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SCREENING OF RESISTANCE OF *PIPER BETLE* LANDRACES AGAINST *SINGHIELLA PALLIDA* (HEMIPTERA : ALEYRODIDAE)

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ABSTRACT

Whitefly, *Singhiella pallida* Singh is a serious pest of betelvine *Piper betle* L. Eleven cultivars of *Piper betle* were screened for resistance against *Singhiella pallida*. The biochemical parameters and nutritional parameters of betel leaf were analysed. The varieties Simurali Sanchi and Halisahar Sanchi showed resistance to pest recording whitefly infestation level from 1.01 to 1.34 adults/leaf/vine. However, Simurali Golebhavana was found highly susceptible observing 6.04 whitefly adults/leaf/vine throughout the period of investigation. The resistant cultivars had more phenol (5.52-5.74 mg/g of fresh leaf) and ortho-dihydroxy phenol content (1.25-1.38 mg/g of fresh leaf) than the susceptible cultivars. In-contrary, susceptible cultivars Simurali Golebhavana contained higher concentration nitrogen (25.72 mg/g of dry leaf) and protein (39.68 mg/g of dry leaf) in its leaf as compared to resistant cultivars. Resistant cultivars Simurali Sanchi and Halisahar Sanchi had more than two times higher polyphenol oxidase (1.03-1.11 Δ495/min/μg protein) and peroxidase (1.12-1.48 Δ436/min/μg protein) activities than the susceptible cultivars. The plant enzyme polyphenol oxidase and ortho-dihydroxy phenol found as major factors, which contributed 96.9% variation in white fly population towards imparting resistance in the cultivars. So, Sanchi type of cultivars are resistant to whitefly for high level of polyphenol oxidase activities and OD-phenol content in its leaves.

INTRODUCTION

Betelvine, *Piper betle* (L.), commonly known as 'Pan' is an important cash crop in India. Betelvine is cultivated for its leaf, which are normally consumed directly along with arecanut by millions of people in the world. It is a valuable foreign exchange earner in Indian trade; worth of which is 9000 million every year (Kaleeswari and Sridhar, 2013). In the Asiatic region, the crop ranks second to coffee and tea in terms of daily consumption (Kumar *et al.,* 2010).

In India, the cultivation of betelvine crop is seriously threatened by several insect pests (Raut and Bhattacharya, 1999). Among the several insect pests infesting betelvine, whitefly, *Singhiella pallida* Singh contributes to considerable yield and quality loss. It reduces leaf yield upto 13.39%, when mean population of flies is 43.4 per vine with an overall monetary loss is 29.63% (Das and Mallik, 2009). The economic threshold level varies from 3-4 pests/leaf based on different climatological week (Pal *et al*., 2013). The population of whitefly on betelvine varies with different climatological factors (Dhar *et al*., 2014) and cultivars used for cultivation, where Bangla types are highly susceptible and sanchi types being moderately resistant to whitefly (Das and Mallik, 2010).The resistance and susceptibility attributes of several crops to different insect-pests and pathogens has been explained by many researchers due to the presence of secondary plant metabolites mainly phenols (Vandana Sukhla *et al*., 2014; Pathipati and Yasur, 2010). Moreover, high levels of two major oxidizing enzyme of plants such as poly phenol oxidase and peroxidase impart induce resistance to insect-herbivores and pathogens (Rachana *et al*., 2015; Bandi and Siva subramanian, 2012; He *et al*., 2011). In-contrary, many herbivores such as mealy bug, thrips have high survival rate, fecundity and body size with high infestation level on higher nitrogen host (Cocco *et al*., 2014 and Keeping *et al*., 2014).

Similarly, there are plentiful reports stating betelvine leaf is a rich source of primary as well as secondary plant metabolites such as carbohydrates, proteins, amino acids, reducing sugars, flavonoids, terpenoids, steroids, volatile oil, mucilage, tannin, saponins (Periyanayagam *et al*., 2012), high amount of phenols and its acetates (Satish *et al*., 2015; Bajpai *et al*., 2012). Phenol content in the betel leaf varies greatly from one land races to another (Bajpai *et al*., 2013; Shivasankara *et al*., 2012).

Acknowledging the importance of white fly constraint in betelvine production and based on the interesting reports on wide-array of primary and secondary plant metabolites present in the betel leaf, emphasis was given to identify the resistant cultivars of betelvine against whitefly, so that the same may be used for future crop improvement programme for avoiding the yield and quality loss.

