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Imtiaz Ali Khan
Department of Entomology, the University of Agriculture, Peshawar, Pakistan.

Komal Habib
Department of Entomology, the University of Agriculture, Peshawar, Pakistan.

Rasheed Akbar
Department of Entomology, the University of Agriculture, Peshawar, Pakistan.

Ashraf Khan
Department of Entomology, the University of Agriculture, Peshawar, Pakistan.

Muhammad Saeed
Department of Agricultural Sciences, University of Haripur, Haripur, Pakistan.

Abid Farid
Department of Agricultural Sciences, University of Haripur, Haripur, Pakistan.

Ijaz Ali
Institute of Biotechnology and Genetic Engineering, the University of Agriculture, Peshawar, Pakistan.

Mukhtar Alam
Faculty of Agriculture, University of Swabi, Pakistan.

Correspondence:
Imtiaz Ali Khan
Department of Entomology, the University of Agriculture, Peshawar, Pakistan.

Proximate chemical composition of brinjal, *Solanum melongena* L. (Solanales: Solanaceae), genotypes and its correlation with the natural enemies in Peshawar

Imtiaz Ali Khan, Komal Habib, Rasheed Akbar, Ashraf Khan, Muhammad Saeed, Abid Farid, Ijaz Ali, Mukhtar Alam

Abstract
Coccinella septumpunctata L. (Coccinellidae: Coleoptera), *Episyrphus balteatus* DeGeer (Syrphidae: Diptera) and *Chrysoperla carnea* Stephens (Chrysopidae: Neuroptera) are the natural enemies of major insect pests of *S. melongena* in Pakistan. Using host plant resistance and natural enemies can be safe and cheap alternatives to insecticidal control of insect pests of *S. melongena*. The experiment was conducted at the New Developmental Farm (NDF), The University of Agriculture, Peshawar (UAP) in 2014. Three Brinjal genotypes Shamli, Pearl Long and Black Beauty were used in the study. Overall mean densities of *C. septumpunctata*, *E. balteatus* and *C. carnea* were higher (0.79, 056, 0.44 individuals leaf^{-1}) on Shamli and lower (0.57, 0.45, 0.22 individuals leaf^{-1}) on Black beauty, respectively. The results of proximate chemical composition of *S. melongena* leaves and fruits varied among the three genotypes. Moisture content was higher (93%) in Black Beauty and lower (92.10%) in Pearl Long. Ash content was higher (6.4%) in Pearl Long and lower (5.4%) in Black Beauty. Crude protein was higher (1.51%) in Shamli and lower (1.30%) in Black Beauty. Crude fat was higher (0.31%) in Shamli and lower (0.28%) in Black Beauty. Total sugars were higher (4.22%) in Shamli and lower (3.96%) in Black Beauty. Fiber content was higher (1.33%) in Shamli and lower (each 1.31%) in Pearl Long and Black Beauty. Densities of the natural enemies were negatively correlated with proximate chemical composition of the three brinjal genotypes except for positive correlation to the moisture, crude fiber and total sugars. These present results will provide baseline for using host plant resistance and natural enemies in insect pest's control of *S. melongena* in Peshawar.

Keywords: Correlation, Host plant resistance, Natural enemies, proximate chemical composition, S. melongena

1. Introduction
Brinjal, *Solanum melongena* L., also called eggplant or aubergine (French name), of uncertain origin, but it is extensively grown in almost all parts of the world [1]. In Pakistan, it occupies 9,044 ha area and its production is 88,1,48 tons. Yield of brinjal in Pakistan has been reported to be 97,466 kg/ ha [2].

Some of the important insect pests of brinjal in Pakistan are brinjal fruit borer, *Leucinodes orbonalis* Guenee (Lepidoptera, Pyralidae), brinjal stem borer, *Euzophera perticella* Ragonot (Lepidoptera, Pyralidae), leaf roller, *Eublemma olivacea* (Walker) (Lepidoptera, Noctuidae), beetle, *Epilachna vigintioctopunctata* Fabr. (Coleoptera, Coccinellidae), aphid, *Aphis gossypii* (Homoptera, Aphididae), Whitefly, *Bemisia tabici* (Gene.) (Hemiptera, Alerodydidae), thrips, *Thrips palmi* Karny (Thysanoptera, Thripidae) [3]. Cotton jassid (CJ), *Amrasca biguttula biguttula* (Ishida) (Hemiptera, Cicadellidae) is also a serious pest in Pakistan [4, 5].

