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Full Length Article

Host-plant-preference and Mortality Analysis of *Phenacoccus solenopsis* in Association with Biochemical Traits of Different Plant Species

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Abstract

Dietary requirement and fitness of phytophagous insect pest depend upon the nutrient chemistry of host plant. The present research was carried out to investigate the association of host-plant-preference and mortality of *Phenacoccus solenopsis* first instar influenced by chemical characteristics of plant species. The experiment was carried out at (25 + 5ºC and 60 + 5% RH) in Integrated Pest Management Laboratory, University of Agriculture, Faisalabad, Pakistan during 2012. First instar exhibited maximum (attractiveness index = 0.75) toward *Gossypium hirsutum* followed by *Trianthema portulacastrum* (0.34) as compared *Hibiscus rosa-chinensis* (1.00) kept as standard. Mortality percentage of 1st instar of CMB among 25 tested plant species ranged from 10% in cotton to 80% in *Conyza bonariensis*. Cluster analysis for biochemical traits showed that cluster-1 comprised of *Lantana camara*, *H. rosa-sinensis*, *Helianthus annuus*, *Parthenium hysterophorus*, *Withania somnifera*, *Euphorbia prostrate*, *Portulaca oleracea*, *Solamum melongena*, *T. portulacastrum*, *G. hirsutum*, *Abelmoschus esculentus* and *Capsicum frutescens* that had maximum attractiveness and less mortality. Cluster-2 *Convolvulus arvensis*, *Eclipta prostrate*, *Amaranthus spinosus*, *Clerodendron inerme* and *Tribulus terrestris* had medium whereas cluster-3 *Launea nudicaulis*, *Chinopodium morale*, *C. album*, *Achyranthes aspera*, *C. bonariensis* and *Digera arvensis* least attractiveness index but highest mortality. Principal component analysis (PCA) depicted that first three PCs expressed 83% of the total variability. Mortality of pest was negatively correlated with nitrogen and but positively with potassium, phosphorus, sodium, reducing sugar and total sugar. In conclusion, chemical contents affected attractiveness index and mortality of *P. solenopsis*; however there is need to explore allelochemicals imparting resistance against *P. solenopsis*. © 2017 Friends Science Publishers

Keywords: Biochemical traits; Cotton mealybug; Cluster and principal component analysis

Introduction

Cotton mealybug (CMB) *Phenacoccus solenopsis* Tinsley (Pseudococcidae: Hemiptera) is an economic insect pest of cotton (Wu and Zhang, 2009; Wang *et al.*, 2009), the invasion of *P. solenopsis* in Asia was attributed to illegal bringing of Bt. cotton bolls by some progressive growers from USA (Deshpande, 2009). In Pakistan, it resulted in cotton production loss up to 1.3 million bales (Abdullah, 2009). Despite cotton, it has also been reported damaging various other crops (Arif *et al*., 2009). Occurrence of *P. solenopsis* among its host plants did not remain similar that may be due to various reasons.

Basic cause may be that insect pests have varying level of dietary requirement and their fitness depends upon the nutrient chemistry of host plant. Nutrients not only affect the growth and development of plant species but also alter the quality of their food source for herbivorous insect pest (Goncalves-Alvim *et al.*, 2004). Quality and quantity of food affects the food selection behavior, survival and reproduction of phytophagous insect pest (Du *et al.*, 2004) due to variation in volatile compounds, phenology, secondary metabolites, tissue solidity and defense mechanisms. These characteristics of plants are critically very important in decision making of insects for feeding, probing and oviposition (Joachim-Bravo *et al.*, 2001; Shahid *et al.*, 2012).

Nutrients play an important role in the physiological activity of the plants and induce resistance against insect pest and diseases (Hu *et al*., 2010). Liu *et al.* (2004) also reported that nutrients in plants play important role in the fitness of feeding insect pest, because of quantitative and qualitative dietary requirement of insect pest for food. Nitrogen being a structural element of chlorophyll and protein molecules helps in the formation of chloroplasts and accumulation of chlorophyll in them that make plant succulent (Daughtry, 2000; Amaliotis *et al*., 2004). Sucking insect pests including CMB are commonly attracted toward succulent plants that are enriched with chlorophyll (Tucker, 2004). Researchers demonstrated that herbivorous insect-pest preference is due to imbalanced nutrition (Neal *et al.*, 1994; Liu and Stansly, 1995). Although several researchers

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worked on the host range of *P. solenopsis* in Pakistan (Arif *et al*., 2009; Abbas *et al*., 2010) but the information regarding host-plants-preference (HPR) regulating biochemical factors (nutrients) in various host plants for *P. solenopsis* is quit lacking. The present study was carried out to investigate the HPR regulating biochemical factors of different host plants against CMB.

Materials and Methods

Population Culture of Cotton Mealybug

The adults of *P. solenopsis* were collected from chineserose (*Hibiscus rosa-sinensis*), brought in Integrated Pest Management Laboratory, University of Agriculture, Faisalabad, and cultured on *H. rosa-sinensis* during 2008–09. The culture was maintained in glass jars with dimension (30 x 30 x 30 cm) at a temperature of 25 ± 2°C and relative humidity 65±5% for further experimentation.