Therefore, attempts have been made to elucidate the infestation pattern of white fly on different cultivars of betelvine for identification of resistant varieties as well as to explain its differential feeding preference on the basis of biochemical and nutritional parameters of betel leaf.

MATERIALS AND METHODS

Screening of betelvine cultivars

The screening of betelvine cultivars against whitefly at field level was carried out in a closed conservatory ('Boroj') at the Instructional farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal. The experimental domain comes under *terai* agroclimatic zone of West Bengal; situated between 25º57`N and 27ºN latitude and 88º25`E longitude.

Two types of cultivars, namely 'Bangla' type including nine cultivars viz. Simurali Chamurdali, Kali Bangla, Simurali Golebhavna, Bagerhat Local, Gayasur, Simurali Bhavna, Ghanagette, Kutki and Simurali Deshi and 'Sanchi' type including two cultivars viz. Halisahar Sanchi and Simurali Sanchi were studied for their relative resistance to whitefly.

The plot size of 2 m x 1 m with paired row, having spacing of 10 cm x 10 cm x 50 cm was maintained in a randomized block design with three replications for each cultivar. Standard agronomic practices and disease control methods were adopted.

Five vines were taken randomly from each row of each replication and adult whitefly population were recorded from top three apical leaves following the method described by Das and Mallik (2010). The observations were taken in every standard week throughout the period of investigation from 2009 to 2011.

Biochemical and nutritional parameters

The following biochemical and nutritional parameters of the leaf of each cultivar were analyzed in laboratories of Department of Agricultural Entomology and Department of Plant Pathology of the University in consecutive three years from 2009 to 2011 in the peak population month of the white fly *i.e.,* December during the period of investigation.

Phenolics

The total phenol content was estimated using Folin-Ciocalteau reagent following the method by Malik and Singh (1980) using spectro photometric method with catechol as standard. The samples were measured at 650 nm wavelength and expressed as mg/g fresh weight of tissue. Similar to phenol, OD phenol was measured at 515 nm and expressed as mg/g fresh weight of tissue following the method described by Mahadevan and Sridhar (1982).

Protein estimation

Quantification of soluble protein and functional protein as polyphenol oxidase and peroxidase was done during the study period. Soluble protein was estimated according to the method by Lowry *et al*. (1951) as modified by Chakraborty *et al*. (1995). Quantitative protein was estimated following Lowry's method (1951) as absorbance at 660 nm against a standard curve of bovine serum albumin and expressed as mg/g fresh weight of tissue.

Method described by Mahadevan and Sridhar (1982) was followed for spectrophotometric estimation of polyphenol oxidase after extraction in sodium phosphate buffer (pH 6.6). Optical density was estimated at 495 nm using 0.05 M pyrogallol solution as substrate at every 5 minutes interval up to 6 reading and expressed as change of optical density in one minute for one microgram of protein at 495 nm absorbance (Δ495/min/μg protein).

Peroxidase activity was measured following the method of Addy and Goodman (1972) by extracting proxidase in phosphate buffer (pH 6) at 4ºC. Pyrogallol and hydrogen peroxide was used as substrate to measure enzyme activity at every 30 seconds up to 180 seconds by measuring absorbance at 436 nm using a spectrophotometer. The peroxidase activity was expressed in change of optical density in one minute for one microgram protein at 436 nm absorbance (Δ436/min/μg protein).

Plant nitrogen

Plant nitrogen (N) was estimated by digesting plant sample with sulphuric-salicylic acid mixture, sodium thiosulphate and catalyst mixture (K_2SO_4 : $CuSO_4$: Se powder = 100:6:1) in the microprocessor based digestion system followed by distillation in microprocessor based distillation system and titration of distillate collected in boric acid mixed indicator solution with N/200 H_2SO_4 until colour changes from green to pink (Bremner and Mulvaney, 1962).

Plant phosphorus and potassium

For estimation of plant phosphorus (P) and potassium (K) content, plant sample was first digested with di-acid mixture (HNO_3:$HClO_4$ = 9:4) in microprocessor based digestion system followed by dilution and filtering. To measure the P content in the solution, aliquot taken from the solution was treated with vanadomolybdate reagent and absorbance was measured at 420 nm using spectrophotometer. On the other for measuring K present in the solution, aliquot taken from the solution was further diluted and K concentration was measured using a flame photometer (Jackson, 1973).

