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Influence of various biochemical factors on the occurrence of *Helicoverpa armigera* (Hubner) in Tomato

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Abstract
Experiment on the influence of various biochemical factors on the occurrence of *Helicoverpa armigera* in tomato was carried out under field conditions, following RCBD on fourteen commercially available tomato genotypes *viz.* Mission 102, Sultan, 027, Chinar, GS 5575, Sourabh, T 7008, R 165, RK 101, Riogrande, Roma, Bambino, Super Classic and Roma VF. Results indicated that genotype chinar was found to be the most resistant while genotype R 165 was found to be most susceptible. Chemical analysis revealed ascorbic acid, phenols and acidity showed negative correlation with fruit infestation, while ash and pH showed positive correlation. Moisture had non significant negative correlation with both larval population and fruit infestation. Multiple linear regression models showed resistance to be influenced by combination of various factors rather than a single factor. Tomato genotypes with high content of both ascorbic acid and phenols, as well as acidy could be used as marker traits for resistance against *H. armigera.*

Keywords: Biochemical plant factors, *H. armigera*, Host plant resistance, Tomato genotypes

1. Introduction
Among the insect pests of tomato, Tomato fruit worm (*Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is the most dreaded one. *H. armigera* feeds on both tomato foliage as well as fruit by feeding inside it thus affecting fruit quality both human consumption and marketing point of view [10]. In Pakistan, fruit losses by *H. armigera* have been estimated to be in the range of 32-53% [14, 12] but the reliable monetary losses in tomato crop are not available. Approximately annual loss of 5 billion US dollars has been attributed to *H. armigera* world wide [20].

Heavy reliance on chemical control of *H. armigera* has resulted in various environment and health problems [11]. Problems associated with chemical control has ultimate shifted the focus towards integrated pest management. Of different management components, resistant varieties have attained much attention in recent years. Several studies have been conducted regarding the significance of host plant resistance in integrated pest management. Plants usually have inbuilt self defence mechanism against insect pests. Certain physical plant characters constitute an important component of such a defence mechanism. Chemistry of host plants, together with the physical plant characters can contribute towards the overall plant resistance against insect pests. Host plant chemistry can play a vital role and has been reported to influence *H. armigera* infestation [6, 8, 17, 21].

The importance of this pest and 80 % share of total pesticide use that it receive in Pakistan [18], has led us to investigate alternative control strategies. To this end, biochemical plant factors need to be explored for its role in host plant resistance of tomato against the said pest. Lack of sufficient information and research work on commercially available genotypes of tomato in Pakistan provides ample justification for this work to be carried out. The present study aims to ascertain the influence of various biochemical factors in both leaves and fruits on the occurrence of *H. armigera.*

2. Materials and Methods
Plant Material
The present study was carried out on commercially available fourteen tomato genotypes including nine F1 hybrids (RK 101, Mission 102, Sultan, 027, Chinar, GS 5575, Sourabh, T 7008 and R 165) and 5 varieties (Riogrande, Roma, Bambino, Super Classic and Roma VF)

during 2010 at New Developmental Farm, The University of Agriculture Peshawar- Pakistan. Healthy seedlings of all the genotypes were transplanted in separate plots on ridges, each measured 5.5 x 2.5 m. Plant to plant and row to row space were 45 cm and 75 cm respectively. The experiment was laid out in RCBD with three replications. Standard agronomic practices (*e.g.,* ploughing, manuring and irrigation) were applied uniformly to all experimental plots. Data on the larval population plant^{-1} was recorded on randomly selected 5 plants per genotype in each replication at weekly interval and their mean was calculated. The percent fruit infestation (presence of holes by *H. armigera* larvae) was recorded for each plot after each picking. The percent fruit infestation was calculated by the formula:

$$\text{Percent fruit infestation} = \frac{\text{Weight of infested fruits} \times 100}{\text{Total weight of fruits (sound + infested fruits)}}$$

Biochemical analysis of tomato leaves and fruits

The leaf samples of all genotypes were plucked from 40 days old plant where as fruit samples were collected randomly on tenth days of fruit setting for biochemical analysis. The moisture and ash content in all samples were determined using standard method of (AOAC 2005) [4]. pH of the tomato leaf and fruit extract was determined by digital pH meter. Before using the pH meter was standardized with the solutions of known pH of 4 and 7. Then 10 ml of the tomato leaf and fruit extract was taken in a clean breaker separately and the electrode was directly dipped into the sample to record the pH value. Total titratable acidity (% citric acid) was calculated by neutralization reaction [19]. Ascorbic acid was estimated by 2, 6- dichlorophenol indophenols dye reduction method as reported in AOAC 1990 [3]. Phenols content of the tomato were determined by Folin Ciocalteau method [7].

