Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(5): 915-920

Received: 09-07-2018
Accepted: 10-08-2018

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Morphological and biochemical characters of eggplant (*Solanum melongena*) conferring resistance against whitefly (*Bemisia tabaci*)

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Abstract
Results showed that maximum whitefly incidence was observed in Black Round and the minimum population was recorded on Black Beauty. Correlation analysis described that the hair length on midrib region, leaf lamina thickness and percentage of manganese contents showed negative and hair density on midrib region, nitrogen contents and protein contents have a positive correlation with whitefly population. When the morphological characters were regressed together, hair density on midrib was a most important parameter with 72.1% impact. The combination of all morphological parameters has no significant impact on the whitefly population. Among all the biochemical parameters nitrogen showed maximum 71.1% impact while all parameters together showed 8.4% impact. Principle component analysis showed that the thickness of the leaf lamina played an important role in the reduction of whitefly when all significantly correlated parameters were calculated together. From the results, it can be concluded that the morphological and biochemical characters of brinjal imparted resistance against whitefly which can be used as an important tool in integrated pest management model.

Keywords: *Solanum melongena*, morphological resistance, whitefly, biochemical traits, host plant resistance

1. Introduction

Brinjal (*Solanum melongena* L.) is an important solanaceous vegetable crop in sub-tropics and tropics. It is extensively grown in India, Pakistan, China, Philippines, Bangladesh, Egypt, France, Italy, Middle East, Far East and the U.S.A. [1]. Brinjal is grown as an annual crop throughout the year in the tropics and subtropics [2]. In Pakistan, the area under this crop was 8,482 hectares and the total production was 84,690 tons with an average yield of 9.98 Kg/ha. [3]. Brinjal crops are damaged by several insect pests that can cause considerable damage, which renders the fruit unfit for human consumption [4]. Some of the important insect pests of brinjal in Pakistan are brinjal fruit borer, *Leucinodes orbonalis Guenee* (Lepidoptera, Pyralidae), brinjal stem borer, *Euzophera perticella* Ragonot (Lepidoptera, Pyralidae), leaf roller, *Eublemma olivacea* (Walker) (Lepidoptera, Noctuidae), beetle, *Epilachna vigintioctopunctata* Fabr. (Coleoptera, Coccinellidae), aphid, *Aphis gossypii* (Homoptera, Aphididae), Whitefly, *Bemisia tabici* (Genn.) (Hemiptera, Aleyrodidae), thrips, *Thrips palmi* Karny (Thysanoptera, Thripidae) and cotton jassid (CJ), *Amrasca biguttula biguttula* (Ishida) (Hemiptera, Cicadellidae) [5]. Whitefly is an important pest of many vegetable crops. Two species of whitefly are important pests in vegetable crops the greenhouse whitefly (*Trialeurodes vaporariorum*) and silverleaf whitefly (*Bemisia tabaci*). Whitefly transmits Begomovirus in tomato and cucumber which causes tomato yellow leaf curl and cucumber yellows [6]. Whitefly also caused a problem in eggplant [7]. Farmers mostly tried to use easy and quick method to control insect pest. To achieve this, farmers mainly use synthetic pesticides [8]. Pesticide not only exerts health issues in human and animals it also pollutes the environment [9]. The unplanned and extensive use of pesticides also causes the development of resistance among insects [10]. HPR (Host Plant Resistance) is a key component of IPM which can make crop unsuitable for pests without the development of any induced and inheritable resistance in the insects. Plant morphological and biochemical traits played an important in resistance. Hair length on leaf negatively correlated with whitefly population.

More the length less will be the population. It is also showed that nitrogen contents of a plant played an important role in whitefly population establishment. Nitrogen contents of cotton positively correlated with whitefly population [11]. Keeping in view the importance of host plant resistance, the present study was designed on six verities of brinjal to determine the role of morphological and biochemical plant traits and to determine the comparative resistance and susceptibility of different verities against *B. tabaci*. So the host plant resistance can be used in IPM to minimize the pesticide usage in the standing crop.

2. Materials and Methods

2.1 Experimental plants and sowing of plants

Six brinjal verities were used in the present studies. Black Beauty, Black Round, New Nobel, BSS-513, BSS-985 and Kalash were planted in the Randomized Complete Block Design (RCBD) with three replications to each verity. The plot size was 3.83m x 8.82m with the plant to plant and row to row distance was maintained as 23 cm and 76 cm respectively. The data was recorded on weekly bases by selecting 15 leaves/plot/variety/replication.