Statistical analysis

Screening of betelvine cultivars

The data, thus obtained were computed and subjected to analysis of variance following RBD after making square root transformations by $\sqrt{N}$, where N (Number of adult whitefly).

Biochemical and nutritional parameters

The data, thus obtained from each of the biochemical and nutritional parameters of leaf in both the year 2009 and 2010 were computed and subjected to analysis of variance following randomized block design (R.B.D.). The correlation followed by step down regression analysis was done to understand the relationship between whitefly population infestation and each of the biochemical and nutritional parameters of the leaf. Data computation and statistical analysis was done in SAS 9.2.

RESULTS AND DISCUSSION

Screening of eleven varieties of betelvine revealed that all the varieties were found to be infested by white fly throughout the period of investigation (Table 1). The overall highest population was observed on Simurali Golebhavna (6.04 adults/leaf/vine). Other Bangla type cultivars such as Simurali Chamurdali and Kali Bangla were highly infested by the pest, where whitefly population was found more or less 5 adults/leaf/vine. Moderate infestation ranging from 4.05-4.56 adults/leaf/vine was observed in Ghanagette, Kutki, Simurali Deshi, Gayasur and Simurali Bhavna. Bagerhat local was least infested by the whitefly (3.16 adults/leaf/vine) among the Bangla type cultivars. However, the lowest whitefly infestation of 1.01 adults/leaf/vine was noticed in Simurali Sanchi, among the eleven screened cultivars followed by Halisahar Sanchi (1.34 adults/leaf/vine).

A negligible pest population was observed in the hot summer and monsoon months *i.e.* April to August. However, the population gradually increased with onset of winter month i.e. November and pest attained peak population in the month of December. The population started declining in peak winter i.e. January. In months when the pest population was at its peak, all the varieties except Simurali Sanchi and Halisahar Sanchi were heavily infested by the pest. The highest population was noticed in Simurali Golebhavana (17.89 adults/leaf/vine). The lowest population was found in Simurali Sanchi (3.45 adults/leaf/vine). It was followed by Halisahar Sanchi, observing 4.56 adults/leaf/vine.

Considering the white fly infestation throughout the period of investigation, especially in the peak population months and the economic threshold level (3-4 adults/leaf/vine) of the pest as stated by Pal *et al*., 2013; it was noticed that the Sanchi type cultivars, Simurali Sanchi and Halisahar Sanchi were found resistant against whitefly. Though, Simurali Sanchi was the only cultivar in which, pest population was found below ETL in peak population month. Among the Bangla type, Bagerhat Local was found least infested by white fly. The other varieties were found moderate to highly susceptible to the pest. Simurali Golebhavna was found most susceptible variety. It was followed by Simurali Chamurdali and Kali Bangla. However, the varieties like Ghanagette, Kutki, Simurali Deshi, Gayasur and Simurali Bhavna were moderately infested by the pest.

Therefore, the screening of eleven varieties revealed that the Sanchi type cultivars viz. Simurali Sanchi and Halisahar Sanchi falls under resistant group, while Bangla type cultivars Bagerhat Local is moderately resistant to the pest. Among rest eight varieties, Gayasur, Kutki, Simurali Deshi, Ghanagette and Simurali Bhavna are moderately susceptible, while Simurali Golebhavna, Simurali Chamurdali and Kali Bangla are highly susceptible to the pest.

Several biochemical parameters of leaves of eleven cultivars were measured and presented in Table 2. A marked difference in some biochemical parameter values were noticed among resistant and susceptible cultivars. The resistant varieties recorded high phenol, ortho-dihydroxy (OD) phenol content as well as polyphenol (PPO) and peroxidase (POX) activities. Simurali Sanchi contained highest phenol (5.74 mg/g leaf) and OD phenol (1.38 mg/g leaf) in its leaf. Similarly, it also

Table 1: Varietal Screening of betelvine (*P. betle*) against whitefly, *S. pallida* in 2009 –2011 (Pooled)