Statistical Analysis: The data were analyzed by statistical software Mstat C and the means were separated through LSD Test at P = 0.05. Simple correlation and multiple linear regression analysis were also carried out to determine the influence of biochemical factors on larval population and fruit infestation by *H. armigera.*

3. Results

Mean larval population plant^{-1} and fruit infestation by *H. armigera* larvae

Table 1 revealed that highest larval population plant^{-1} (2.41) was recorded on genotype R 165 followed by the genotype Super classic (2.24) and T 7008 (2.22) while minimum larval population plant^{-1} was recorded on genotype Chinar (1.89). As far as the fruit infestation by *H. armigera* larvae is concerned, the genotype R 165, GS 5575 and Super classic were found to be most the susceptible with maximum fruit infestation 41.55, 39.35 and 37.19 percent respectively while the genotype chinar, Sourabh and sultan were declared as most resistant with 20.32, 22.15, 23.61 percent fruit infestation respectively. Among the remaining genotypes Mission 102 Bambino and Roma VF showed intermediate response with 30.35, 30.72 and 31.08 percent fruit infestation respectively.

Table 1: *H. armigera* larval population and fruit infestation of various tomato genotypes

Genotypes	Larval population plant^{-1}	Percent fruit infestation
GS 5575	2.21 ab	39.35 ab ***
R 165	2.41 a	41.55 a ***
27	2.08 b-d	35.84 b-d
Souhrab	1.99 cd	22.15 f *
Sultan	1.95 cd	23.61 f *
T-7008	2.22 ab	36.03 bc
Chinar	1.89 d	20.32 f *
Mission 102	1.97 cd	30.35 de **
RK101	2.07 b-d	25.35 ef
S.Classic	2.24 ab	37.19 ab ***
Bambino	2.08 b-d	30.72 c-e **
Riogrande	2.14 bc	30.09 b-d
Roma VF	2.09 b-d	31.08 cd **
Roma	2.05 b-d	33.69 b-d
LSD (.05)	**0.20**	**5.49**

Means followed by different letters are significantly different at .05% level of probability followed by LSD test.
* resistant
** intermediate resistant
*** susceptible

Biochemical analysis of tomato leaves and fruits

Significant variation was observed in all biochemical traits of the tested genotypes. Biochemical factors like pH, phenols content, acidity, ascorbic acid ash and moisture was observed in both fruits and leaves. Fruits had higher observation for all biochemical factors except pH (Table 2 and 3). Among leaves of the tested genotypes, higher phenolic content were observed in Chinar (0.202 mg g^{-1}) and Sourabh (0.197mg g^{-1}) while lower phenolic content was recorded in genotype GS 5575 (0.154 mg g^{-1}) as shown in Table 2. The acidity was found to be significantly higher in leaves of the genotypes RK 101 (0.580%), Sultan (0.577%) and Chinar (0.568%), all being non significantly different from each other. While the acidity was significantly lower (0.370%) in leaves of genotype Super Classic. Significantly higher content of ascorbic acid (10.63 mg/100 g) was found in genotype Sourabh while lower content was observed in genotypes R 165 (5.53 mg/100 g) and GS 5575 (5.87 mg/100 g), (both being non significantly different from each other). Genotype Super Classic had highest pH (4.74) while Chinar (4.16), Roma (4.17), Bambino (4.20),

Mission 102 (4.20) and Roma VF (4.20) had lowest pH values. The genotype R 165 contained higher ash content (0.500%) followed by Super Classic (0.486%), Bambino (0.453%) and GS 5575 (0.450%) while lower ash content was found in Mission 102 (0.320%), Sourabh (0.356%) and RK 101 (0.333%), all being non significantly different from each other. The moisture content was significantly higher in genotype T 7008 (68.33%) which being non significantly different from all genotypes except Super Classic (65.28%) and Roma VF (64.76%) which had lower moisture content.