Plant Material

A total of 25 host plant species viz., *Lantana camara* (Verbenaceae), *Chinopodium morale* (Chenopodiaceae), *H. rosa-sinensis* (Malvaceae), *Convolvulus arvensis* (Convulvulaceae), *Launea nudicaulis* (Euphorbiaceae), *Withania somnifera* (Solanaceae), *Coronopus didimus* (Brassicaceae), *Euphorbia prostrate* (Euphorbiaceae), *Conyza bonariensis* (Asteraceae), *Solamum melongena* (Solanaceae), *Abelmoschus esculentus* (Malvaceae), *Cirsium arvense* (Asteraceae), *Chenopodium album* (Chenopodiaceae), *Gossypium hirsutum* (Malvaceae), *Capsicum frutescens* (Solanaceae), *Amaranthus spinosus* (Amaranthaceae), *Clerodendron inerme*, *Trianthema portulacastrum* (Aizoaceae), *Portulaca oleracea* (Portulacaceae), *Tribulus terrestris* (Zygophyllaceae), *Digera arvensis* (Amaranthaceae), *Eclipta prostrate* (Asteraceae), *Parthenium hysterophorus* (Asteraceae), *Achyranthes aspera* (Amaranthaceae) and *Helianthus annuus* (Asteraceae) commonly available in the agro-climate of Faisalabad, Punjab, Pakistan were evaluated against 1st instar cotton mealybug following multi choice experiment under laboratory conditions at temperature of 25± 2°C and relative humidity 65 ± 5%. The selection of 1st instar was based on the fact that this instar is the most active and could be most suitable for host plant preference study as compared to rest of the stages which are mostly sessile.

Determination of Attractiveness Index of First Instar of *P. solenopsis*

In order to study the host-plant preference of mealybug toward the selected plant species, healthy twigs of these plants with at least five tender leaves, those were neither exposed to any pesticide applications nor had mealybug infestation, were picked and brought in laboratory. The collected twigs were cleaned, washed and air-dried to remove moisture from the surface of leaves. Attractiveness of first instar of *P. solenopsis* was determined in a host-plant-preference-study-wheel (HPPSW) that was constructed from thermopore sheet of diameter 120 cm. In the center of sheet a hole of 7.5 cm diameter was made where a petridish of diameter 7.5 cm was adjusted for the release of specimens of first instar; whereas, it's outer boundary of width 15 cm was made gyrating. The gyrating part of HPPSW was used to adjust the glass vials with twigs inserted in the nutrient solution in the vials. The vials having plant's twigs were adjusted at equidistance from each other (15 cm) as well as from the petridish adjusted in the central hole of sheet. A counted numbers of newly emerged 1st instar mealybug (400) were collected from the culture with the help of camel hair brush into plugged test tube, starved for two hours and then are released in the petridish adjusted in the center of HPPSW. The outer gyrated part of the HPPSW was rotated at ninety degree angle after every fifteen minutes for 12 h. Then whole of the experimental setup was left for next 12 h period and then the data collection was initiated 24 h after the release of test insect. The numbers of the 1st instar mealybug settled on the twigs of each plant species were counted. In this way the plants were evaluated against most active and mobile first instar of *P. solenopsis* (as compared with all other instars) on the basis of attractiveness index by using the equation described by Lin *et al.* (1990).

$$\text{Attractiveness index} = \frac{2G}{G+S}$$

G: Number of mealybug on tested plant, S: Number of mealybug on control plant:

Determination of Mortality of First Instar of *P. solenopsis*

In order to study the nymphal mortality of first instar, ten ovisaced females of cotton mealybug were confined in ten glass vails separately having moistened filter paper at the bottom of vails and leaf disc above it. The ovisaced females were released on the leaf disc and confined there till the emergence of 1st instar nymphs. For the conduct of this study, Seventy five experimental units were prepared and maintained for twenty five treatments (plant species) replicated thrice in completely randomized design. Each experimental unit consisted of two transparent cups of plastic glass inverted over each other. Nutrient solution was poured and one plant's twig was adjusted in the lower cup, while a hole was made in the bottom of 2nd inverted cup of each experimental unit to introduce the 1st instar nymphs of mealybug. The purpose of using nutrient solution was to maintain the turgidity and nutrients in the leaves of the twigs for facilitating feeding. The twigs of selected plants were made free of any kind of contamination by rinsing in water and air-drying before adjusting in experiment unit. After the emergence of first instar crawlers from the ovisac, twenty five first instar nymphs were isolated and brushed by camel hair brush over the twig of each experiment unit through the hole made in the 2nd inverted cup. The hole was then plugged with cotton soaked in water. Each experiment unit was observed on daily basis till their molting into 2nd instar. Similarly plants were evaluated against most active and mobile first instar of *P. solenopsis* (as compared with all other instars) on the basis of its mortality percentage. The 1st instar nymphs successfully molted to 2nd instar were counted and percent mortality of 1st instar nymphs was calculated by using following formula:

$$\text{Mortality\%} = \frac{A - B}{A} \times 100$$

A: Total 1st instar nymphs released, B: Number of 1st instar mealybug successfully molted to second instar.

Determination of Chemical Contents of Leaves of Selected Plant Species

For the determination of mineral content in the leaves of each plant species, fresh and un-infested leaves were collected, rinsed in water, oven dried and grinded with the help of electric grinder into fine powder. The prepared powder was digested by following the procedure described by Loska and Wiechula (1975). Sodium and potassium contents of leaves were determined on flame photometer (Jenway PFP7) following the methodology as described by Blake *et al*. (1969). Nitrogen contents were estimated by using micro-kjeldhals according to the methodology described by Bremmer and Keeney (1965). Phosphorous contents were estimated using the procedure elaborated by Wolf (1982) on spectrophotometer. Total soluble sugars and reducing sugars were determined according to the method described by Raizi *et al.* (1985). For determination of reducing Sugar (Glucose), 1 mL of the plant extract/digested solution was taken in 25 mL glass test tube. 5 mL of O-tolidine was added and heated at 97ºC. Then the test tubes were cooled in ice cold water. Absorbance of the supernatant was taken at 630 nm on spectrophotometer. Similarly, for total soluble sugars/total Carbohydrates 0.1 mL of extract was taken in 25 mL test tube and 3 mL of freshly prepared anthrone was added to it. The mixture was heated at 97°C on water bath for 10 min. Test tubes were then cooled in ice bath. Absorbance of the supernatant was taken at 630 nm on spectrophotometer. Standard curve was developed with different concentrations of glucose and sugars values were calculated by comparing sample absorbance of the supernatant with standards.

