BT-121	4.14B	183.90AB	199.23A	180.47B	2.88A	3.16A	3.05A	40.67B	9.08B	28.90B	1.54B	112.59B
SITARA-009	2.77D	166.27BC	167.20C	182.07B	2.52AB	2.77A	3.31A	43.56B	9.91AB	38.17A	1.75A	75.54F
PB-38	1.76G	165.80BC	157.23CD	143.10C	2.07BC	2.40AB	3.37A	51.18A	10.59A	36.95A	1.84A	85.58E
F- Value	916.85	7.88	10.60	9.65	4.20	2.30	1.20	37.80	57.10	27.90	40.80	94.20
P- Value	0.00	0.00	0.00	0.00	0.01	0.09	0.36	0.00	0.00	0.00	0.00	0.00

Table 2. Data regarding various chemical plant traits of Bt cotton

Genotypes	Thrips Population per leaf	Zn ppm	Fe ppm	Cu ppm	Mn ppm	P ppm	K (%)	N (%)	Crude protein (%)	Chlorophyll contents CCI
IR-NIBGE-4	1.87F	24.28D	291.03E	9.32E	28.21B	2808.7D	3.14B	2.78BC	19.43A	45.53B
IR-NIBGE-5	2.00E	26.86C	353.97B	15.19C	32.71A	2529.3F	3.21B	3.02A	18.30B	45.67B
SITARA 10-M	5.03A	22.85E	328.27C	14.99C	23.86D	2139.0G	3.66A	2.87B	17.20C	55.80A
SITARA 11-M	2.95C	20.57F	281.67E	11.90D	26.54C	2719.4E	2.66C	2.69CD	16.25D	52.47AB
BT-121	4.14B	31.17A	363.30B	17.19B	29.38B	2923.3C	2.54CD	2.56DE	15.88DE	51.51AB
SITARA-009	2.77D	30.23A	309.77D	18.10AB	32.64A	3823.1A	2.38D	2.48EF	16.20D	56.77A
PB-38	1.76G	28.23B	423.23A	19.07A	25.46C	3730.9B	2.14E	2.41F	15.12E	53.53AB
F-value	916.85	82.40	70.50	97.80	49.20	611.0	63.90	23.20	32.20	2.24
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08

Table 3a. Correlation coefficient values between physical factors and thrips population on *Bt* cotton

	Thickness of leaf lamina	Gossypol glands per cm²			Hair density per cm²			Hair length			Leaf area
		Vein	Midrib	Lamina	Vein	midrib	Lamina	Vein	Midrib	Lamina	
r- value	0.085	-0.368	-0.349	-0.447*	0.806**	0.395	0.574**	0.138	-0.056	0.451*	0.021
S.E.	0.013	0.472	0.597	0.623	0.422	0.756	0.787	0.175	0.130	0.177	0.640
P- value	1.000	0.101	0.120	0.042	0.000	0.075	0.006	1.000	1.000	0.040	1.000

Table 3b. Correlation coefficient values between chemical factors and thrips population on *Bt* cotton

	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Protein	Zinc (Zn)	Manganese (Mn)	Copper (Cu)	Chlorophyll contents	Iron (Fe)
r- value	0.092	-0.490*	0.362	-0.225	-0.089	0.057	0.146	0.387	-0.150
S.E.	0.062	4.776	0.135	0.182	0.376	0.354	0.499	0.397	1.285
P- value	1.000	0.024	0.106	0.325	1.000	1.000	1.000	0.083	1.000

Table 4a. Multivariate regression analysis of thrips population and physical plant traits

Regression model (Thrips Vs physical characters)	R^2	$100R^2$	Impact	SE	F	P
Y= 3.090 – 0.205 X1	0.200	20.0	20.0	0.286	4.75	0.04
Y= -1.611 - 0.167X1 - 0.343X2**	0.782	78.2	58.2	0.153	32.23	0.00
Y= 1.581 - 0.210X1 – 0.248X2** + 0.114X3**	0.851	85.1	6.90	0.131	32.28	0.00
Y= - 1.435 – 0.220 X1+ 0.229 X2**+ 0.109X3**+ 0.131X4 ^{NS}	0.854	85.4	0.30	0.133	23.44	0.00

X1= Gossypol gland on lamina, X2= Hair density on vein, X3= Hair density on lamina, X4= Hair length on lamina

Table 4b. Multivariate regression analysis of thrips population and chemical plant traits

Regression model (Thrips Vs chemical characters)	R^2	$100R^2$	Impact	SE	F	P
*Y= 3.372 – 0.0285X1	0.240	24.0	24.0	0.278	6.01	0.02