2.2 Determination of morphological plant traits

Three random plants were selected from each plot in such a way that one leaf from the top portion of the plant, a middle leaf from the second plant and lower leaf from the third plant was taken. Hair density and hair length were counted from leaf lamina, vein and midrib region. These parameters were counted from the lower side of the leaf. 1 cm length of the midrib and leaf vein area was taken out with the help of a fine razor, while 1 cm^2 of leaf lamina was taken out with the help of 1 cm^2 iron dye. Hair length and hair density were counted under CARL ZEISS binocular microscope. The thickness of the leaf lamina was taken from three different places of leaf lamina. To determine the leaf lamina thickness ocular micrometer was used. Leaf area was measured by leaf area meter L-3100 [12].

2.3 Biochemical analysis

Phytochemical plants traits were examined at crop maturity. Different micro and macronutrient analysis were done from the leaves of brinjal plant viz., nitrogen was estimated by Kjeldhal apparatus [13] and phosphorus percentage was determined by using a calorimetrically Novaspace-II spectrophotometer. Potassium percentage was estimated by a flame photometer. Protein, iron and manganese contents were determined by methods mentioned by Khan *et al*., 2017 [11]. Chlorophyll contents of brinjal leaves were measured by using chlorophyll meter model SPAD-502plus made in Japan [11].

To prepare the sample for biochemical plant traits determination, digestion of leaves was done. For digestion, the leaves were dried and ground. 1g of the grounded sample was taken and ashed for 2 hours at 500 ºC after ashing let the material be cooled. 10 drops of distilled water added in the ash and 3-4ml of HNO_3 was added. Let the HNO_3 evaporate on a hot plate at 100-200 ºC. Dissolve the ash in 10ml HCl and transfer 50ml into the volumetric flask. For wet ashing, 1g of the ground sample. After that 10ml HNO_3 was added and let the sample soak it thoroughly. 3ml of 60% $HClO_4$ was added and heated on hot plate slowly until the bubbling was stopped. Keep heating it until the HNO_3 was evaporated. After drying of HNO_3 the material was cooled and 10ml HCl was added and 50ml of the substrate was taken into a volumetric flask.

2.4 Statistical Analysis

Data were computed statistically with the help of Statistix 8.1 and Microsoft office Excel 2013. LSD (Least Significant Difference) with $P< 0.05$ was used to determine the significance of results and mean comparisons. The correlation was computed to determine the relation between whitefly population and with different morphological and biochemical plant traits of brinjal. To determine the impact of the significantly correlated parameters, a multiple linear regression model was constructed with the help of Statistix 8.1. Graphical work was done on R i386 3.5.0 and Minitab 16

3. Results

3.1 Population comparison of whitefly on different *Brinjal* cultivars

Results showing that the whitefly population differs significantly in different Brinjal genotypes per leaf as shown in Table 1a. (F=657.40; DF=6; P=0.000). Maximum population was recorded in Black Round with a mean of 1.600 per leaf which significantly differ from other genotypes. The minimum population was recorded with a mean population of 0.346 per leaf on Black Beauty. Analysis of variance of phenotypic characters is shown in Table 1a, while the variation among the biochemical traits is shown in Table 1b. All the phenotypic and biochemical parameters showed significant variation among all the brinjal verities under study except chlorophyll contents where the difference among the verities was non-significant.

3.2 Influence of phenotypic and phytochemical plant traits on whitefly population on Brinjal.

3.2.1 Effect of Morphological traits on whitefly population

Results in Table 2a showed that hair length on leaf midrib has a significantly negative impact on whitefly population. Hair density on midrib region also showed the positive and significant impact on whitefly population. Results also revealed that population of whitefly will be affected significantly if the leaf lamina is thicker, while leaf area has a non-significant but positive correlation with on the basis of a number of whitefly individuals per leaf.

3.2.2 Effect of biochemical plant traits on whitefly population

As far as biochemical traits are concerned results in Table 2b showed that nitrogen percentage in the brinjal plant played a significant and the positive role in whitefly population dynamics. Manganese has a negative but significant impact on whitefly population. While the crude protein contents showed significant and positive while chlorophyll contents showed positive but not significant influence on whitefly population. Fig 1 showing the correlation matrix of different morphological and biochemical characters.