Statistical Analysis

The data collected were subjected to uni- and multivariate analyses to select plant species bearing resistance against *P. solenopsis.* Pearson Correlation Coefficient values were used to estimate the association of biochemical plant traits with attractiveness and mortality of mealybug. Dendrogram and genetic similarity among the plant species were also generated using the Jaccard's coefficient of similarity expressed as Euclidean genetic distances. Similarly, cluster analysis was used to sort the plant species into their appropriate groups with minimum error by using Statistical Package for Social Sciences (SPSS) and Minitab softwares (Sneath and Sokal, 1973). The data was also subjected to principle component analysis using Minitab software for determining the contribution of plant's chemical traits towards the variation in attractiveness and mortality of mealybug.

Results

Chemical Variation in Leaf of Selected Host Plants of *P. Solenopsis*

There were significant differences in the biochemical leaf traits of the tested host plants ($P < 0.05$). Similarly host plants also had significant effects on the attractiveness index and mortality of *P. solenopsis* ($P < 0.05$) (Table 1). Potassium contents ranged from 1.28 to 4.79%, being significantly lower in *C. frutescens* and higher in *C. morale.* Nitrogen contents ranged from 2.29 to 6.25%, being the minimum in *L. nudicaulis* and the maximum in *H. rosa-sinensis.* Phosphorus contents ranged from 0.17 to 0.62% bearing lower limit in *C. bonariensis* and higher limit in *C. arvensis*. Sodium contents were found in the range of 0.46–3.18%, being significantly lower in *A. spinosus* and higher in *C. morale*. The contents of reducing sugar ranged from 0.11% in *S. melongena* to 0.34% in *L. camara.* Total sugars ranged from 0.10% in *D. arvensis* to 0.38% in *C. bonariensis* (Table 2).

Attractiveness Index in First Instar of *P. solenopsis*

Attractiveness of *P. solenopsis* for different host plants ranged from 0.31 to 1.00. The attractiveness index of mealybug was maximum for *H. rosa-sinensis* (kept as control), which proved that it was the most preferred host plant. Following *H. rosa-sinensis*, *G. hirsutum* demonstrated the maximum preference for *P. solenopsis* exhibitibg attractiveness index value of 0.75. Seventeen plant species including *L. camara, C. morale, C. arvensis, P. hysterophorus*, *A. spinosus*, *T. portulacastrum*, *T. terrestris*, *A. esculentus*, *E. prostrate*, *C. bonariensis S. melongena*, *P. oleracea*, *D. arvensis*, *E. prostrate*, *A. aspera, C. frutescens* and *C. inerme* demonstrated attractiveness index values in the range of 0.2–0.4 and exhibited 60–80% less preference for mealybug as compared to *H. rosa-sinensis* (standard). However, six plant species including *H. annuus*, *L. nudicaulis, W. somnifera, C. didimus*, *C. arvense* and *C. album* proved less attractive to mealybug as their attractiveness index value ranged from 0.04–0.19 (Fig. 1).

Mortality Percentage in First Instar of *P. solenopsis*

In contrast *L. camara*, *P. hysterophorus*, *H. rosa-sinensis*, *H. annus, G. hirsutum, C. arvensis, W. somnifera, T. portulacastrum, A. esculentus, E. prostrate, S. melongena, P. oleracea and E. prostrate* demonstrated 0–25% mortality, being minimum in *H. rosasinensis* and *P. hysterophorus*. However, *L. nudicaulis*, *A. spinosus*, *T. terrestris*, *C. frutescens caused* 26–50%, while *C. morale*, *C. didimus*, *C. inerma*, *C. arvense*, *C. album*, *A. aspera*, *C, bonariensis* and *D. arvensis* administrated more than 50% mortality in *P. solenopsis* (Fig. 2).

Association between Biochemical Traits and *P. solenopsis*

Simple correlation coefficients between the biochemical traits of the plants and attractiveness as well as mortality of the first instar of *P. solenopsis* (Table 3). Mortality of pest was positively correlated with phosphorus, potassium, sodium, reducing sugar and total soluble sugars. The probability value (P < 0.05) shows that significant relationship existed between chemical plant traits and attractiveness index of CMB kept as dependent variables. The correlation coefficient values (r), regression equation and coefficient of determination (R^2) reveal that chemical traits including phosphorus, sodium and potassium levels had a high negative correlation with attractiveness index of CMB (Table 3). However, probability value (P<0.05) depicted that positive correlation existed between nitrogen contents and attractiveness index of CMB. Reducing sugar and total soluble sugars had positive association with attractiveness index of CMB however nature of association was weak (Table 3).

The probability value (P < 0.05) shows non-significant relationship between chemical plant traits and mortality percentage of CMB kept as dependent variable. The correlation coefficient values (r) and regression equation and Coefficient of determination (R^2) reveal that chemical traits including phosphorus, sodium, potassium, reducing sugar and total soluble sugar levels had positive correlation with mortality percentage of CMB.