Variety	No. of whitefly/leaf/vine in the month of												
	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Mean
Simurali Chamurdali (V1)	4.61(2.15)*	3.04(1.74)	2.34(1.53)	3.15(1.77)	1.66(1.29)	2.64(1.62)	3.05(1.74)	3.64(1.89)	4.33(2.08)	5.28(2.30)	16.00(4.00)	11.08(3.33)	5.07(2.12)
Kali Bangla (V2)	3.80(1.95)	2.27(1.50)	2.63(1.62)	3.72(1.92)	1.92(1.38)	2.63(1.61)	3.42(1.84)	3.99(1.99)	4.93(2.22)	4.91(2.22)	15.33(3.91)	10.11(3.18)	4.97(2.11)
Simurali Golebhavna (V3)	6.20(2.49)	3.41(1.82)	3.38(1.83)	3.76(1.93)	2.31(1.52)	2.73(1.65)	3.92(1.98)	4.07(2.01)	6.12(2.47)	6.64(2.58)	17.89(4.22)	12.02(3.47)	6.04(2.33)
Bagerhat Local (V4)	3.13(1.76)	1.52(1.23)	2.22(1.49)	1.99(1.41)	1.53(1.23)	1.90(1.37)	2.34(1.52)	2.11(1.45)	2.09(1.44)	3.30(1.81)	10.74(3.28)	5.08(2.25)	3.16(1.69)
Halisahar Sanchi (V5)	1.27(1.12)	1.18(1.08)	0.78(0.88)	0.82(0.89)	0.88(0.94)	0.74(0.85)	0.94(0.96)	1.02(1.01)	0.98(0.99)	1.38(1.17)	4.56(2.13)	1.50(1.22)	1.34(1.10)
Gayasur (V6)	4.14(2.03)	1.32(1.15)	2.19(1.48)	2.54(1.59)	1.81(1.34)	2.41(1.55)	3.08(1.75)	2.68(1.63)	2.13(1.46)	3.31(1.82)	13.47(3.67)	10.11(3.18)	4.10(1.89)
Simurali Sanchi (V7)	0.97(0.98)	1.01(1.00)	0.43(0.65)	0.66(0.81)	0.50(0.70)	0.48(0.69)	0.81(0.89)	0.70(0.83)	0.94(0.97)	1.16(1.08)	3.45(1.86)	1.07(1.03)	1.01(0.96)
Simurali Bhavna (V8)	5.12(2.26)	2.46(1.57)	2.70(1.64)	1.57(1.25)	1.65(1.28)	1.97(1.40)	2.91(1.70)	3.94(1.98)	2.17(1.47)	3.97(1.99)	10.95(3.31)	9.19(3.03)	4.05(1.91)
Ghanagette (V9)	5.89(2.42)	2.72(1.64)	2.95(1.72)	3.05(1.73)	2.49(1.57)	2.26(1.50)	2.84(1.68)	3.00(1.72)	2.60(1.61)	4.93(2.22)	12.77(3.57)	9.22(3.04)	4.56(2.04)
Kutki (V10)	5.70(2.38)	3.13(1.77)	2.94(1.71)	3.33(1.89)	2.73(1.64)	2.20(1.48)	2.88(1.69)	3.07(1.74)	2.34(1.53)	3.33(1.81)	12.19(3.49)	9.33(3.06)	4.43(2.01)
Simurali Deshi (V11)	6.39(2.49)	3.61(1.89)	3.48(1.86)	3.01(1.73)	3.02(1.72)	2.47(1.58)	2.26(1.47)	3.01(1.72)	1.98(1.40)	3.39(1.84)	12.76(3.57)	7.65(2.77)	4.42(2.00)
Mean	4.29(2.00)	2.33(1.49)	2.37(1.49)	2.51(1.53)	1.86(1.33)	2.04(1.39)	2.59(1.57)	2.84(1.63)	2.78(1.60)	3.78(1.89)	11.83(3.36)	7.85(2.69)	-

Sources of variation		Variety (V)	Month (M)	V x M
SEm (±)	:	0.01	0.01	0.04
CD at 5%	:	0.03	0.04	0.12

(* Figures in parentheses are square root transformed values)

Table 2: Biochemical parameters of the leaves of different *Piper betle* L. varieties (Fresh weight basis) in the peak population period of *Singhiella pallida* Singh