Adults and nymphs of *A. biguttula biguttula* feed on the underside of the leaves by sucking plant sap, which results in yellowing and curling of leaves. It also injects toxic material into the leaves, which causes necrosis. The blades of severely infested leaves show burn symptom and such leaves may ultimately drop down [6]. Damage caused by the jassid to brinjal could be up to 54 percent [7].

Insect resistance in crop plants is an important component of Integrated Pest Management (IPM) and it is considered as non-monetary input at farmers end. Resistant and tolerant cultivars form the basic component of Integrated Pest Management (IPM) over which other

Components are to be built up. It contributes helpfully in IPM in two ways: reduces the quantum of insecticides and improves performance of natural enemies in plants. Even a low level of tolerance in plants has a dramatic effect, which in fact reduces the need of insecticides [8].

Host plant resistance is a preventive control measure, which is compatible with integrated pest management (IPM) strategy. Growing resistant varieties, such as ISD006, BL114 and BL095 has been recommended as a control method for the jassid on brinjal [9]. Screening of brinjal varieties have been done by a number of researchers. Brinjal varieties KB9, Pusa Purple Long, KP10 and BB1 were tolerant to CJ [10]. In another study, a large number of varieties were reported to be resistant against the jassids [11]. In a study on resistance of brinjal to jassid identified 19 brinjal accessions, which exhibited high level of resistance to it [12]. Varieties a 300 (Mistasa), Abar, Parat, EG 2003, Mara and Acc 612 were resistant to jassid in a four year resistance study in Philippines [13].

L. orbonalis has remained a major pest of brinjal since two decades. Fruit infestation by this pest ranges from 20.70 to 88.70% in various parts of India [14]. Twenty varieties of brinjal were tested for resistance to *L. orbonalis*, but none was found resistant to it [15]. The lower fruit infestation may be found due to the presence of smaller fruit diameter and weight and more seed presence in the fruit. The presence of thin stem, more branches, lower third leaf length and width, more spines, rough leaf surface area, heavily lignified thick cuticle, broad and thick hypodermis, closely packed vascular bundle and small pith area may be responsible for lower infestation and vice versa in case of higher infestation [16].

Keeping in view the importance of brinjal crop and the losses caused by various insect pests to it, the present research project aimed to determine proximate chemical composition of brinjal genotypes and its correlation with the natural enemies of insect pests of *S. melongena* in Peshawar.

2. Materials and Methods

2.1 Field layout

Seedlings of three brinjal genotypes, namely Shamli hybrid, Pearl Long (long brinjal) and Black Beauty (round brinjal) were purchased from the local market of Gurh Mandi in Peshawar and transplanted in March, 2014 in the NDF, UAP. The experiment continued till the end of September, 2014. The experiment was laid out in Randomized Complete Block Design. Plant to plant and row to row distance was kept at 30 cm and 65 cm, respectively. There were five rows of ten plants each per treatment and each treatment replicated three times. The size of each treatment was 26 m^2. The crop was given standard agronomic practices throughout the growing season. Fertilization was done through broadcast method after three weeks of transplantation. Weeds were removed when necessary.

Application of a synthetic insecticide Advantage 20% EC (Carbosulfan) was done twice when the pest's status reached to economic threshold level. Data of the natural enemies were recorded 24h before and then 24, 48 and 72 h and at weekly intervals after each insecticidal application.

2.2 Natural enemies of insects' pests of S. melongena

2.2.1 C. septumpunctata, C. carnea and E. balteatus

Larvae and adults of the three natural enemies were recorded on three randomly selected leaves per 10 randomly selected plants per treatment. The data was converted into combined means of larvae and adults. *E. balteatus* adults were collected through hand net. *C. carnea* and *E. balteatus* larvae were found feeding on the aphids and adults feeding on plant exudates. The data was converted into combined means of larvae and adults.

2.2.2 Chemical Analysis

Proximate chemical composition of brinjal leaves and fruits were conducted. Standard methods for finding % moisture content, % ash, crude protein, crude fiber, total reducing sugars and crude fat per 100 grams of brinjal leaves and fruits were used, which are as follows

2.2.3 Moisture content

To find out moisture contents the experiment was carried out at Dept. of Biochemistry, The University of Agriculture, Peshawar, Pakistan. Moisture content was determined by taking 1 gm of well mixed sample in a dried and weighted China dish. The sample was kept in oven at 105 ºC until a constant weight was obtained [17]. Moisture content was calculated as following

$$\text{Moisture (\%)} = \frac{W1-W2}{W3} \times 100$$

Where,

W1 = Initial weight of sample
W2 = final weight of sample
W = Weight of sample

2.2.4 Ash

For the determination of ash content, about 1 gm of sample form each treatment was taken in a dried and cleaned China dish and charred over a slow burning flame. The samples were then placed in the muffle furnace at 550 ºC until constant weight was obtained [16]. The ash content was calculated on percentage as under.

