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Assessment of susceptibility of *Ocimum tenuiflorum* to lace wing bug [*Cochlochila bullita* (Stal.)]: Host's physico-morphic and biochemical properties

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Abstract
Two promising subtypes of tulsi, *Ocimum tenuiflorum* were field screened under natural infestation of lace wing bug, *Cochlochila bullita* to assess its reaction. Seven biophysical and ten biochemical attributes of leaves were studied in relation to the expression of their reaction towards lace wing bug. It was observed that highly susceptible *O. tenuiflorum* subtype Rama had relatively higher leaf length (4.04 cm), leaf width (2.36 cm), leaf area (5.99 cm^2), petiole length (1.79 cm), plant height (90.67 cm), wider leaf angle (35°) and canopy diameter (78.33 cm) than the relatively tolerant subtype Krishna. Similarly, with respect to biochemical parameters, chlorophyll B (r value = 0.785, 0.567), total chlorophyll (0.848, 0.703) and carotenoids (0.702, 0.471) showed significant positive correlation with tinged bug incidence and its damage. Susceptible subtype Rama possessed lower reducing (0.189 g/100 g), non-reducing (2.651 g/100 g) and total sugars (2.840 g/100 g) as compared to tolerant subtype Krishna (0.280, 0.906 and 1.185 g/100 g, respectively). In contrast, higher amount of total phenol (6.79 mg/g) and anthocyanin (0.00058 mg/g) contents in leaves were recorded in subtype Krishna and the observed correlations were negative and significant with pest population and its damage. In terms of succulence, leaves of subtype Rama had higher moisture content (80.8%) than the subtype Krishna (78.1%) and showed significant positive correlation with tinged bug population (r value = 0.830) and leaf damage (0.968).

Keywords: *Ocimum tenuiflorum,* subtype, tinged bug, host plant, biophysical traits, biochemical attributes

1. Introduction

Ocimum tenuiflorum L. or *O. sanctum* L. popularly known as holy basil, *tulasi* or *tulsi* is indigenous to the Indian subcontinent. It is an aromatic, aesthetic plant belongs to the family Lamiaceae and now widespread as a cultivated medicinal plant throughout the Southeast Asian tropics. The plants of the genus *Ocimum* are famous for their essential oils containing a number of aromatic compounds and for this reason Tulsi is very correctly ascribed as the "Queen of Herbs" [38]. Basil is a multipurpose group of plants widely known for their aromatic and medicinal properties which comprises of about 160 species [5]. The genus *Ocimum* exhibited considerable intra and inter specific diversity. Though *O. teniuflorum*, *O. basilicum*, *O. gratissimum*, *O. kilimandscharicum*, *O. micranthum*, *O. campechianum*, *O. americanum*, *O. minimum*, and *O. citriodorum*, are found to be occurred in Indian sub continent, *O. americanum*, *O. minimum*, and *O. citriodorum* are exotic species [39].

In India, there are two types of tulsi under cultivation *viz*., the green type or Sri tulsi or Ram tulsi and Krishna tulsi bears purple leaves and is preferred in the trade for its higher potency of drug and former is the most common [36]. In India, the estimated area under Basil is about 25,000 ha which account for annual production of about 250-300 tonnes of oil [36]. The leaves and tender parts of the shoots and seeds are the economically important plant parts, which have enormous commercial significance due to their potential to yield an array of essential oils [16, 27]. Ocimum oil is often used in the perfumery, cosmetic industries and flavoring of food stuffs in confectionery. The extract from tulsi is also reported to have fungicidal, botanical insecticidal, antifeedant properties [14]. In Ayurveda, tulsi has been used for thousands of years for its diverse healing properties and used in remedies for a variety of ailments. Pharmacologically, tulsi is rich in number of chemical metabolites *viz*., eugenol, oleanolic acid, ursolic acid, rosmarinic acid, linalool, carvacrol, β-caryophylleneetc [21]. In plant protection, a natural attractant was isolated from tulsi and used as an attractant for fruit flies [29]. This multipurpose plant is often attacked by a soft, black coloured lace wing bugs (*Cochlochila bullita*) in large numbers found throughout its growth period and causing havoc damage to the leaves and is also known as Ocimum tinged bug [31]. It was reported as most destructive pest of Ocimum in India [16] which sucks the cell sap from the tender leaves, shoots and inflorescence. This pest was first time reported in basil from the eastern part of India [15]. Curling and drying of leaf tips of basil are typical symptoms of the lace wing bug infestation [31].