3.3 Multivariate regression analysis of whitefly population with morphological and biochemical plant traits

Morphological traits of brinjal plants which were significantly correlated with the whitefly population were evaluated to construct a regression model. Results (Table 3a) showed that the impact of hair length on midrib have 72.1% significant impact on whitefly population, while the regression model was working significantly. The model regressed hair length on midrib and hair density on midrib showed 26.8% significant impact on whitefly population. Combination of hair length on midrib, hair density on midrib and thickness of leaf lamina showed no impact on whitefly population. From these results, it can be concluded that the hair density alone has a maximum impact on whitefly population. Biochemical traits of brinjal plants which were significantly correlated with the whitefly population were evaluated to construct a regression model. Results in Table 3b showed that manganese exerts 71.1% significant impact on whitefly population. The regression of manganese and nitrogen combined showed that the impact was 15%. The combination of all three parameters manganese, nitrogen and protein when regressed, results showed a non-significant impact of 8.4% It can also be concluded that nitrogen is an important factor in the fluctuation of the whitefly population.

3.4 Principle component analysis of significantly correlated parameters

Results in Fig. 2 showed that nitrogen, protein and hair density on midrib have a negative impact on the population of whitefly, Meanwhile, the thickness of leaf lamina, Hair length of midrib and manganese have a positive impact on whitefly population, when these parameters analyzed on the basis of the first component. The impact of all negatively correlated components showed that the whitefly population is increasing and positive impact showed the role of morphological and biochemical plant traits in the reduction of the whitefly population. According to the second factor, the range of negative parameters is less than as compared to the positive parameters. So it can be concluded that positive parameters have more impact on whitefly population than negative parameters. In positive parameters, the thickness of leaf lamina and hair length on midrib region have more impact than manganese, while the thickness of leaf lamina has more impact than hair length on midrib. So after analyzing the results, it can be concluded that the thickness of leaf lamina is a most important morphological parameter in the fluctuation of whitefly.

Table 1a: Analysis of variance of whitefly population and different morphological parameters in different brinjal cultivars.

Varieties	Mean population per leaf	Trichome density			Trichome length mµ			Thickness of leaf lamina mµ /cm²	Leaf area/cm²
		Lamina / cm²	Vein/cm	Midrib/cm	Lamina/cm²	Vein/cm	Midrib/cm		
Black Beauty	0.346F	14.080DE	11.080DE	12.713E	1.896D	1.476D	2.853A	1.856A	119.01A
Black Round	1.600A	18.157A	15.157A	17.387A	2.603B	2.183B	1.796D	1.446C	100.03B
New Noble	0.850D	17.410AB	14.410AB	14.233CD	2.953A	2.533A	2.133C	1.730B	93.88C
Bss-513	1.100C	16.373BC	13.373BC	15.603BC	1.550E	1.130E	1.450E	1.523C	80.58D
Bss-985	0.600E	13.483E	10.483E	13.310DE	2.233C	1.813C	2.503B	1.667B	70.54E
Kalash	1.346B	15.003CD	12.003CD	16.640AB	1.480E	1.060E	1.380E	1.283D	116.67A
F-Value	35.8	15.97	14.86	15.53	93.24	95.21	86.52	33.64	122.22
P-value	0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.000

Analysis of variance of six brinjal verities with three replications was conducted with RCBD with at 95% of confidence interval. Values of $P<0.05$ showed significant variation among the treatments.

Table 1b: Analysis of variance of different biochemical plant traits of different brinjal cultivars

Varieties	Zn ppm	Fe ppm	Cu ppm	Mn %	P ppm	K %	N %	Crude Protein %	Chlorophyll Contents CCI
Black Beauty	31.703A	356.41B	17.317B	34.827A	2382.6CD	2382.6CD	3.427D	17.083D	46.997B
Black Round	24.357C	330.71C	17.123B	25.977D	2658.7BC	2658.7BC	3.890A	20.637A	57.130A
New Noble	22.077C	284.11E	14.030C	28.660C	3580.9A	3580.9A	3.560C	17.407D	53.797AB
Bss-513	29.740AB	425.67A	21.197A	27.577C	3673.1A	3673.1A	3.653BC	18.407C	54.863AB
Bss-985	31.743A	312.21D	20.230A	34.757A	1989.0D	1989.0D	3.283E	16.323E	58.097A
Kalash	25.793BC	293.47E	11.447D	30.333B	2902.7B	2902.7B	3.737B	19.503B	52.840AB
F-Value	7.65	152.96	81.94	62.21	23.57	60.04	30.29	161.07	1.63
P-Value	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.238

Analysis of variance of six brinjal verities with three replications was conducted with RCBD with at 95% of confidence interval. Values of $P<0.05$ showed significant variation among the treatments.