Table 1: Analysis of variance for the biochemical contents of leaf and response of *P. solenopsis*

Dependent variable	DF	F-value	P-value
Potassium	24^a/50^b	90.5	0.0000
Nitrogen	24^a/50^b	18.8	0.0000
Phosphorus	24^a/50^b	42.7	0.0000
Sodium	24^a/50^b	30.6	0.0000
Reducing sugar	24^a/50^b	247	0.0000
Total sugar	24^a/50^b	119	0.0000
Attractiveness index	23^a/48^b	71.1	0.0000
Mortality (%)	23^a/48^b	70.5	0.0000

^{a}Degree of freedom for plant species, ^{b}Error degree of freedom

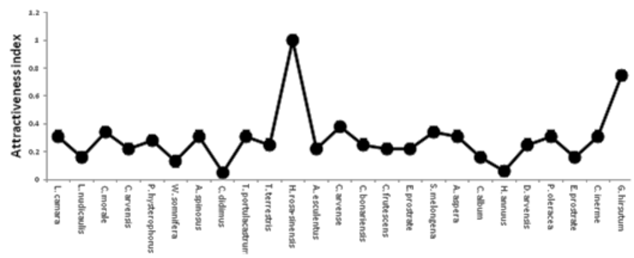

Fig. 1: Attractiveness index of *P. solenopsis* among selected plant species

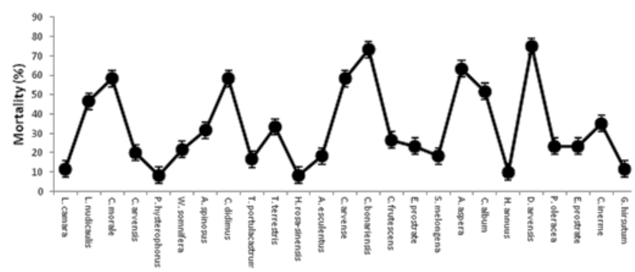

Fig. 2: Mortality of first instar of *P. solenopsis* among selected plant species

However, probability value (P <0.05) depicted that negative correlation existed between nitrogen contents and mortality of *P. solenopsis* (Table 3).

Cluster Analysis among Studied Traits

Twentyfive plant species were categorized into three different clusters with the help of cluster analysis (Fig. 3). Clusters were made on the basis of attractiveness index, the mortality of *P. solenopsis* and biochemical traits of the selected plant species. Cluster ANOVA revealed that there were significant differences in the biochemical leaf traits of the tested host plants (P < 0.05) and the attractiveness index and mortality of *P. solenopsis* (P < 0.05) (Table 3). Cluster-1 comprised of twelve plant species including *L. camara, H. rosa-sinensis, H.annuus, P. hysterophorus, W. somnifera, E. prostrate, P. oleracea, S. melongena, T. portulacastrum, G. hirsutum, A. esculentus and C. frutescens* having final cluster center

Table 2: Mean comparison regarding biochemical contents percentage in different host plants of *P. solenopsis*

Plant species	Potassium (%)	Nitrogen (%)	Phosphorus (%)	Sodium (%)	Reducing sugar (%)	Total sugar (%)
L. camara	1.38ij	4.44bc	0.23jk	0.97h	0.34a	0.28c
L. nudicaulis	3.70b	2.29i	0.52abc	3.10a	0.32a	0.30bc
C. morale	4.79a	3.91de	0.38fg	3.18a	0.32a	0.33b
C. arvensis	3.78b	3.58de	0.62a	2.81a	0.19ef	0.12fg
P. hysterophorus	2.86cd	4.46b	0.50cd	1.63ef	0.12hi	0.20d
W. somnifera	3.94b	2.60hi	0.59ab	1.71ef	0.28b	0.16ef
A. spinosus	2.93c	3.85de	0.46de	0.46de	0.20de	0.21d
C. didimus	3.73b	2.92gh	0.58ab	2.07cd	0.18ef	0.11g
T. portulacastrum	2.48cd	4.08cd	0.35gh	2.08cd	0.22c	0.28c
T. terrestris	1.78hi	3.22fg	0.35gh	1.23gh	0.13hi	0.20d
H. rosa-sinensis	1.81hi	6.25a	0.24jk	1.43fg	0.18ef	0.10g
A. esculentus	1.86hi	3.29fg	0.32hi	1.49fg	0.13gh	0.30bc
C. arvense	2.39ef	2.35i	0.38fg	2.12bc	0.21cd	0.20de
C. bonariensis	2.26fg	4.28cd	0.17k	1.91cd	0.28b	0.38a
C. frutescens	1.28j	4.07cd	0.28ij	1.21gh	0.29b	0.32bc
E. prostrate	1.88gh	3.86de	0.27ij	1.63ef	0.12hi	0.12fg
S. melongena	2.92cd	3.78de	0.26ij	1.85cd	0.11i	0.18de
A. aspera	1.70hi	4.62b	0.32hi	1.82cd	0.27b	0.28c
C. album	2.47cd	4.42bc	0.36gh	1.77cd	0.21cd	0.21d
H. annuus	1.83gh	3.57ef	0.28ij	1.65ef	0.17f	0.21d
D. arvensis	2.49de	2.84gh	0.59ab	1.74de	0.12hi	0.10g
P. oleracea	3.53b	3.64ef	0.40ef	2.24b	0.21cd	0.30bc
E. prostrate	2.04fg	2.65hi	0.43de	1.82cd	0.27b	0.29bc
C. inerme	2.25fg	2.49hi	0.32hi	1.65ef	0.27b	0.31bc
G. hirsutum	1.62ij	3.76de	0.33hi	1.56ef	0.14g	0.22d
LSD (0.05)	0.52	1.15	0.10	0.53	0.02	0.04

Table 3: Correlation and regression for chemical nutrients in leaf and association between attractiveness index and mortality (%) of *P. solenopsis*

	Attractiveness index		Mortality %	
Chemical nutrients	Regression	Correlation	Regression	Correlation
Phosphorus	$y = -0.3175x + 0.3653; R^2 = 0.294$	r (p) =-0.46 (0.02)	$y = 34.339x + 20.108; R^2 = 0.0343$	r (p) =0.21(0.3)
Nitrogen	$y = 0.0547x + 0.03; R^2 = 0.5902$	r (p) =0.75 (0.00)	$y = -7.5596x + 61.249; R^2 = 0.0992$	r (p) =-0.33 (0.1)
Potassium	$y = -0.0614x + 0.3756; R^2 = 0.444$	r (p) =-0.24 (0.22)	$y = 4.8009x + 20.636; R^2 = 0.0402$	r (p) =0.23 (0.26)
Sodium	$y = -0.0401x + 0.4664; R^2 = 0.4027$	r (p) =-0.16 (0.41)	$y = 12.384x + 9.7138; R^2 = 0.0873$	r (p) =0.24 (0.23)
Total soluble sugar	$y = 0.0654x + 0.0822; R^2 = 0.3038$	r (p) =-0.03 (0.87)	$y = 20.585x + 28.169; R^2 = 0.006$	r (p) =0.12 (0.53)
Reducing sugar	$y = 0.4183x + 0.1461; R^2 = 0.1105$	r (p) =0.05 (0.78)	$y = 71.057x + 18.053; R^2 = 0.0583$	r (p) =0.36 (0.07)