Since, the commercial and organized farming of this "queen of herb" is sporadic, though it is grown in almost every Hindu household in India, infestation of this lace wing bugs on tulsi was remain unattended. Damage is more serious during winter months (November to January) when almost all the plants are affected by this nefarious sucking pest. In view of its seriousness, efforts have been directed to study the variation of the pest population and damage severity amongst its two popular subtypes and elucidating the mechanisms of extent/variation of infestation, if any. Management of most sucking pests in agricultural and horticultural ecosystems has largely relied on chemical control. However, the demands for clean and ecologically sound environment envisages, careful planning for rationalizing insecticide interventions are need of the hour [7, 12]. Within the environmental friendly pest management approach, Host Plant Resistance (HPR) is one of the most cost-effective and safe methods. Development of suitable resistant/tolerant varieties is an ideal component at no additional cost, compatible with other methods of pest control and free from environmental pollution against buildup of pest population. Various biophysical and biochemical characters of the plants play an important role by providing resistance against number of insect pests [9, 10].Therefore, an attempt was made to identify the response of two popular subtypes in order to determine resistance/ susceptibility towards this bug.

2. Materials and methods

2.1 Experimental site and Plant materials

The field experiments were conducted in Varanasi, Uttar Pradesh, India during 2016-17. The adult bugs were collected and preserved in 75% alcohol and sent to National Pusa Collection, Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India for taxonomic identification. The plant materials for this experiment consist of two subtypes of tulsi *viz*., Rama (*O. tenuiflorum* subtype Rama) which is green in colour and Krishna (*O. tenuiflorum* subtype Krishna) which is black/purple in colour. Periodical observations on lace wing bug infestation were taken at weekly intervals from randomly selected fifty leaves and expressed as number of bugs/leaf.

2.2 Biophysical parameters

Different plant parameters *viz*., leaf length (cm), leaf width (cm), leaf area (cm^2), leaf angle (°), length of petiole (cm), number of branches per plant and plant height (cm) were studied for their role in expression of varietal reaction to lace wing bug. Data on per cent leaf damage from total (healthy + damaged) leaves were recorded from randomly selected ten plants in each subtype by using the following formula:

$$\text{Leaf damage (\%)} = \frac{\text{Number of damaged leaves}}{\text{Total number of leaves}} \times 100$$

Angle between the leaf and the main stem/ branch was measured by using a protractor. Total number of branches were counted twice *i.e*., 180 DAT and at physiologically matured stage. Similarly, 30 fully developed mature leaves of uniform age related to each test entry were collected and their lengths from base to apical tip as well as leaf width and area were measured with the help of a graph paper.

2.3 Biochemical parameters

Ten biochemical parameters, *viz*. chlorophyll A and B, total chlorophyll, carotenoids, reducing, non-reducing and total sugars, total phenols, anthocyanin and moisture content of leaves were estimated for their role in expression of reaction to lace wing bugs. Leaf samples of each subtype (seven days after opening) were collected and analyzed for the aforesaid biochemical parameters to indicate existence of any variation among the duo entries. The extraction of chlorophyll and carotenoid were carried out in 80% acetone as per the procedure [26] and absorption of the sample extracts were recorded at 663, 645 and 480, 510 nm wavelengths for chlorophyll and carotenoid respectively, using Implen Nanophotometer 1256. Similarly, The chlorophylls (Chlorophyll- a and Chlorophyll- b), and carotenoids concentrations on leaves of duo subtypes were calculated by procedure laid out [2] and concentrations were expressed in milligrams per gram fresh weight. Estimation of total phenols was done using Folin-Ciocalteau reagent (FC reagent) [22]. Estimation of total sugar, reducing and non-reducing sugars were done by using Benedicts quantitative reagent [13]. Similarly, assay for anthocyanin was conducted [25] and expressed as mg/g of leaf sample. For analysis of moisture content, five samples, each of 10g of fresh leaves from the top, middle and bottom of different plants, were taken from each subtype. All the leaves, under experiment, were cleaned with a muslin cloth, weighed and kept into a drying oven (NSW 144), at 65°C for 72 hours. The leaves were taken out from the oven and kept in a desiccator for 10 minutes and weighed. After the weight of the dry material become constant, the moisture percentage was calculated, according to the following formula:

$$\text{Moisture percentage} = \frac{\text{Wt. of fresh leaves} - \text{Wt. of dry leaves}}{\text{Wt. of fresh leaves}} \times 100$$