$$\text{Ash (\%)} = \frac{W1-W2}{W3} \times 100$$

Where,

W1 = Initial weight of sample
W2 = final weight of sample
W = Weight of sample

2.2.5 Crude protein

Kjeldahl method was used for the determination of crude protein at Dept. of Biochemistry, The University of Agriculture, Peshawar, Pakistan. To find out protein the samples were digested by heating with concentrated Sulphuric acid in the presence of digestion mixture. The mixture was then made alkaline. Ammonium sulphate thus form released ammonia which was collected in 2% boric acid and titrated against standard HCL. Total protein was calculated by multiplying the amount of nitrogen with constant factor (5.70) and the amount of protein was calculated.

Reagents: the following reagents were used to carry out the procedure for finding out the protein.

i. 0.005 HCL (standard)
ii. Concentrated sulphuric acid
iii. Sodium hydroxide solution
iv. Digestion mixture: Potassium sulphate (K_2So_4) and copper sulhate (CuSo4)
v. Boric acid: dissolved 40 gm of boric acid in sufficient distilled water made the volume upto 100 ml.

vi. Mixed indicator: Dissolved 0.078g of Bromocresole green indicator and 0.1 g of methyl red indicator in ethanol. Then took 20 ml of this solution and dissolved in1L distilled water containing 20 gm of boric acid (adjust the color to dark green by NaOH and HCL).

Procedure: Digestion for finely ground samples was carried out with 1.5 gb (approx.) of digestion mixture, and 7 ml of concentrated H_2SO_4. The volume of the digested sample was made upnto 100 ml. all the digested mixture was added to Kjeldahl distillation flask, then made alkaline by the addition of sufficient 40% NaOH solution and distilled into a flask containing boric acid solution and mixed indicator. This distillate was then titrated against 0.005N HCl. A blank was run simultaneously through all the steps, so as to bring into account any amount of nitrogen being added from the chemical.

Percent protein content of the sample was calculated by the formula:

$$\%\text{Nitrogen} = \frac{(S-B) \times 0.1N \times 0.014 \times 100}{\text{Weight of the sample (g)} \times V(ml)} \times 100$$

$$\%N = Y$$

Now,

$$\% \text{ protein} = Y \times 5.7$$

$$\% \text{ protein} = X$$

Where,

S = Titration sample
B = Blank Titration
N =Normality of HCl used (ml)
V = Volume of sample taken from distillation (ml)
0.014= Molecular weight of nitrogen
100 = Dilution water
5.7 = the factor for wheat fillover was to find out % protein which is constant.

2.2.6 Crude fat

Extraction of crude fat was performed through Soxhlets Apparatus. 2-4 g moisture free sample was taken in a clean previously dried extraction thimble. The thimble was placed in an extraction tube. A previously clean and dried 200 ml round bottom flask weight. Then it was filled up to one third with solvent (petroleum ether 40-60) and connected with the extraction tube. The apparatus turned on the flow of top water and burner. Extraction was continued for 3-4 hours. Siphoning was occurred after every 5-10 minutes at the condensation rate of 3 to 4 drops per second. After completion of the process, thimble was removed from the extractor and flask was heated so that all the solvent was collected for future use. The apparatus was allowed to cool down. Then the flask was dried at 105 °C for 1 hour. Finally it was cooled and weight again [17]. The percent oil content of the sample was calculated as per following formula

$$\% \text{ oil} = \frac{\text{wt of flask +oil – wt of empty flask}}{\text{Wt of sample}} \times 100$$

2.2.7 Crude Fiber

Chemicals

2% NaOH =10g/500ml water solution
2% HCl= 10ml/500ml water solution

Procedure: First two g sample was taken. It was put in a beaker containing 200ml HCl. The sample in acid was placed on water bath. It was boiled for 30 minutes. Filtered the residue and was transferred to a beaker containing 200ml NaOH and boiled for 30 minutes on a water bath. Then filtered and washed with 100mL hot water. The residue was transferred to crucible put in a oven for 4 hours at 105 °C cool and weighed. The crucible was put in furnace for 4 hrs at 550 °C, cooled and reweighted [17].

$$\%\text{Crude Fiber} = \frac{\text{(weight of oven dried residue- weight after ignition)} \times 100}{\text{Weight of sample}}$$

2.2.8 Statistical analysis

The data recorded for each parameter was be analyzed statically by using Statistix 8.1 Software and means were separated by using Fisher Protected Least Significance Difference Test at 5% level of significance [18].