X1= Phosphorus contents

Table 4c. Multivariate regression analysis of variance between thrips population and various chemical and morphological plant traits

Regression model (Thrips Vs physical/chemical characters)	R^2	$100R^2$	Impact	SE	F	P
Y= -1.703 - 0.309X1 – 0.228X2** + 0.122X3**+ 0.138X4^{NS} – 0.0121X5^{NS}	0.862	86.2	86.2	0.134	18.68	0.00

X1= Gossypol gland on lamina, X2= Hair density on vein, X3= Hair density on lamina, X4= Hair length on lamina, X5= Phosphorus contents

and number of gossypol glands on midrib, veins and leaf lamina, leaf area and thickness of leaf lamina differed highly significantly among various genotypes of cotton (Table 1). As far as correlation between thrips population and morphological plant traits is concerned, correlation coefficient values (r-values) given in Table 3 revealed that gossypol glands on leaf lamina showed negative and significant correlation with the thrips population with an r-value of -0.447*. It means more the gossypol gland on leaf lamina less the thrips population. Hair density on vein and lamina showed positive and highly significant correlation with the thrips population with R values of 0.806** and 0.574** respectively. It is representing that more the number of hairs on vein and lamina region more the population of thrips. Hair length on leaf lamina also showed positive and significant correlation with the thrips population with an r-value of 0.451*. By the soul of the statement if hair length on lamina is more the infestation of the pest in crop is more. While all other morphological parameters expressed non-significant correlation towards resistance against thrips population.

The various biochemical plant traits viz., nitrogen, phosphorus, potassium, manganese, protein, copper, zinc, chlorophyll contents of leaves and iron differ significantly among various genotypes of cotton as given in Table 2. As far as the correlation between thrips population and biochemical traits is concerned r-values given in Table 3 revealed that negative and highly significant correlation was observed between thrips population and phosphorus contents of leaves with an r-value of -0.490*. If a crop have sufficient amount of phosphorus the crop will be considered as resistant against thrips population. While all other biochemical parameters expressed non-significant correlation towards resistance against thrips population.

***Multivariate linear regression models between thrips population and physical and chemical plant characters*:** The factors those were showed significant correlation with the thrips population were processed for multiple regression analysis of variance. The results (Table 4a) reveal that hair density on vein was the most important factor which contributed maximum i.e. 58.2% in pest population fluctuation of the pest. Gossypol gland on lamina was the second important factor showing impact of 20%, Hair density on lamina and hair length on lamina showed 6.90% and 0.30 % impact in per unit change in pest population. The value R^2 observed to be 0.854 when the effect all the physical plant characters was computed together. All the regression model were however fitted to be good because the $P<0.05$ and giving significant multivariate linear regression models.

The results (Table 4b) regarding multivariate regression analysis of variance between thrips population and chemical plant characters reveal that phosphorus contents showed 24% impact in per unit change in the pest density. The regression model was well fitted and showing significant multivariate linear regression models. Amongst various factors only the character exerts significant correlation with thrips population.

The data on the physical and chemical plant characters showing results (Table 4c) reveal that R^2 value was observed to be 0.862 while giving the impact of 86.2% when the effect of all the factors were computed together. In regression model equation, it was found that gossypol on lamina hair density on vein and hair density on lamina has significant impact on population fluctuation in combination. However hair length and phosphorus contents exerted no impact. Regression was found to be the best due to $P<0.05$.

***Principal component analysis of morphological and chemical characters of Bt cotton*:** Data regarding the effect of physical and chemical characters on population fluctuation of pest were computed for PCA to determine the effect of individual parameter on thrips population (Table 5).

Morpho-chemical components: All components showed 95.159% influence. Hair density on vein showed contrasting behavior. Phosphorus was most important factor with maximum value of 0.993 and hair density of lamina can be considered non important factor in first component due to value of 0.000.

***Hair density on lamina vs Morpho-chemical components*:** This factor exerted 3.741% influence on pest population. Phosphorus showed contrasting behavior but counted as non-important factor with minimum value of -0.001. The hair density on lamina had maximum value 0.848 and classified as important factor in second component.

***Hair density on vein vs Morpho-chemical components*:** This factor exerted 0.804% influence on thrips. Hair density on lamina and gossypol gland on lamina other factors showed contrasting behavior. Phosphorus showing minimum value of 0.045 and said to be unimportant factor in third component. Hair density of vein was classified as important factor due to the value of 0.846.

***Gossypol gland on lamina and hair density on lamina*:** Both factors had 0.234% influence on pest population. Hair density on lamina and phosphorus were important factors showed contrasting behavior. Gossypol gland on lamina contained value of 0.959. By gaining 0.086 value hair density on vein classified as unimportant factor.