2.4 Protein profiling through SDS page

Total protein form the leaves were extracted in 0.1M phosphate buffer (pH7) and subjected to SDS page analysis at different acrylamide / bisacrylamide concentrations (usually 12% resolving / 4% stacking gels) using a vertical slab gel electrophoresis apparatus (GE Health care Life Sciences, UK). Each lane was loaded with 50 µl of protein. Electrophoresis was conducted at 50-100v with four replications. Proteins separated on gel were stained with coomassie brilliant blue R250 for 2-4 hours followed by destaining gel in destaining solution for 1 to 2 hours until clear background was obtained [18].

2.5 Native PAGE analysis of peroxidases (POs)

To study the expression pattern of different isoforms of POs in two genotypes (black and green), activity gel electrophoresis was carried out as described [34].

2.6 Statistical analysis

The data were subjected to analysis of variance using SAS (version 9.2) package and the means were separated by LSD test at 5% level of probability. The data regarding morpological and biochemical characters of the plant were correlated with lace wing bug population to find out their relation with its incidence. Similarly, a final regression model was developed with the tingid bug population *vis-à-vis* various physico-chemical properties of the leaf.

3. Results and discussion
The lace wing bug is an important pest of tulsi and other basil crops. It can cause curling and drying of leaf tips which directly influence the quality and yield of leaf and essential oil. Taking into consideration the economic importance of this pest in Indian sub continent, there was an urgent need to determine resistance/ susceptibility of tulsi towards this sucking pest. In this experiment our approach was to assess the extent of lace wing bug infestation and to establish a relationship with various physic-morphic and biochemical parameters of host plant in tulsi.

3.1 Pest identification and extent of damage
The insect specimens sent to National Pusa Collection, Division of Entomology, Indian Agriculture Research Institute, New Delhi were taxonomically identified as *Cochlochila bullita* (Stål) (Hemiptera: Tingidae). Damage was more severe during last October to mid-January and a yield loss of 27.84% was recorded due to the damage of the tingid bug on tulsi [1]. *C. bullita*, also known as Ocimum tinged [19, 31], sucks the cell sap from the tender leaves, shoots and inflorescence. Recently, *C. bullita* was reported as a destructive pest of *Ocimum sanctum* from Jharkhand, India [16]. Observation revealed that both nymphs and adults feed mostly on the underside of leaves by sucking the sap from plants' photosynthetic tissues resulting in irregular pale stippling, mottling and bleaching of the leaves. Heavy infestation resulted in drying of affected leaves which later dropped prematurely and a modest reduction in plant growth rate. The role of different biophysical and biochemical characters on lace wing bug incidence on *Ocimum* were studied and described as under.

3.2 Physico-morphic characters
The seven biophysical parameters viz., leaf length, leaf width, leaf area, leaf angle, petiole length, plant height and canopy diameter of two *Ocimum* subtypes were discussed in relation to expression of its reaction towards lace wing bug, *C. bullita*
Among the two *Ocimum* subtypes, highest tingid bug incidence (68.33% leaf damage) as well as its occurrence (4.44 tingid bugs/leaf) were recorded on *Ocimum* subtype Rama followed by *Ocimum* subtype Krishna (58.75% leaf damage and 3.39 tingid bugs/leaf). Highest leaf length (4.04 cm), its width (2.36 cm) and leaf area (5.99 cm^2) were recorded in subtype Rama having light green coloured leaves while leaf length, width and area were lower in subtype Krishna with purple leaves (Table 1). Significant positive correlations (r) were observed between pest population and leaf length (r = 0.958), width (0.936) and total leaf area (0.853). Similar trend was observed between per cent leaf damage and leaf length (0.994), width (0.857) and leaf area (0.978). A significant positive correlation between leaf length and jassid incidence in okra. Broad and lengthy leaf might be providing more area/more food to this sap sucker as compared to narrow and small leaves [12, 28].
The data also revealed that higher petiole length and wider leaf angle were observed in the subtype which suffered more tingid incidence and vice versa. The *Ocimum* subtype Rama which showed average 4.44 tingids/leaf had the higher petiole length of 1.79 cm (Table 1). Similarly, lower numbers of lace wings (3.39/leaf) as well as its damage (58.75%) were noted on relatively tolerant subtype Krishna. Thus the correlations between the petiole length and total number of tingids/leaf and leaf damage were positive and significant (0.989 and 0.966, respectively). Similarly, leaf angle also showed significant and positive correlation (r = 0.986 and 0.931) with lace wing bug preference and its damage. From the present findings, it is evident that the subtype with longer petiole length and wider leaf angle favored incidence of this pest.
Varied differences were observed in the plant height of duo *Ocimum* subtypes and it ranged from 56.67 in subtype Krishna to 90.67 cm in subtype Rama. A significant and positive correlation (r = 0.687) was found between plant height and tingid incidence. Cotton accessions taller in height (166.5 - 168 cm) harbored more jassid infestation [33].
It is also evident that canopy diameter had positive and significant correlation with the tingid bug population and leaf damage (r values = 0.831 and 0.967, respectively) (Table 1). Subtype Rama having broader canopy diameter suffered more tingid bug incidence than subtype Krishna which had narrow canopy diameter. Wider canopy diameter changes the microclimate that might be favorable for the survival and rapid multiplication of the pest. Present findings are in accordance with the recommendations where canopy of grape clusters were exposed to increase wind and sunlight for quick drying of the spores of *Botrytis* [4].