Table 4: Anova Parameter for Cluster and Cluster Membership among Biochemical Traits of some Host Plants toward Mortality and Attractiveness Index of *p. Solenopsis*

Independent variables	*df*	*F*	*P*	Final Cluster Centers		
				CLUSTER-1 *L. camara, H. rosa-sinensis, H.annuus, P. hysterophorus, W. somnifera, E. prostrate, P. oleracea, S. melongena, T. portulacastrum, G. hirsutum, A. esculentus* and *C. frutescens*	CLUSTER-2 *C. arvensis, E. prostrate, A. spinosus, C. inerme, T. terrestris*	CLUSTER-3 *L. nudicaulis, C. didimus, C. arvense, C. morale, C. album, A. aspera, C. bonariensis, D. arvensis*
N	2/22	1.2	0.00	1.72	2.5	2.8
P	2/22	0.56	0.00	0.72	0.64	0.6
K	2/22	0.42	0.00	0.5	0.63	0.45
Na	2/22	0.5	0.00	0.25	0.7	0.77
Reducing sugar	2/22	0.2	0.00	0.06	0.19	0.29
Total soluble sugar	2/22	0.32	0.00	1.16	1.42	2.1
Attractiveness index	2/22	0.40	0.00	0.1	0.3	0.6
Mortality (%)	2/22	5.94	0.009	20.3	40.5.	62.1

readings for nitrogen (1.72), phosphorus (0.72), potassium (0.5), sodium (0.25), reducing sugar (0.06), total soluble sugar (1.16), preference (0.1) and mortality (20.3) percent respectively. Cluster-2 consisted of a group of five plant species including *C. arvensis*, *E. prostrate*, *A. spinosus*, *C. inerme, T. terrestris* with attractiveness index ranging from 0.31 to 1.00 having final clusters of nitrogen (2.5), phosphorus (0.64), potassium (0.63), sodium (0.7), reducing sugar (0.19), total soluble sugar (1.42), preference (0.3) and mortality (40.5) percent respectively while cluster-3 possessed eight plant species including *L. nudicaulis, C. didimus, C. arvense, C. morale,* *C. album, A. aspera, C. bonariensis, D. arvensis* with cluster center for nitrogen (2.8), phosphorus (0.6), potassium (0.45), sodium (0.77), reducing sugar (0.29), total soluble sugar (2.1), preference (0.6) and mortality (62.1) percent respectively (Table 3). Cluster-1 comprised of the most preferred host plant species with minimum mortality of *P. solenopsis* and highest food distribution as compared with cluster-2 and cluster-3. The plant included in cluster-2 demonstrated intermediate preference and mortality to *P. solenopsis.* However, the plant species included in cluster-3 explained the maximum mortality of pest and exhibited the least preference for *P. solenopsis.* The pair wise Mahalanobis distances (D^2statistics) among three clusters of 25 plant species (Table 4, 5) revealed that plant species of cluster 2 demonstrated maximum diversity against the members of cluster 3 for the most of the studied traits. Not a single cluster showed obvious separation. The tree diagram showed more or less similar results exhibiting similarity among clusters (33.3%) comprising of two main groups A and B each of which is further sub-divided into two clusters (Fig. 3).

Principal Component Analysis

In this study, three out of seven Principal components (PCs) were taken having Eigenvalues ≥1. Results depicted that first three PCs expressed 83% of the total variability amongst the selected plant species of *P. solenospsis* (Table 6) other PCs contributed only about 17% of the total variability. PC1 contributed maximum variability (40%) following PC2 (26%) and PC3 (17%). The biochemical traits like sodium, potassium, phosphorus and reducing sugar explained positive but nitrogen and total sugars demonstrated negative factor loadings on PC1. Biochemical traits like nitrogen, potassium, sodium, reducing and total soluble sugars revealed positive but nitrogen expressed negative factor loading on PC2. The first principal component is required to have the largest possible variance (i.e., inertia and therefore this component demonstrated the largest part of the inertia in the data table). The second component is computed under the constraint of being orthogonal to the first component and to have the largest possible inertia. Nitrogen, phosphorus, potassium, sodium and reducing demonstrated positive but total sugar explained negative factor loadings on PC3.

Discussion

The experiment was conducted to determine antibiotic based mechanism of resistance in different plant species against cotton mealybug *P. solenopsis*. Biological parameters of mealybug, and biochemical traits of the tested plants and their association were determined to investigate the possible biochemical based antibiotic mechanism of resistance**.** Due to the polyphagous nature of *P. solenopsis*, it was imperative to study the effect of different food plants on its biology because food quality is known to be an important factor affecting demography, survival, fecundity and life expectancy of insect pest (Dixon, 1987). Present studies revealed that significant variation in the life cycle of *P. solenopsis* was observed among tested plant species. Plant species including *L. camara, C. arvensis, P. hysterophorus, A. spinosus, T. partulacastrum, T. terrestris, H. rosa-sinensis, A. esculentus, C. frutescence, E. prostrate, S. melongena, H. annuus, P. oleraceae, E. prostrate, C. inerme* and *G. hirsutum* were preferred host plants of *P. solenopsis*, because they induced less than 30% mortality. Plant species comprising of *C. morale, C. didimus, C. arvense, C. bonariensis, A. aspera* and *D. arvensis* were considered as least preferred host plants of *P. solenopsis*, because they induced more than 50% mortality, indicating greater suitability of that plant, because antibiotic symptoms of plants vary from acute or lethal to sub-chronic or mild effects that may be of permanent or temporary in nature.