Variety	Total Phenol (mg/g fresh leaf)	OD Phenol (mg/g fresh leaf)	Polyphenol Oxidase (Δ495/min/μg protein)	Peroxidase (Δ436/min/μg protein)	Protein (mg/g fresh leaf)
Simurali Chamurdali (V1)	3.67	0.75	0.44	0.41	31.94
Kali Bangla (V2)	4.45	0.74	0.46	0.30	28.23
Simurali Golebhavna (V3)	2.86	0.62	0.23	0.26	39.68
Bagerhat Local (V4)	5.54	1.03	0.62	0.87	22.97
Halisahar Sanchi (V5)	5.52	1.25	1.03	1.12	20.03
Gayasur (V6)	5.20	0.96	0.53	0.59	26.27
Simurali Sanchi (V7)	5.74	1.38	1.11	1.48	19.78
Simurali Bhavna (V8)	4.98	1.01	0.51	0.67	34.20
Ghanagette (V9)	4.97	0.74	0.54	0.65	20.45
Kutki (V10)	4.70	0.98	0.52	0.65	27.92
Simurali Deshi (V11)	4.78	0.95	0.48	0.50	29.93
SEm (±)	‘0.02	0.01	0.003	0.005	0.10
CD at 5%	0.07	0.03	0.01	0.01	0.29

Table 3: Nutritional parameters of the leaf of different *Piper betle* varieties (Dry weight basis) in the peak population period of *Singhiella pallida* Singh

Variety	Nitrogen Concentration (mg/g dry leaf)	Phosphorus concentration (mg/g dry leaf)	Potassium Concentration (mg/g dry leaf)
Simurali Chamurdali (V1)	23.38	3.30	17.09
Kali Bangla (V2)	21.52	2.90	16.68
Simurali Golebhavna (V3)	25.72	2.86	16.05
Bagerhat Local (V4)	18.50	2.61	15.47
Halisahar Sanchi (V5)	17.53	2.86	17.59
Gayasur (V6)	18.59	2.72	19.30
Simurali Sanchi (V7)	16.65	2.70	18.35
Simurali Bhavna (V8)	24.34	2.43	17.28
Ghanagette (V9)	17.53	2.94	17.25
Kutki (V10)	20.44	2.78	16.26
Simurali Deshi (V11)	22.21	2.75	16.78
SEm (±)	0.49	0.02	0.09
CD at 5%	1.46	0.05	0.26

Table 4: Relation between biochemical parameters of betelvine leaf with whitefly population

	IP	TP	ODP	PPO	POX	Prot	N	P	K
IP	1	-0.81815	-0.95466	-0.96948	-0.96622	0.73221	0.70264	0.37987	-0.36207
		0.0021	<.0001	<.0001	<.0001	0.0104	0.0159	0.2492	0.2739
TP	-0.81815	1	0.81409	0.77218	0.78437	-0.83348	-0.81964	-0.5219	0.38711
	0.0021		0.0023	0.0054	0.0043	0.0014	0.002	0.0996	0.2395
ODP	-0.95466	0.81409	1	0.91186	0.92896	-0.62446	-0.62419	-0.46728	0.39532
	<.0001	0.0023		<.0001	<.0001	0.04	0.0401	0.1473	0.2288
PPO	-0.96948	0.77218	0.91186	1	0.95201	-0.79103	-0.76356	-0.18897	0.43227
	<.0001	0.0054	<.0001		<.0001	0.0037	0.0062	0.5779	0.1842
POX	-0.96622	0.78437	0.92896	0.95201	1	-0.74249	-0.73985	-0.34358	0.35902
	<.0001	0.0043	<.0001	<.0001		0.0089	0.0092	0.3009	0.2782
Prot	0.73221	-0.83348	-0.62446	-0.79103	-0.74249	1	0.97708	0.0513	-0.33361
	0.0104	0.0014	0.04	0.0037	0.0089		<.0001	0.8809	0.316
N	0.70264	-0.81964	-0.62419	-0.76356	-0.73985	0.97708	1	0.09967	-0.40794
	0.0159	0.002	0.0401	0.0062	0.0092	<.0001		0.7706	0.213
P	0.37987	-0.5219	-0.46728	-0.18897	-0.34358	0.0513	0.09967	1	-0.03619
	0.2492	0.0996	0.1473	0.5779	0.3009	0.8809	0.7706		0.9159
K	-0.36207	0.38711	0.39532	0.43227	0.35902	-0.33361	-0.40794	-0.03619	1
	0.2739	0.2395	0.2288	0.1842	0.2782	0.316	0.213	0.9159	

IP : Insect Population; TP : Total Phenol, ODP : OD-Phenol, PPO : Polyphenol Oxidase, POX : Peroxidase; Prot : Protein; N; Nitrogen: P : Phosphorus; K : Potassium

recorded highest PPO (1.11 Δ495/min/μg protein) and POX (1.48 Δ436/min/∝g protein) activities. Other Sanchi type cultivar, Halisahar Sanchi had also higher PPO (1.03 Δ495/min/∝g protein) and POX (1.12 Δ436/min/∝g protein) activities followed by moderately resistant cultivar Bagerhat Local 0.62 Δ495/min/∝g protein and 0.87 Δ436/min/∝g protein respectively. Whereas, the most susceptible variety, Simurali Golebhavna had very low phenol (2.86 mg/g leaf), OD phenol