3.3 Biochemical characters
The data with regard to total sugar content, reducing and non-reducing sugar content, phenol content, chlorophylls content, anthocyanin and moisture contents of the leaf of *Ocimum* subtypes Rama and Krishna are discussed in relation to expression of its reaction towards *Ocimum* tinged bug.
The differences in the total sugar content among the two tulsi subtypes were significant and higher sugar content (2.84 g/100g) was recorded in subtype Rama whereas lower sugar content was recorded in subtype Krishna (1.185 g/100 g) (Table 2). The relation between total sugar and leaf damage and pest occurrence were positive and significant (0.894 and 0.943, respectively). It was reported that low sugar content (1.2 mg/g) on tolerant urd bean cultivar LBG-611 than susceptible LBG-17 (1.42 mg/g) against *Maruca vitrata* [8, 20].
Considerable and significant variations in reducing and non-reducing sugar contents existed among the leaves of different entries of *Ocimum*. The subtype Rama possessed highest non-reducing (2.651 g/100g) sugar, while the corresponding low quantum was in subtype Krishna (0.906 g/100 g). In contrast, reducing sugar content of the test materials ranged from 0.189 – 0.280 g/100 g (Table 2) with maximum in subtype Krishna and lowest in subtype Rama. Thus present study indicated that increase in non-reducing sugar content increased the pest incidence [3, 6]. High percentage of non-reducing sugars was found in susceptible genotypes (ML-337, ML-423 and Ml-428) compared to the resistant genotypes (ML-5 and Ml-131) of mung bean against pest complex including *M. testulalis*.
Marked differences in phenol contents on different subtypes of *Ocimum* (6.12 – 6.79 mg/g of fresh sample) were noticed. The phenol content (6.79 mg/g) in subtype Krishna was relatively high than the subtype Rama. Observed relationship among phenol content in leaves with leaf damage and lace wing bug population were negative. Phenolics in a fairly large concentration could ward off insect pests because of direct toxicity [23].

Like other biochemical constituents, chlorophylls of leaves also imparted resistance/ susceptibility to *Ocimum* against *C. bullita*. Significant and positive correlations were recorded amongst chlorophyll-B and total chlorophyll content in leaves and leaf damage (r = 0.567 and 0.703, respectively) and pest occurrence (r = 785 and 0.848, respectively). Subtype Rama having green leaves had higher amount of chlorophyll A (1.95 mg/g), chlorophyll B (0.96 mg/g) as well as total chlorophyll (2.91 mg/g) compared to subtype Krishna with purple coloured leaves and the corresponding values were 1.79, 0.89 and 2.62 mg/g, respectively. Chlorophyll contents in leaves had negative correlation with leafhopper, *Amrasca devastans* in different cotton accessions indicating light green coloured leaves (low chlorophyll content) are less preferred by the sap sucker [24]. In present study, two tulsi subtypes had distinctly different leaf colours *viz*., green in subtype Rama and purple in subtype Krishna. As the sap suckers including tingid bug prefers green coloured leaves, therefore subtype Rama was preferred by the tingid bug compared to the subtype Krishna.
Total anthocyanin content of leaves were also analyzed and correlated with lace wing bug population and their damage. From the Table 1 it is clear that *Ocimum* subtype Krishna had higher quantity of anthocyanin (0.00034 mg/g) compared to subtype Rama (0.00058 mg/g). Thus the observed correlation between anthocyanin content in leaves and pests population (r = – 0.675) and leaf damage (– 0.623) were significant and negative. Statistical analysis of the data for the two tulsi subtypes revealed a positive and significant correlation between moisture content in leaves, lace wing bug population (r = 0.830) and damage (r = 0.968) indicating more succulence favoured more tinged bug incidence leading to more damage. Present findings are in agreement with the finding that there was a positive correlation of moisture contents with the incidence of *A. biguttula biguttula* [35].