Table 5: D^2 distance among different clusters

	Cluster-1	Cluster-2	Cluster-3
Cluster-1	0.000		
Cluster-2	25.39	0.000	
Cluster-3	11.8	15.58	0.000

Table 6: Principal component analysis of biochemical traits in different plant species of *P. solenopsis*

Eigenvalue	PC1	PC2	PC3	PC4	PC5	PC6	PC7
	2.84	1.81	1.18	0.48	0.34	0.18	0.15
% of total variance	0.40	0.26	0.17	0.07	0.05	0.02	0.02
Cumulative variance %	0.40	0.66	0.83	0.90	0.95	0.98	1.00
Factor loading by various biochemical traits							
Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Attractiveness index	-0.38	0.06	0.61	-0.19	0.45	-0.10	-0.46
Nitrogen	-0.45	0.03	0.46	0.05	-0.57	0.38	0.31
Phosphorus	0.49	-0.23	0.15	-0.44	-0.06	0.64	-0.27
Potassium	0.47	0.09	0.42	0.03	-0.49	-0.54	-0.20
Sodium	0.40	0.27	0.39	0.54	0.39	0.24	0.32
Reducing sugar	0.07	0.64	0.004	-0.63	0.08	-0.08	0.39
Total soluble sugar	-0.07	0.66	-0.24	0.26	-0.20	0.27	-0.55

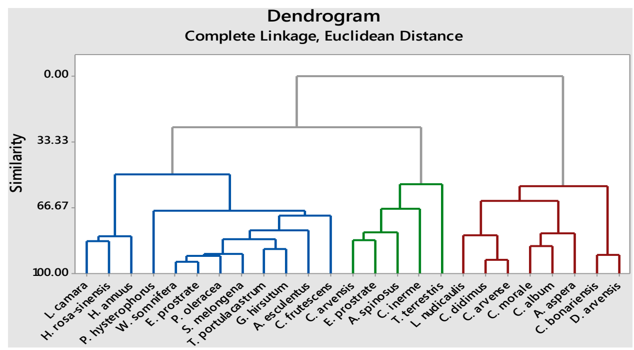

Fig. 3: Cluster analysis regarding similarity between biochemical traits versus attractiveness index and mortality of first instar of *P. solenopsis*

The most common symptoms in insect pests include larval death in the early instars, irregular growth rates, decline in size and weight of the larvae or nymphs, prolongation of the larval period, failure to pupate, failure of adults to emerge from the pupae, inability to concentrate on food reserves, followed by a failure to hibernate, abnormal adults, decreased fecundity, reduction in fertility, restlessness and abnormal behavior, reduced honey dew secretions by sucking insect pests (Hartnett and Abrahamson, 1979; van Lenteren and Noldus, 1990; Pedigo, 1996; Dhaliwal and Arora, 2003). Reason was due to imbalanced food nutrients for the proper growth and development of cotton mealybug. Dietary requirement and fitness of insect pests depends upon the nutrient chemistry of host plant. The additive effects of biochemical factors especially dietary requirements influence the life parameters of herbivorous insect pest (Van-Emden and Peakall, 1996; Goncalves-Alvim *et al*., 2004; Mierziak, *et al*., 2014). These finding depict the role of biochemical factors in antibiotic mechanism against sucking insect pest and support our finding against *P. solenopsis* which is also a sucking insect pest (Pedigo, 1996; Felkl *et al*., 2005). because nutrients directly affect the metabolic activities of pest and indirectly alter the quality of food source for herbivorous insect pest (Kogan, 1982; Pedigo, 1996; Tsai and Wang, 2001; Kim and Lee, 2002; Li *et al*., 2004; Goncalves-Alvim *et al*., 2004; Arif *et al*., 2013; Mierziak, *et al*., 2014). Insect performance, their development, feeding preference, food consumption, survival, growth, reproduction and population density alongwith weight of youngones of insectpest are highly influenced by mineral contents especially nitrogen and crude protein (Prosser *et al.*, 1992; Abisgold *et al.*, 1994; Girousse and Bournoville, 1994; Febvay *et al.*, 1988; Sarmah *et al.* (2011), because nitrogen is the basic component of amino acids and crude protein thus normally increase herbivore preference (Singh and Taneja, 1989; Zhong-xian *et al.*, 2007). Another reason is that nitrogen is a structural element of chlorophyll molecules thus helps in the formation of chloroplasts and accumulation of chlorophyll in them that make plant succulent. Sucking insect pests are commonly attracted toward succulent plants that are enriched with chlorophyll (Daughtry, 2000; Amaliotis *et al*., 2004; Tucker, 2004).

Sugar concentration also affected the attractiveness of *P. solenopsis*, results revealed that attractiveness was influenced positively with total soluble sugars. Higher population of another type of mealybugs on different plant species due to increased carbohydrate sugars has also been reported (Karar *et al.,* 2015). Results further explained negative association for attractive index of cotton mealybug with reducing sugars of plants. Results further demonstrated that reducing sugars had positive interaction with mortality percentage of cotton mealybug. From the results it was clear that inhibition in attractiveness of *P. solenopsis* was also due to increased reducing sugar contents that serve as olfactory and gustatory stimuli and imbalanced nutrition for insect pest (Neal *et al*., 1994; Liu and Stansly, 1995; Hu *et al*., 2010).