Table 2: Biochemical analysis of tomato leaves of 14 genotypes

Genotype	Phenols (mg g^{-1})	Acidity %	Ascorbic acid (mg $100g^{-1}$)	pH	Ash Content (%)	Moisture Content (%)
GS 5575	0.189 i	0.420 h	25.26 ef	4.53 ab	0.750 ab	78.16 a
R 165	0.192 i	0.400 h	22.43 g	4.63 a	0.743 a-c	77.07 a
027	0.201 gh	0.741 c	28.77 bc	4.10 d	0.726 b-d	79.66 a
Sourabh	0.225 a	0.783 b	29.13 bc	4.13 cd	0.643 e	79.09 a
Sultan	0.227 a	0.840 a	30.00 b	4.06 d	0.686 de	78.89 a
T 7008	0.207 d-f	0.544 f	24.61 ef	4.16 cd	0.736 a-c	77.87 a
Chinar	0.229 a	0.846 a	32.46 a	4.06 d	0.700 cd	78.39 a
Mission 102	0.213 bc	0.708 cd	26.43 d-f	4.12 cd	0.723 b-d	79.42 a
RK101	0.217 b	0.690 d	27.54 cd	4.13 cd	0.776 a	80.33 a
Super Classic	0.200 h	0.460 g	24.50 f	4.49 ab	0.746 a-c	77.35 a
Bambino	0.212 b-d	0.586 e	26.46 d-f	4.26 c	0.730 a-d	80.36 a
Riogrande	0.206 e-g	0.425 gh	26.55 de	4.50 ab	0.726 b-d	78.24 a
Roma VF	0.210 c-e	0.537 f	24.63 ef	4.21 cd	0.750 ab	79.11 a
Roma	0.203 f-h	0.461 g	25.91 d-f	4.45 b	0.736 a-c	78.39 a
$LSD_{(0.05)}$	**0.0063**	**0.0396**	**2.010**	**0.1523**	**0.0496**	**ns**

Means in columns sharing similar letters are non significantly different at α = 0.05 (LSD Test).

Higher phenol content was calculated in fruits of genotype Chinar (0.229 mg g^{-1}), Sultan (0.227 mg g^{-1}) and Sourabh (0.225 mg g^{-1}) while lower in GS 5575 (0.154 mg g^{-1})and R 165 (0.167 mg g^{-1}). Fruits of genotype Chinar and Sultan showed highest acidity content (0.846% and 0.840 % respectively), while acidity value was lowest for genotype R165 (0.400 %) and GS 5575 (0.420%). Ascorbic acid content was found significantly higher in fruits of genotypes Chinar (32.46 mg/100 g) and lowest in fruits of genotype R 165 (22.43 mg/100 g).

Fruits had lowest pH value in genotypes Chinar (4.06), Sultan (4.06) and 027 (4.10), while higher pH values were recorded in genotypes R 165 (4.63), GS 5575 (4.53), Riogrande (4.50) and Super Classis (4.49). Significant variation was found in ash content of fruits. The genotype RK 101 had higher ash content (0.776%) while Sourabh had lowest ash content (0.643%). No significant differences were found in moisture content in fruits of all tested tomato genotypes. However, higher moisture content was recorded in fruits of genotype Bambino (80.36%) while lower in Super Classic (77.35%) as shown in Table 3.

Table 3: Biochemical analysis of tomato fruits of 14 genotypes

Genotype	Phenols (mg g^{-1})	Acidity %	Ascorbic acid (mg $100g^{-1}$)	pH	Ash Content (%)	Moisture Content (%)
GS 5575	0.154 h	0.445 f	5.87 jk	4.45 cd	0.450 a-c	67.88 a
R 165	0.167 g	0.418 g	5.53 k	4.60 b	0.500 a	68.03 a
027	0.175 d-f	0.401 gh	7.80 ef	4.58 b	0.436 bc	67.36 ab
Sourabh	0.197 a	0.540 bc	10.6 a	4.41 c	0.356 ef	67.84 a
Sultan	0.190 b	0.577 a	9.60 b	4.26 d-f	0.433 c	67.19 a-c
T 7008	0.171 e-g	0.527 c-e	6.30 ij	4.40 c	0.416 cd	68.33 a
Chinar	0.202 a	0.568 a	9.10 c	4.16 g	0.380 de	66.57 a-c
Mission 102	0.183 c	0.535 b-d	8.60 d	4.20 e- g	0.320 f	66.76 a-c
RK101	0.179 cd	0.580 a	8.26 de	4.27 de	0.333 ef	66.42 a-c
Super Classic	0.171 e-g	0.370 i	6.13 j	4.74 a	0.486 ab	65.28 bc
Bambino	0.183 c	0.520 de	7.20 gh	4.20 e-g	0.453 a-c	67.11 a-c
Riogrande	0.170 fg	0.400 h	6.73 hi	4.63 b	0.433 c	67.98 a
Roma VF	0.176 de	0.550 b	6.90 gh	4.20 e-g	0.376 de	64.76 c
Roma	0.174 d-f	0.512 e	7.33 fg	4.17 fg	0.423 cd	65.92 a-c
$LSD_{(0.05)}$	**0.0059**	**0.0177**	**0.4903**	**0.0936**	**0.0508**	**2.507**

Means in columns sharing similar letters are non significantly different at α = 0.05 (LSD Test).