3.4 Protein profile and peroxidase expression

The use of SDS page in profiling of proteins expressed that, several proteins ranging from less than 20 kDa to higher than 120 kDa were visualized in the black/purple genotype *i.e.*, subtype Krishna whereas in green genotype (subtype Rama) three proteins in the size ranges of 27-36 kDa only were visualized (Fig 1). In other words, large number of proteins resolved on the gel from the black/purple genotypes might be involved in conferring resistance in subtype Krishna. In this subtype, expression of peroxidase isoform (PO) was observed whereas in the green genotype no such isoforms of PO were visualized in the native page analysis (Fig 2). Expression of peroxidase might indirectly confer resistance to the tingid bug in black/purple genotype. Several reports revealed that, peroxidase enzyme had a major role in imparting resistance to sucking insect pests such brown plant hopper in rice [32], aphids in black gram [37].

3.5 Development of a model

Apart from these, a final model was developed considering tinged bug population on leaves as well as its different biophysical and biochemical parameters (Table 3). No other variable met the 0.500 significance level for entry into the model, so the final model is

$$Y = -25.86 + 0.17x_1 - 1.32x_2 + 0.27x_3 + 5621.26x_4 - 1.77x_5 + 0.23x_6 + 1.13x_7 - 1.08x_8 - 0.60x_9 + 4.22x_{10} + 1.31x_{11} + 0.37x_{12} - 0.47x_{13} + 0.18x_{14}.$$

Form the final Forward stepwise regression model it is clear that among all the physioco-morphic and biochemical parameters, fourteen parameters were considered for developing a predicting model. From the Table 2 it was evident that leaf area had highest impact (13.09%) on susceptibility/resistance to the *C. bullita* followed by total chlorophyll content of leaves (9.99%) whereas plant height contributed minimum of 6.68% in plants' tolerance towards the occurrence of tingid bug.

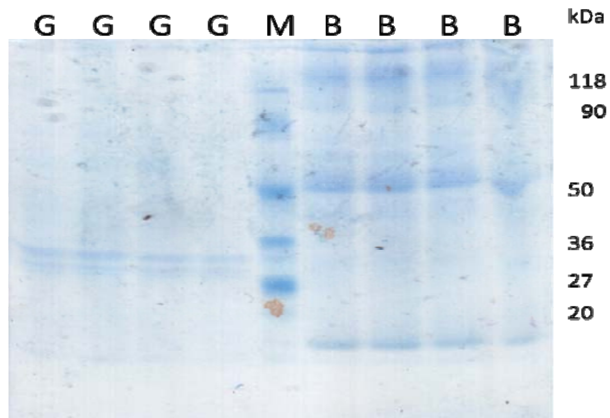

Fig 1: Profiling of total protein on SDS PAGE from tulsi genotypes (B-black, G- Green)

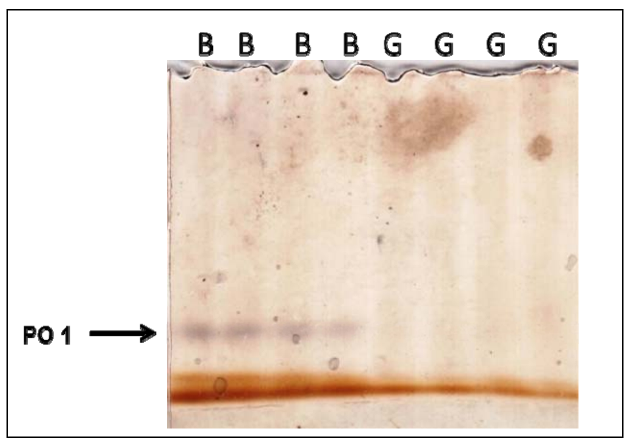

Fig 2: Expression of PO isoforms in tulsi genotypes (B-black, G-Green)