Results of present studies revealed that potassium contents had negative effect on the attractiveness of mealybug, the reason was due to physiological activity of the plants and increase in defense system of plant species against insect pest and diseases (Du *et al*., 2004; Liu *et al*., 2004). From the biochemical results it was observed that among phosphorus, nitrogen, potassium, sodium, total soluble sugar, reducing sugar contents, the nitrogen contents played maximum role toward attractiveness of *P. solenopsis*.

Cluster analysis is data reduction technique used for categorizing lot of combinations into desired groups on the basis of similarities and variations. In the present study tested host plants of *P. solenopsis* were categorized into the three combinations. Cluster 1 consisted of favorable host plants bearing more attractiveness but less cotton mealybug mortality. In contrast cluster 3 demonstrated resistant plant source with less attractiveness and more mortality whereas cluster 2 contained plants showing intermediate attractiveness and mortality of *P. solenopsis.*

Conclusion

Association between chemical contents of tested plants had significant effect on the preference and mortality of mealybug. Mortality of pest was positively correlated with, potassium, but preference was positively associated with nitrogen, crude protein and potassium. From the results it was clear that close association exits between chemical contents of plants with the biological activities of *P. solenopsis*.

References

Abbas, G., M.J. Arif, M. Ashfaq, M. Aslam and S. Saeed, 2010. Host plants distribution and overwintering of cotton mealybug (*Phenacoccus solenopsis*; Hemiptera: Pseudococcidae). *Int. J. Agric. Biol.*, 12: 421–425

Abisgold, J.D., S.J. Simpson and A.E. Douglas, 1994. Nutrient regulation in the pea aphid *Acyrthosiphon pisum*: application of a novel geometric framework to sugar and amino acid consumption. *Physiol. Entomol.*, 19: 95–102

Abdullah, A., 2009. Analysis of mealy bug incidence on the cotton crop using ADSS-ODLAP (online analytical processing) tool. *Comp. Elect. Agric.*, 69: 59–72

Amaliotis, D., I. Therios and M. Karatissiou, 2004. Effect of nitrogen fertilization on growth, leaf nutrient concentration and photosynthesis in three peach cultivars. *ISHS, Acta Hort.*, 449: 36–42

Arif, M.I., M. Rafiq and A. Ghaffar, 2009. Host plants of cotton mealybug (*Phenacoccus solenopsis*): a new menace to cotton agro-ecosystem of Punjab, Pakistan. *Int. J. Agric. Biol.*, 11: 163–167

Arif, M.J., M.R. Shahid, M.D. Gogi, M. Arshad and M.A. Khan, 2013. Studiesonbiologicalparametersofaninvasivemealybug, *Phenacoccus solenopsis* Tinsely (Pseudococcidae: Hemiptera) on different host plants under laboratory Conditions. *Acad.. J. Entomol.*, 6: 55–60

Blake, W.E., M.W.R. Bryant and A. Waters, 1969. The flame-photometric determination of sodium in solutions of high calcium content. *Analyst*, 94: 49–53

Bremmer, J.M. and D.R. Keeney, 1965. Steam distillation methods for determination of ammonium, nitrate and nitrite. *Anal. Chim. Acta*, 32: 485–489

Daughtry, C.S.T., C.I. Walthall, M.S. Kim, E. Brown, D.E. Colstoun and J.E. Mcmurtrey, 2000. Estimating corn leaf chlorophyll concentracion from leaf and canopy reflectance. *Rem. Sens. Environ.*, 74: 229–239

Deshpande, V., 2009. *Mealybug Species, the Bt Cotton Killer, is Exotic.* Indian Express, July. http://www.indianexpress.com/news/mealybug-species-the-bt-cotton-killer-is-exotic-experts/490540/

Dhaliwal, G.S. and R. Arora, 2003. *Principles of Insect Pest Management*, 2nd edition, pp: 90-94. Kalyani Publishers, Ludhiana, India

Dixon, A.F.G., 1987. Cereal aphids as an applied problem. *Agric. Zool. Rev*., 2: 1–57

Du, L., F. Ge, S. Zhu and M.N. Parajulee, 2004. Effect of the cotton cultivar on development and reproduction of *Aphis gossypii* (Homoptera: Aphididae) and its predator *Propylaea japonica* (Coleoptera: Coccinellidae). *J. Econ. Entomol*., 97: 1278–1283

Febvay, G., B. Delobel and Y. Rahbe, 1988. Influence of the amino acid balance on the improvement of an artificial diet for a biotype of *Acyrthosiphon pisum* (Homoptera: Aphididae). *Can. J. Zool*., 66: 2449–2453

Felkl, G., E.B. Jensen, K. Kristiansen and S.B. Andersen, 2005. Tolerance and antibiosis resistance to cabbage root fly in vegetable Brassica species. *Entomol. Experim. Applic*., 116: 65–71

Girousse, C. and R. Bournoville, 1994. Role of phloem sap quality and exudation characteristics on performance of pea aphid grown on lucerne genotypes. *Entomol. Exp. Appl*., 70: 227–235

Goncalves-Alvim, S.J., R.G. Collevatti and G.W. Fernandes 2004. Effects of genetic variability and habitat of Qualea parviflora (Vochysiaceae) on Herbivory by Free-feeding and Gall-forming Insects. *Ann. Bot.*, 94: 259–268

Hartnett, D.C. and W.G. Abrahamson, 1979. The effects of stem gall insects on life history patterns in *Solidago canadensis*. *Ecology*, 60: 910–917

Hu, J.S., D.B. Gelman, M.E. Salvucci, Y.P. Chen and M.B. Blackburn, 2010. Insecticidal activity of some reducing sugars against the sweet potato whitefly, *Bemisia tabaci, Biotype B.J. Insect Sci.*, 10: 203 available online: insectscience.org/10.203

Joachim-Bravo, I.S., O.A. Fernandes, S.A. DE-Bortoli and F.S. Zucoloto, 2001. Oviposition behavior of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae): association between oviposition preference and larval performance in individual females. *Neotropic Entomol.*, 30: 559–564