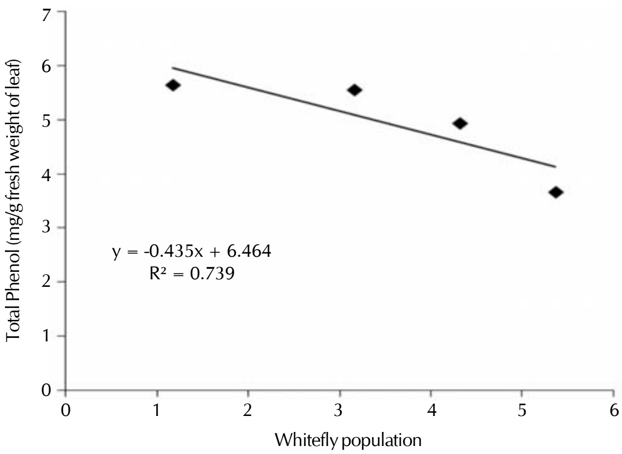

Figure 1: Total phenol and whitefly interactions

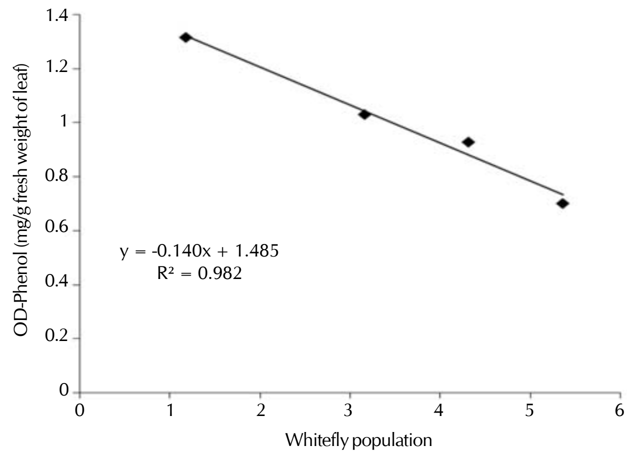

Figure 2: OD-Phenol and whitefly interactions

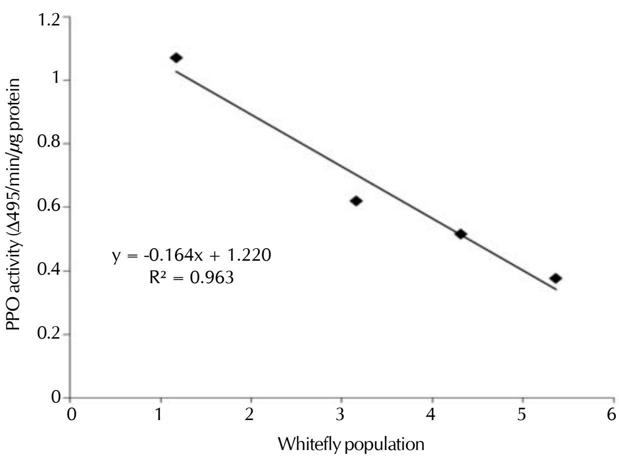

Figure 3: PPO activity and whitefly interactions

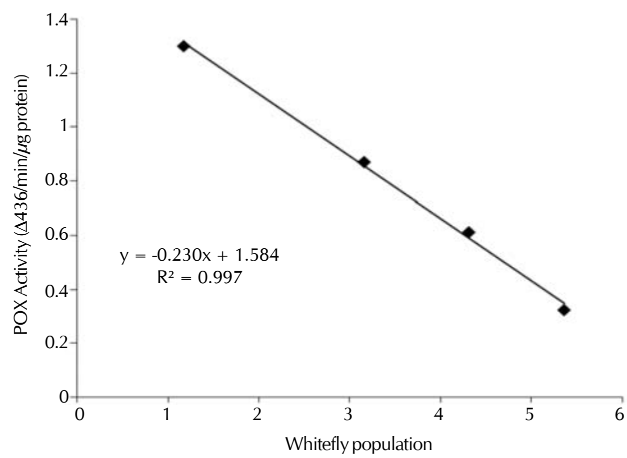

Figure 4: POX activity and whitefly interactions

(0.62 mg/g leaf), PPO (0.23 Δ495/min/μg protein) and POX (0.26 Δ436/min/μg protein) activities. Other susceptible cultivars as well as moderately susceptible cultivars had even low PPO and POX activities ranging from 0.44 to 0.54 Δ495/ min/μg protein and 0.30 to 0.67 Δ436/min/μg protein respectively. It seems from the result that resistant cultivars such as Simurali Sanchi and Halisahar Sanchi had twice phenol and OD phenol content as compared to most susceptible cultivar Simurali Golebhavna. The moderately resistant variety, Bagerhat Local had distinctly higher phenol and OD phenol content as well as PPO and POX activities over moderately and highly susceptible varieties.

The nutritional parameters of the leaves of betelvine landraces (Table 3) showed that the susceptible varieties had high protein content and nitrogen concentration in leaf than the resistant cultivars. The susceptible variety Simurali Gole bhavna had very high protein content (39.68 mg/g fresh leaf) and nitrogen concentration (25.72 mg/g dry leaf) as compared to the most resistant variety Simurali Sanchi, recording 19.78 and 16.65 mg/g leaf respectively. Another