Table 1: Biophysical parameters of two subtypes of *Ocimum* with lace wing bug incidence

Subtype	Mean ± SEm								
	Number of Tingids / Leaf	Leaf damage (%)	Biophysical parameters (Mean ± SEm)						
			Leaf length (cm)	Leaf width (cm)	Leaf area (sq cm)	Petiole length (cm)	Leaf angle (º)	Plant height (cm)	Canopy diameter (cm)
Ocimum subtype Rama	4.44 ± 0.73	68.33 ± 3.87	4.04 ± 0.26	2.36 ± 0.28	5.99 ± 0.67	1.79 ± 0.29	35 ± 1.86	90.67 ± 0.89	78.33 ± 0.83
Ocimum subtype Krishna	3.39 ± 0.75	58.75 ± 3.29	3.30 ± 0.32	1.60 ± 0.23	3.58 ± 0.31	1.37 ± 0.21	33 ± 1.06	56.67 ± 0.88	73.33 ± 0.83
r = (with tinged bug population)	--	--	0.958	0.936	0.853	0.989	0.986	0.687	0.831
r = (with leaf damage)	--	--	0.994	0.857	0.978	0.966	0.931	0.888	0.967
F test	--	--	S	S	S	S	S	S	S

S = Significant; NS = Non-significant

Table 2: Biochemical properties of two subtypes of *Ocimum* with lace wing bug incidence

Subtype	(Mean ± SEm)											
	Number of Tingids / Leaf	Leaf damage (%)	Biochemical parameters*									
			CA	CB	TC	C	RS	NRS	TS	TP	A	MC
Ocimum subtype Rama	4.44 ± 0.73	68.33 ± 3.87	1.95 ± 0.07	0.96 ± 0.12	2.91 ± 0.13	1.00 ± 0.08	0.189 ± 0.03	2.651 ± 0.06	2.840 ± 0.06	6.12 ± 0.15	0.00034 ± 0.002	80.8 ± 0.01
Ocimum subtype Krishna	3.39 ± 0.75	58.75 ± 3.29	1.79 ± 0.47	0.89 ± 0.34	2.62 ± 0.58	0.97 ± 0.32	0.280 ± 0.34	0.906 ± 0.16	1.185 ± 0.05	6.79 ± 0.31	0.00058 ± 0.0013	78.1 ± 0.01
r = (with tinged bug population)	--	--	0.018	0.785	0.848	0.702	– 0.195	0.728	0.943	– 0.303	– 0.675	0.830
r = (with leaf damage	--	--	0.197	0.567	0.703	0.471	– 0.411	0.911	0.894	– 0.512	– 0.623	0.968
F test	--	--	NS	S	S	S	NS	S	S	NS	S	S

* On fresh weight basis; S = Significant; NS = Non-significant

CA: Chlorophyll A (mg/g); CB: Chlorophyll B (mg/g); TC: Total chlorophyll (mg/g); C: Carotenoids (mg/g); RS: Reducing sugar (g/100 g); NRS: Non-reducing sugar (g/100 g); TS: Total sugar (g/100 g); TP: Total phenol (mg/g); A: Anthocyanin (mg/g); MC: Moisture content (%)

Table 3 Forward stepwise regression model showing effect of different leaf characters on number of lace wing bug