Table 2a: Correlation of morphological plant traits with whitefly population on Brinjal

Variable	Hair Length			Hair Density			Thickness of leaf lamina	Leaf area
	Lamina	Vein	Midrib	Lamina	Vein	Midrib		
R value	-0.010	-0.010	-0.849**	0.686	0.674	0.994**	-0.876**	0.081
SE	0.500	0.500	0.264	0.364	0.364	0.054	0.241	0.498
P value	0.985	0.985	0.032	0.133	0.133	0.000	0.022	0.879

Correlation was calculated with P=0.05, ** showed the significant correlation values, SE= Standard error

Table 2b: Correlation of Biochemical plant traits with whitefly population on Brinjal

variable	N	P	K	Zn	Cu	Mn	Fe	Chlorophyll Content	Crude Protein
R value	0.925**	0.339	0.064	-0.616	-0.291	-0.843**	-0.044	0.490	0.935**
SE	0.191	0.470	0.499	0.394	0.478	0.269	0.500	0.436	0.177
P value	0.008	0.510	0.905	0.193	0.576	0.035	0.934	0.324	0.006

Correlation was calculated with P=0.05. ** showed the significant correlation values. SE= Standard error

Table 3a: Regression model (Whitefly Vs physical characters)

Regression Equation	R^2	$100R^2$	Impact	SE	F	P
$Y^{**}= 2.341-0.677X1^{**}$	0.72	72.10	72.10	0.11	10.35	0.03
$Y^{**}= -2.939+0.029X1^{NS}+0.257X2^{**}$	0.99	98.90	26.80	0.03	131.96	0.00
$Y^{**}= -3.209+0.008X1^{NS}+0.264X2^{**}+0.129X3^{NS}$	0.99	98.90	0.00	0.31	61.32	0.02

X1= Hair density on Midrib, X2= Hair length on Midrib, X3= Thickness of leaf lamina, NS= Non-significant, * showed the significance of regression model and significance of morphological trait in a model. Linear regression was calculated at $P<0.05$, SE= Standard error, Asterisk indicates a significance of model and related biochemical trait, while NS= Non-significant.

Table 3b: Regression model (Whitefly Vs Biochemical characters)

Regression Equation	R^2	$100R^2$	Impact	SE	F	P
$Y^{**}= 4.196-0.106X1^{**}$	0.71	71.10	71.10	0.11	9.86	0.03
$Y^{**}= -4.450-0.020X1^{NS}+1.683X2^{NS}$	0.86	86.10	15.00	0.09	9.30	0.05
$Y^{NS}= 5.686-0.092X1^{NS}-3.204X2^{NS}+0.528X3^{NS}$	0.95	94.50	8.4	0.07	11.40	0.08

X1= Nitrogen, X2= Manganese, X3= Crude protein, NS= Non-significant, * showed the significance of regression model and significance of biochemical trait in a model. Linear regression was calculated at $P<0.05$, SE= Standard error, Asterisk indicates a significance of model and related biochemical trait, while NS= Non-significant.