resistant cultivar, Halisahar Sanchi also had low protein content (20.03 mg/g fresh leaf) and nitrogen concentration (17.53 mg/g dry leaf) in its leaf. A significant difference in protein content and nitrogen concentration of leaf was also observed between moderately resistant and susceptible cultivars., Moderately resistant cultivar Bagerhat Local had low protein content (22.97 mg/g fresh leaf) and nitrogen concentration (18.50 mg/g dry leaf) as compared to the moderately susceptible cultivars except cultivar Ghanagette. The protein content of Bagerhat Local was significantly higher than Ghanagette, while nitrogen concentration was at par with each other in both the varieties. However, no distinguished difference was observed in case of phosphorus and potassium concentration of leaf among the resistant and susceptible cultivars. Highest phosphorus concentration was found in susceptible variety Simurali Chamurdali (3.30 mg/g dry leaf), while lowest concentration was noticed in moderately susceptible variety Simurali Bhavna (2.43 mg/g dry leaf). On the other hand, the potassium concentration was found higher in resistant cultivars than the susceptible cultivars. Though, it was found highest in Gayasur (19.30 mg/g dry leaf), which is moderately susceptible cultivars, whereas low concentration was observed in moderately resistant cultivar, Bagerhat Local (15.47 mg/g dry leaf). Therefore, the potassium concentration in leaf exhibited a haphazard trend among the cultivars.