3. Results and Discussion

Mean density of *C. septumpunctata* leaf^{-1} was non-significantly different on the three brinjal genotypes throughout the data collection period (Table 1). However, its density was comparatively higher on Shamli than the other two genotypes on all data collection dates except 72h after 1st chemical treatment, where density of the beetle was higher on Pearl Long than on the other two genotypes. Density of the beetle remained lowest on Black Beauty than the other two genotypes throughout the data collection dates. Overall mean density of the beetle was higher (0.79 beetles leaf^{-1}) on Shamli with and lower (0.57 beetles leaf^{-1}) on Black Beauty with**.**

Table 1: Mean density of C. septumpunctata leaf^{-1} on three brinjal genotypes during 2014.

Geno Type	Mean density of C. septumpunctata leaf^{-1} on														Overall Mean
	Pre-spray	After 1st Spray						Pre-spray	After 2nd spray						
	6-May	24h	48h	72h	1 wk	2 wk	3 wk	2-Jun	24h	48h	72h	1 wk	2 wk	3 wk	
Shamli	1.0	0.7	0.6	0.6	0.5	0.7	1.2	1.4	1.1	0.8	0.6	0.4	0.6	0.9	0.79
Pearl Long	0.7	0.5	0.73	0.5	0.4	0.6	1.1	1.3	0.9	0.7	0.6	0.4	0.6	0.8	0.69
Black Beauty	0.8	0.4	0.3	0.2	0.2	0.5	0.9	1.4	0.8	0.5	0.4	0.3	0.5	0.8	0.57
LSD Value	ns	Ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns	ns	ns	ns

Means in columns followed by different letters are significantly different at 5% level of significance (LSD test). ns = non-significant.

Mean density of the *E. balteatus* leaf^{-1} was non-significantly different on the three brinjal genotypes throughout the data collection period (Table 2). However, density of the fly was comparatively higher on Shamli than the other two genotypes throughout the data collection period, except that it was higher on Pearl Long on Pre-spray and equal to that on Shamli on 24h, 72h after 1st spray; and on pre-spray, 48h, 72h and 1 wk after 2nd spray. Overall mean density of the fly was higher on shamli (0.56 fly leaf^{-1}) and lower on Black Beauty (0.45 fly leaf^{-1}).

Table 2: Mean density of E. balteatus leaf^{-1} on three brinjal genotypes during 2014.

Geno-type	Mean density of E. balteatus leaf^{-1} on														Over-all Mean
	Pre-spray	After 1st Spray						Pre- spray	After 2nd spray						
	6-May	24h	48h	72h	1 wk	2 wk	3 wk	2-Jun	24h	48h	72h	1 wk	2 wk	3 wk	
Shamli	0.9	0.7	0.6	0.4	0.2	0.5	0.7	0.9	0.6	0.4	0.2	0.4	0.6	0.8	0.56
Pearl Long	1.1	0.7	0.5	0.4	0.1	0.4	0.6	0.9	0.5	0.4	0.2	0.4	0.5	0.7	0.53
Black Beauty	1.0	0.6	0.5	0.3	0.2	0.3	0.6	0.8	0.4	0.2	0.1	0.3	0.4	0.6	0.45
LSD Value	Ns	ns	ns	ns	Ns	Ns	ns	ns	ns	ns	ns	Ns	ns	ns	ns

Means in columns followed by different letters are significantly different at 5% level of significance (LSD test).
ns = non-significant.

Mean density of *C. carnea* density leaf^{-1} was non-significantly different on the three brinjal genotypes throughout the data recording period (Table 3). However, density of the *C. carnea* was comparatively higher on Shamli than the other two genotypes, except on 72h after 1st treatment. Density of the *C. carnea* remained lower on Black Beauty than the other genotypes throughout the data recording period. Overall mean density of the *C. carnea* was higher on Shamli (0.44 individual's leaf^{-1}) and lower on Black Beauty (0.22 individual's leaf^{-1}).

Table 3: Mean density of C. carnea leaf^{-1} on three brinjal genotypes during 2014.