Karar, H., M.J. Arif, M. Arshad, A. Ali and Q. Abbas, 2015. Resistance/ susceptibility of different mango cultivars against mango mealybug, *Drosicha mangiferae* G. *Pak. J. Agric. Sci.,* 52: 365–375

Kim, D.S. and J.H. Lee, 2002. Egg and larval survivorship of *Carposina sasakii* (Lepidoptera: Carposinidae) in apple and peach and their effects on adult population dynamics on orchards. *Environ. Entomol.,* 31: 686–692

Kogan, M., 1982. Plant resistance in pest management. *In*: *Introduction to Insect Pest Management*, pp: 93–134. Metcalf, R.L. and W.H. Luckmann (eds.). John Wiley and Sons, New York, USA

Li, Y., C.B. Hill and G.L. Hartman, 2004. Effect of three resistant soybean genotypes on the fecundity, mortality and maturation of soybean aphid (Homoptera: Aphididae). *J. Econ. Entomol.,* 97: 1106–1111

Lin, H., M. Kogan and D. Fischer, 1990. Induced resistance in soybean to the Mexican bean beetle (Coleoptera: Coccinelidae): comparisons of inducing factors. *Environ. Entomol.,* 19: 1852–1857

Liu, T.X. and P.A. Stansly, 1995. Toxicity and repellency of some biorational insecticides to *Bemisia argentifoliion* tomato plants. *Entomol. Exp. Appl*., 74: 137–143

Liu, J.C., I. Ockenden, M. Truax, J.N.A. Lott, 2004. Phytic acid-phosphorus and other nutritionally important mineral nutrient elements in grains of wild-type and low phytic acid (lpa1-1) rice. *Seed Sci. Res.*, 14: 109–116

Loska, K. and D. Wiechula, 1975. Comparison of Sample Digestion Procedures for the Determination of different metal ions. *Microchim Acta*., 154: 235–240

Mierziak, J., K. Kostyn and A. Kulma, 2014. Flavonoids as Important Molecules of Plant Interactions with the Environment. *Molecules*, 19: 16240–16265

Neal, J.W.J., J.G. Buta, G.W. Pittarelli, W.R. Lusby and J.A. Benz, 1994. Novel sucrose esters from *Nicotiana gusset*: effective biorationals against selected horticultural insect pests. *J. Econ. Entomol.*, 87: 1600–1607

Pedigo, L.P., 1996. *Plant Resistance to Insects*, *In: Entomology and Pest Management*, pp: 413–424. Prentice hall of India Private Limited, New Delhi, India

Prosser, W.A., S.J. Simpson and A.E. Douglas, 1992. How an aphid (*Acyrthosiphon pisum*) symbiosis responds to variation in dietary nitrogen. *J. Insect Physiol.*, 38: 301–307

Raizi, N., J. Kreuger, L. Lay, G. Russo, L. Panza, B. Lindahl and U. Lindahl, 1985. Identification of O-sulphate substituents on D-glucuronic acid units in heparin-related glycosaminoglycans using novel synthetic disaccharide standards. *Glyco. Biol*., 5: 807–811

Sarmah, M.C., M. Chutia, K. Neog, R. Das, G. Rajkhowa and S.N. Gogoi, 2011. Evaluation of promising castor genotype in terms of agronomical and yield attributing traits, biochemical properties and rearing performance of eri silkworm, *Samia ricini* (Donovan). *Industr. Crop Prod.*, 34: 1439–1446

Shahid, M.R., J. Farooq, A. Mahmood, F. Ilahi, M. Riaz, A. Shakeel, I.V. Petrescu-Mag and A. Farooq, 2012. Seasonal occurrence of sucking insect pest in cotton ecosystem of Punjab, Pakistan. *Adv. Agric. Bot.*, 4: 26–30

Singh, R. and A.D. Taneja, 1989. Influence of phytochemicals and leaf pubescence of some malvaceous plants on development, survival and oviposition of cotton leaf hopper. *Z. Angew. Zool*., 76: 357–368

Sneath, P.H.A. and R.R. Sokal, 1973. *Numerical Taxonomy: The Principles and Practices of Numerical Classification*. San Francissco: Freeman

Tsai, J.H. and J.J. Wang, 2001. Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera: Aphididae). *Environ. Entomol.*, 30: 45–50

Tucker, M., 2004. *Primary Nutrients and Plant Growth In: Essential Plant Nutrients* SCRIBD (ed.). Dept. of Agriculture, North Carolina, USA

Van-Emden, H.F. and D.B. Peakall, 1996. *Beyond Silent Spring: Integrated Pest Management and Chemical Safety*. Chapman and Hall, London

Van Lenteren, J.C. and L.P.J.J. Noldus, 1990. Whitefly-plant relationship: behavioral and biological aspects. *In*: *Whitefly: Their Bionomics, Pest Status and Management*, pp: 47–89. Gerling, D. (ed.). Intercept, Andover, UK

Wang, Y.P., S.A. Wu and R.Z. Zhang, 2009. Pest risk analysis of a new invasive pest, *Phenacoccus solenoipsis*, to China. *Chin. Bullet. Entomol.*, 46: 101–106

Wolf, A., 1982. Determination of phosphorus by sample combustion followed by non-suppressed ion chromatography. *J. Chromatogr*., 706: 199–207

Wu, S.A. and R.Z. Zhang, 2009. A new invasive pest, *Phenacoccus solenopsis* threatening seriously to cotton production. *Chin. Bullet. Entomol*., 46: 159–162

Zhong-xian, L., Y. Xiao-ping, Kong-luen Heong and H. Cui, 2007. Effect of nitrogen fertilizer on herbivores and its stimulation to major insect pest in rice. *Rice Sci.*, 14: 56–66

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