The data thus obtained was put into correlation and regression

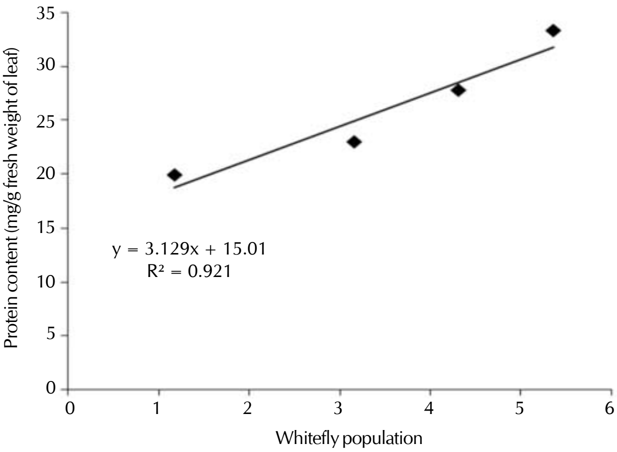

Figure 5: Protein content and whitefly interactions

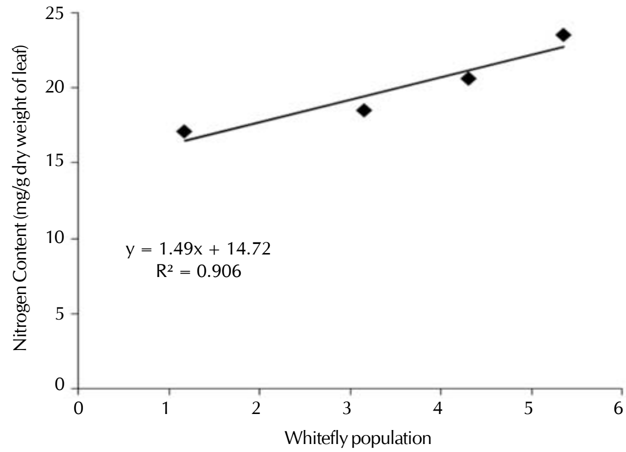

Figure 6: Nitrogen content and whitefly interactions

analysis for conformity of the trend found between biochemical parameters and whitefly infestation (Table 4). The correlation studies showed that there was a significant negative relationship between phenol content of leaf and whitefly infestation. The correlation co-efficient value was found as 0.818, which was significant at 1% level. The same trend was observed in case of OD-phenol, polyphenol oxidase and peroxidase, where significant negative correlation was found at 1% level with the correlation co-efficient 0.955, 0.969 and 0.966 respectively. In-contrary total soluble protein and nitrogen concentration showed a positive correlation at 5% level having the correlation co-efficient 0.732 and 0.703 respectively. However, the phosphorus and potassium concentration showed a non-sense positive and negative correlation with whitefly population respectively.

Outcome of the correlation studies indicates that the higher amounts of phenol and OD phenol as well as increased PPO and POX activities in leaf were important factors in decreasing white fly infestation level. In-contrary, higher protein and nitrogen concentration of leaf were found positively correlated with white fly infestation level. All these factors imparted an influence on population dynamics of white fly.

The biochemical and nutritional parameters, which are either positively or negatively correlated with the white fly infestation at individual level, were put to step down multiple correlation studies to identify the most important factor(s) that mostly influenced the white fly population build up.

The step wise multiple regression equation was found as $Y = 25.45 - 10.05\ X_1 - 8.15\ X_2$; where X_1 = PPO activity and X_2 = OD phenol of leaf. The multiple correlation co-efficient (R^2) value was 0.969, explaining 96.9% variation in whitefly population by these two factors.

The values of different biochemical parameters were grouped under resistant (R), moderately resistant (MR), moderately susceptible (MS) and highly susceptible (HS) categories, and then depicted in Figure 1 to Figure 6. The trend showed that the resistant characteristic of the betelvine land races to whitefly depends on low phenol and OD-phenol content as well as high polyphenol oxidase and peroxidase activities. On the other hand, susceptibility of the land races increases with the increase of nitrogen and protein content of the leaves. Literatures cited on the screening of betelvine variety are in agreement with the findings of the investigation. Sanchi type cultivars viz. Simurali Sanchi, Kalipatti and Halisahar Sanchi show their resistance against white fly, while Bangla type cultivars are moderate to high susceptible to the pest (Das and Mallik, 2010).

Role of phenolic compounds and its oxidative enzymes as well as plant proteins on whitefly infestation are well documented for whitefly species and other sucking pest in different hosts. Total phenols and OD-phenols showed significant negative co-relation with *Bemisia tabaci* infestation imparting induced resistance to black gram host. Phenol and OD-phenol content had increased upto 50% at 30 days after sowing, which protected the plant from the pest by imparting high level of resistance (Taggar *et al*., 2014). Similarly, polyphenol oxidase and peroxidase activities were higher in resistant genotypes compared to susceptible genotypes of *Capsicum annum* infested by *Bemisia tabaci* (Latournerie-Moreno *et al*., 2015). Higher activity of peroxidise and polyphenol oxidase in faba bean cultivar Gazira 2 was strongly associated with its resistant character against aphid *Aphis craccivora* (Soffan *et* al., 2014). Induction of polyphenol oxidase activity in potato leaves resulted due to aphid *Myzus persicae* infestation led to enhancement of resistance in potato against the pest (Xiao-Lin *et* al., 2013). In-contrary, increased level of nitrogen application enhanced the biosynthesis of protein and amino acids resulted into more infestation of whitefly in okra (Athar *et al*., 2011).

Therefore, Sanchi types of cultivars as, Simurali Sanchi and Halisahar Sanchi were found to be the resistant cultivars against whitefly species *Singhiella pallida* Singh. OD-phenol and polyphenol oxidase activity, coupled with lower nitrogen and protein content might be the biochemical basis for resistance in both the cultivars against whitefly species. Nitrogen management in the susceptible or moderately susceptible cultivars may be given due importance for management of whitefly in betel vine. Higher level of resistance in Simurali Sanchi and Halisahar Sanchi cultivars can serve as base for crop improvement programme for developing resistant varieties to *Singhiella pallida*.

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