***Hair length on lamina vs Morpho-chemical components*:** Hair length on lamina exerted 0.064% influence on thrips. All other morphological factors showed contrasting behavior except hair length on vein along with chemical factor phosphorus contents which counted as non-important factor with 0.006 value. Most important factor was hair length on vein with 0.980 value.

DISCUSSION

Quisenberry and Rummel (1979) observed cotton resistance against thrips on the basis of leaf area. He reported that more the leaf area more the infestation of thrips. These results conform the results of presents study. Akram (1984) reported that greater the number of hairs per cm of leaf, greater the amount of resistance against jassid. Lesser the number of hairs in leaves lesser the number of thrips on the leaves. Shvetsova and Alibekova (1984) said that protein heterogeneity, proteinase activity and crude protein contents could be efficient criteria foe determining the resistance of cotton varieties to thrips. Riaz *et al.* (1987) reported that hair density on leaf vein positively and significantly correlated with thrips population. These results are conforming the results of the present studies. He also reported that nitrogen and protein positively correlate with thrips population. In case of nitrogen the results are confirmatory as well as protein in concern the results are contradictory with the results of present studies. Yousaf and Ahmed (1990) reported that chemical contents of cotton had significant impact on thrips population and hair density on leaves positively correlated with thrips population. Javed *et al.* (1992) reported that physical and chemical plant traits contributing resistance to insect pests. A negative correlation was found between jassid infestation and nitrogen contents of leaves while a positive correlation was found between thrips attack and nitrogen contents of leaves. Zia and Chaudhary (1994) reported that Protein content of the cotton showed negative correlation of with respect of thrips population. These results are confirmatory with the results of

Table 5. Principle component analysis of thrips

Factor	Components				
	1	**2**	**3**	**4**	**5**
Gossypol gland on lamina	0.113	0.126	-0.191	0.959	-0.120
Hair density on vein	-0.027	0.502	0.846	0.086	-0.154
Hair density on lamina	0.000	0.848	-0.485	-0.211	-0.022
Hair length on lamina	0.004	0.114	0.099	0.127	0.980
Phosphorus	0.993	-0.001	0.045	-0.107	0.006
Variance %	95.159	3.741	0.804	0.234	0.064

present studies. Ali *et al.* (1995) reported that nitrogen and manganese showed positive response regarding to thrips population in accordance with the present studies. He also reported that Zinc showed negative correlation with thrips which is also a confirmatory result. Ali (1999) reported that nitrogen, potassium, zinc and copper were positively correlated with thrips population. Results of nitrogen, Potassium and copper contents are confirmatory with the results of present studies on other hand zinc results are contradictory with the results of present study. Raza (2000) reported that hair density on leaf lamina and midrib is negatively correlated with thrips population these are contradictory results with the present results this may be due to difference in material under study. Hassan *et al.* (2000) narrated that low level of nitrogen, phosphorus, potassium, zinc and copper contribute in the resistance in cotton against thrips infestation. Bashir *et al.* (2001) observed that jassid population on cotton leaves had negative correlation with hair density on cotton leaf midrib while white fly and thrips showed positive correlation with these factors. Arif *et al.* (2004) concluded that hair density on midrib played positive and significant role in resistance against thrips. This is confirmatory result. Length of hair on vein had negative and significant correlation with thrips. These are also confirming the results. Gossypol gland on midrib and vein were negatively correlated with thrips. These are also confirming the results. Arif *et al.* (2006) reported that hair density on midrib and vein of upper leaves showed of significant and negatively correlated with thrips. Length of hair on midrib of upper leaves and midrib and lamina of middle leaves and midrib vein and lamina of lower leaves showed negative correlation. Number of gossypol glands on upper, middle and lower leaves proved to be negatively correlated with thrips population. Brown and Simmonds (2006) reported that leaf morphology of host and non-hosts of thrips. This study showing that plant invaded by thrips possessed glandular trichones and coriaceous leaf surface while vice versa in case on non-host plant species. Ali and Ghulam (2007) reported that different cotton verities differ significantly regarding to the thrips population. Murugesan and Kavitha (2010) reported that thrips and all other sucking insects had significant and positive correlation with chlorophyll contents of the leaves. Tahir (2013) showed confirmatory results of the all the parameters. He reported that gossypol gland on lamina showed negative correlation with thrips population. Hair length on lamina, hair density on vein and hair density on lamina showed positive correlation with thrips population which is the conformation of the morphological parameters. As far as chemical characters are concerned the phosphorus showed negative correlation with respect to thrips population. These results are in accordance with results of this study.

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