Geno-type	Mean density of C. carnea leaf^{-1} on														Overall Mean
	Pre-spray	After 1st Spray					Pre- spray		After 2nd spray						
	6-May	24h	48h	72h	1 wk	2 wk	3 wk	2-Jun	24h	48h	72h	1 wk	2 wk	3 wk	
Shamli	0.8	0.5	0.4	0.2	0.2	0.5	0.7	0.8	0.4	0.3	0.2	0.2	0.4	0.6	0.44
Pearl Long	0.7	0.4	0.3	0.2	0.1	0.2	0.5	0.7	0.3	0.2	0.1	0.1	0.2	0.4	0.31
Black Beauty	0.6	0.2	0.1	0.1	0.1	0.3	0.4	0.4	0.1	0.0	0.1	0.2	0.2	0.3	0.22
LSD Value	ns	Ns	ns	ns	ns	ns	ns	Ns	ns	ns	ns	Ns	ns	ns	ns

Means in columns followed by different letters are significantly different at 5% level of significance (LSD test).
ns = non-significant.

Ladybird beetle both as larvae and adult consume aphids, whiteflies, small insect and insect eggs, while chrysopa as larvae (and adults in some species also) consume aphids, spider mites (especially red mites), thrips, whitefly, eggs of leafhoppers, moths, and leafminers, small caterpillars, beetle larvae on eggplant [19].

The results of correlation between chemical composition of the three brinjal genotypes and population density of the natural enemies is given in Table 4. It is clear from the results that density of the natural enemies was negatively correlated with chemical composition of the three brinjal genotypes except for positive correlation to the moisture, crude fiber and total sugars.

Table 4. Correlation matrix of natural enemy's density and biochemical factors of three brinjal genotypes during 2014.

Shamli	*C.septempunctata*	0.30,1.02*	0.76,-0.48	0.40,-0.91	0.30,0.30	-0.62,0.63	-0.31,0.31
	E. balteatus	0.78,-0.22	-0.82,0.93	0.97*,-0.05	- 0.77,0.77	0.91,-0.94	0.77*,0.78*
	C. carnea	0.46,0.64	-0.70,0.91	0.94*,-0.24	- 0.88,0.87	0.81,-0.82	0.87*,0.88*
Pearl Long	*C.septempunctata*	0.64*,-0.62	-0.15,-0.88	0.64,0.31	0.64,0.31	0.72,-0.31	0.33,-0.64
	E. balteatus	0.29,-0.25	-0.51,-0.98*	0.22,0.67	-0.26,0.66	0.97*,-0.65	0.66*,-0.24
	C. carnea	1.02,-1.03	-0.09,-0.86	0.68,0.28	-0.67,0.29	0.69*,-0.29	-0.23,-0.64
Black Beauty	*C.septempunctata*	-0.12,-0.96	-0.22,-0.71	0.12,0.30	0.32,0.36	0.93*,0.64*	
	E. balteatus	0.90*,-0.15	-0.09,-0.81	0.006,0.18	0.18,0.17	0.99*,0.76*	
	C. carnea	0.67*,-0.48	-0.46,0.52	-0.42,-0.56	-0.56,-0.58	-0.81,-0.44	

*= Significant at 5%

There is plenty of research findings available on host plant resistance against insect pests of brinjal [10, 11, 12, 15]. However, limited number of researches have been conducted on the correlation of chemical composition of brinjal genotypes with the natural enemies.

High population of coccinellids, syrphids and spiders were recorded on Bejo Sheetal and Pusa hybrid-6 brinjal hybrids. This might be due to volatile chemicals from susceptible plants promoting the population of natural enemies by hardening high pest's load [20].

A field experiment was conducted to study the response of cultivars/ hybrids/ germplasm of brinjal to major insect pests and their natural enemies. The results showed that the hybrid, Sweta was the best in reducing the shoot and fruit damage by *Leucinodes orbonalis* Guen. recording the mean shoot and fruit damage of 8.0 and 8.7 per cent (number basis) and population of spotted leaf beetle, *Henosepilachna vigintioctopunctata* Fab., ash weevil, *Myllocerus* spp. Guerin, mealybug, *Coccidohystrix insolitus* Green, aphid, *Aphis gossypii* Glover, leafhopper, *Amrasca devastans* Ishida and whitefly, *Bemisia tabaci* (Gennadius) recording 8.0, 0.0, 6.5, 6.3, 0.0 and 0.0 nos./ three leaves, respectively. The hybrids, Bejo Sheetal and Pusa hybrid-6 recorded high population of coccinellids, syrphids and spiders. The biochemical characters such as total sugars, total chlorophyll and moisture content were positively correlated with shoot damage while total phenols and ash content have negative correlation [21].

Conclusion and Recommendation

All the three genotypes confer some kind of attraction to the natural enemies of insect pests of *S. melongena*. However, density of all the three natural enemies was comparatively higher on Shamli brinjal genotype than the other two genotypes. The present findings may provide basis for using

natural enemies and host plant resistance in management of brinjal insect pests.

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