Steps of forward selection	Variable Entered	Model R^2	Partial R^2	C(p)
$Y = 2.99 + 0.24x_1$	Leaf area (x_1)	0.1077	0.1077	13.092
$Y = 3.48 + 0.29x_1 + -0.96x_2$	Total chlorophyll (x_2)	0.2242	0.1165	9.990
$Y = -1.19 + 0.31x_1 - 0.91x_2 + 0.19x_3$	Leaf angle (x_3)	0.3029	0.0787	8.540
$Y = -8.08 + 0.28x_1 - 0.91x_2 + 0.22x_3 + 2251.79x_4$	Anthocyanin content (x_4)	0.3614	0.0585	7.978
$Y = -1.27 + 0.26x_1 - 0.998x_2 + 0.21x_3 + 2458.09x_4 - 0.64x_5$	Leaf width (x_5)	0.4286	0.0673	7.032
$Y = -18.68 + 0.22x_1 - 0.97x_2 + 0.23x_3 + 2755.80x_4 - 0.78x_5 + 0.15x_6$	Moisture content (x_6)	0.4750	0.0463	7.002
$Y = -21.40 + 0.17x_1 - 0.95x_2 + 0.24x_3 + 3370.17x_4 - 0.98x_5 + 0.20x_6 + 0.57x_7$	Total phenol (x_7)	0.5154	0.0404	7.231
$Y = -16.87 + 0.21x_1 - 1.18x_2 + 0.18x_3 + 3736.54x_4 - 1.05x_5 + 0.17x_6 + 0.78x_7 - 0.91x_8$	Petiole length (x_8)	0.5712	0.0558	6.785
$Y = -15 + 0.19x_1 - 1.52x_2 + 0.14x_3 + 4474.42x_4 - 1.09x_5 + 0.19x_6 + 0.88x_7 - 1.18x_8 - 1.36x_9$	Plant height (x_9)	0.6193	0.0480	6.680
$Y = -15 + 0.20x_1 - 1.66x_2 + 0.13x_3 + 4681.86x_4 - 1.19x_5 + 0.20x_6 + 0.79x_7 - 1.14x_8 - 1.48x_9 + 2.80x_{10}$	Reducing sugar (x_{10})	0.6440	0.0248	7.595
$Y = -12.69 + 0.21x_1 - 1.45x_2 + 0.13x_3 + 5429.95x_4 - 1.37x_5 + 0.15x_6 + 0.86x_7 - 1.35x_8 - 1.20x_9 + 3.13x_{10} + 0.94x_{11}$	Carotenoids (x_{11})	0.6699	0.0259	8.460
$Y = -20.58 + 0.16x_1 - 1.52x_2 + 0.17x_3 + 5823.98x_4 - 1.42x_5 + 0.20x_6 + 1.02x_7 - 1.13x_8 - 1.09x_9 + 3.55x_{10} + 1.04x_{11} + 0.21x_{12}$	Canopy diameter (x_{12})	0.6844	0.0144	9.828
$Y = -23.91 + 0.14x_1 - 1.25x_2 + 0.23x_3 + 5631.55x_4 - 1.61x_5 + 0.22x_6 + 1.03x_7 - 0.97x_8 - 0.87x_9 + 4.32x_{10} + 1.09x_{11} + 0.32x_{12} - 0.39x_{13}$	Leaf length (x_{13})	0.7032	0.0188	11.005
$Y = -25.86 + 0.17x_1 - 1.32x_2 + 0.27x_3 + 5621.26x_4 - 1.77x_5 + 0.23x_6 + 1.13x_7 - 1.08x_8 - 0.60x_9 + 4.22x_{10} + 1.31x_{11} + 0.37x_{12} - 0.47x_{13} + 0.18x_{14}$	Non reducing sugar (x_{14})	0.7171	0.0139	12.396

No other variable met the 0.500 significance level for entry into the model, so the final model is:

$$Y = -25.86 + 0.17x_1 - 1.32x_2 + 0.27x_3 + 5621.26x_4 - 1.77x_5 + 0.23x_6 + 1.13x_7 - 1.08x_8 - 0.60x_9 + 4.22x_{10} + 1.31x_{11} + 0.37x_{12} - 0.47x_{13} + 0.18x_{14}$$

4. Conclusion

From the present study it can be concluded that *O. tenuiflorum* subtype Rama was more susceptible to lace wing bug, *Cochlochila bullita*. This susceptible subtype had relatively higher leaf length, leaf width, leaf area, petiole length, plant height, wider leaf angle and canopy diameter and more succulent than the relatively tolerant subtype Krishna. Similarly, with respect to biochemical parameters, chlorophyll B, total chlorophyll and carotenoids showed significant positive correlation with tinged bug incidence and its damage. Susceptible subtype Rama possessed lower reducing, non-reducing and total sugars as compared to tolerant subtype Krishna. In contrast higher amount of total phenol and anthocyanin contents in leaves were recorded in subtype Krishna and the observed correlations were negative and significant with pest population and its damage. So, following physico-chemical attributes of the plant should be kept in mind during the breeding programme to get rid of the incidence from this nefarious pest.

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