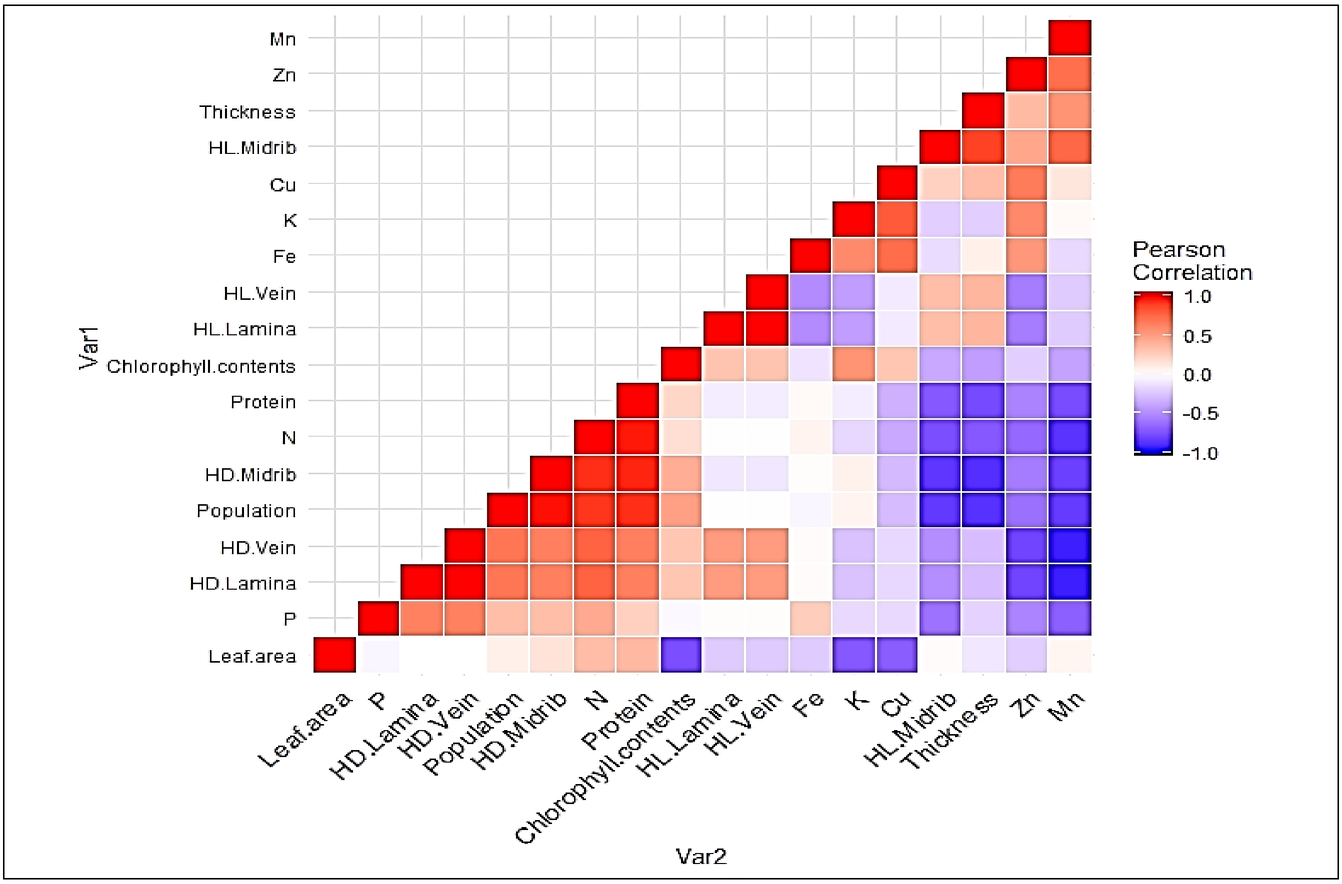

Fig 1: Correlation matrix of morphological plant and biochemical traits with whitefly population and with each other. The intensity of color showing the how strong is the correlation. HD= Hair density, HL= Hair length, Thickness= Thickness of leaf lamina, N= Nitrogen, P= Phosphorus, K= Potassium, Zn= Zinc, Mn= Manganese, Cu= Copper, Fe= Iron.

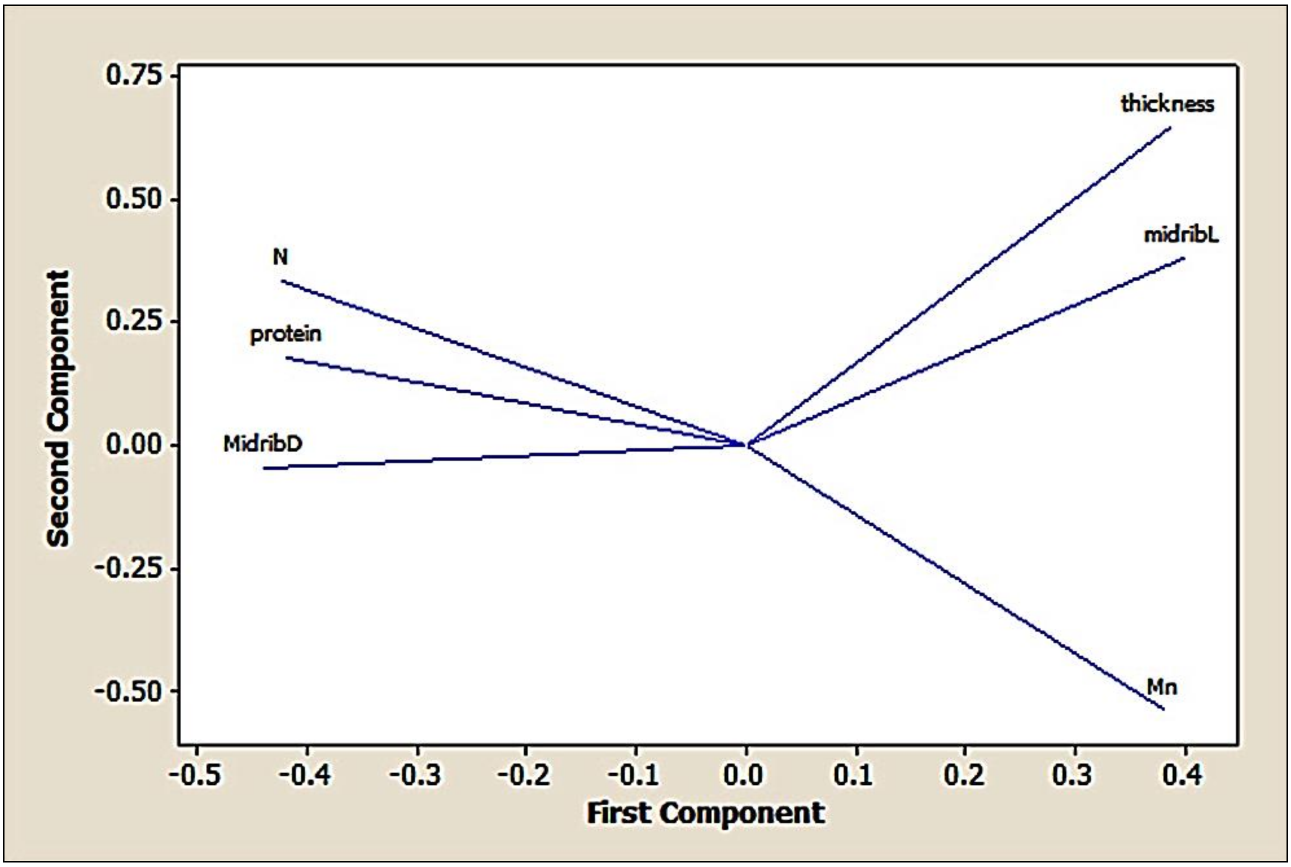

Fig 2: Principle component analysis of significantly correlated morphological and biochemical plant traits of brinjal. N= Nitrogen, HD= hair density, Mn= Manganese, HL= Hair length and Thickness= Thickness of leaf lamina.

4. Discussion

Many researchers assessed the effect of morphological traits of plants against different sucking pests. The Hair length has a negative correlation with the whitefly population [14]. The reported results confirmed that results of a present study where results showed that hair length on midrib that it is negatively and significantly correlated with whitefly population while hair length on lamina and vein region positively but non-significantly correlated with whitefly population. Another researcher reported that hair density on lamina, vein, and midrib region have a significant and positive impact on whitefly population[15], which is also observed in the present study that the hair density also affects the whitefly population positively but the results of vein and lamina are non-significant and midrib have a significant impact. Hair density on a leaf also has a significant and positive correlation with the whitefly population [16]. Literature also revealed that thickness of leaf lamina has a negative but non-significant effect on whitefly population [11]. Some contradictory results were also found in literature, where the reported results stated that the leaf area in sugarcane negatively correlated with whitefly population [17]. The present study showed that nitrogen have a significant effect on whitefly population, but previously reported work showed that the effect is non-significant [17]. Presence of different proteins has a different effect on whitefly population. Some proteins help the population to flourish, while some proteins inhibit the population of whitefly [18]. In the present study overall effect of protein is in favor of population. Manganese has a positive but non-significant effect on whitefly population as reported in previous studies [11], while results here showed that the manganese inhibit the pest population. Overall in this study, it was observed that most of the morphological and biochemical plant traits of eggplant imparted resistance against whitefly population under field condition.

5. Conclusion

From the results, it can be concluded that black beauty is most resistant and black round is most susceptible brinjal plant genotype against whitefly. Hair length and hair density on midrib region, leaf lamina thickness, protein contents, nitrogen and manganese can play a significant role in whitefly resistance. Further exploration also revealed that thickness of leaf lamina is a key character which can impart resistance against whitefly population.

6. Acknowledgement

We would like to appreciate the continuous support of Ms. Lina Cui from Daqing Foreign Language School, Daqing, Heilongjiang, China, for reviewing the manuscript. This research did not receive any specific grant from funding agencies in public, commercial, or not-for-profit sector. Authors declare no conflict of interest in this manuscript.

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