Correlation analysis (Table 4) revealed significantly negative correlation of larval population and fruit infestation with ascorbic acid, acidity and phenol contents while significantly positive correlation with pH and ash content. The negative correlation of moisture content with both larval population and fruit infestation was found non-significant.

Step wise multivariate regression models (Table 5) between larval population and various biochemical plant factors indicated that ascorbic acid was found to be the most important biochemical factor, with maximum contribution (77.6%) toward the larval population of *H. armigera* followed by pH (6%), acidity (4.1%), phenols (2.3%) and ash (2.1 %.) The moisture content had minimum contribution (0.7 %) towards larval population of *H. armigera.*

Table 4: Correlation among various biochemical factors with *H. armigera* larval population and tomato fruit infestation

Biochemical factors	Correlation coefficient (r value)	
	Larval population	Fruit infestion (%)
Ascorbic acid	-0.8802**	-0.9140**
pH	0.8084**	0.7917**
Acidity	-0.7415**	-0.9123**
Phenols	-0.8278**	-0.9524**
Ash	0.7870**	0.8262**
Moisture	-0.2118^{ns}	-0.2887^{ns}

** Significant at $P \leq 0.01$
* Significant at $P \leq 0.05$
ns Non significant

Table 5: Multivariate regression models, along with coefficient of determination (R^2) between *H. armigera* larval population plant $^{-1}$ and various biochemical factors.

Regression Equation	R^2	100 R^2	Role of individual chemical factor (%)
**$Y = 3.117 - 0.059X_1$	0.776	77.6	77.6
** $Y = 1.604 - **0.041X_1 + **0.277 X_2$	0.836	83.6	6.00
**$Y = 0.2441 - ** 0.067 X_1 + * 0.578 X_2 + 0.917 X_3$	0.877	87.7	4.1
**$Y = 0.434 - * 0.063X_1 + * 0.574 X_2 + 0.995 X_3 - 1.434 X_4$	0.900	90.0	2.3
**$Y = 0.353 - **0.061 X_1 + 0.545 X_2 + 0.963 X_3 - 1.235 X_4 + 0.265 X_5$	0.921	92.1	2.1
^{ns} $Y = - 3.532 - ** 0.073X_1 + * 0.614 X_2 + 0.960 X_3 + 1.03 X_4 + 0.388 X_5 + 0.045 X_6$	0.928	92.8	0.7

** Significant at $P \leq 0.01$, * Significant at $P \leq 0.05$, ns non significant

Where Y = larval population /plant, X_1 = ascorbic acid, X_2 = pH, X_3 = acidity, X_4 = Phenols, X_5 = ash content, X_6 = moisture content

Multivariate regression analysis between biochemical plant factors and fruit infestation revealed maximum individual role for ascorbic acid (83.5%), followed by phenols (5.7%) and pH (4%) regarding percent fruit infestation. The acidity and ash content showed similar contribution of 0.7% while moisture content had no contribution towards percent fruit infestation (Table 6).

Table 6: Multivariate regression models, along with coefficient of determination (R^2) between percent fruit infestation and various biochemical plant factors.

Regression Equation	R^2	100 R^2	Role of individual chemical factor (%)
**$Y = 83.272 - ** 3.031 X_1$	0.835	83.5	83.5
**$Y = 26.807 - **2.359X_1 + 10.353 X_2$	0.875	87.5	4.00
*$Y = 57.333 - 1.778 X_1 + 3.596 X_2 - 20.591 X_3$	0.882	88.2	0.7
**$Y = 97.990 - 0.993 X_1 + 2.722 X_2 - 3.913 X_3 - **306.910 X_4$	0.939	93.9	5.7
*$Y = 89.403 - 0.764 - 0.418 X_1 - 0.385 X_2 - 7.351 X_3 - 285.755 X_4 + 28.037 X_5$	0.946	94.6	0.7
^{ns} $Y = 87.565 - 0.770 X_1 - 0.385 X_2 - 7.352 X3 - 284.683 X_4 + 28.095 X_5 + 0.021 X_6$	0.946	94.6	0.00

** Significant at $P \leq 0.01$, * Significant at $P \leq 0.05$, ns non significant

Where Y = percent infested fruits, X_1 = ascorbic acid, X_2 = pH, X_3 = acidity, X_4 =Phenols X_5 = ash content, X_6 = moisture content

4. Disussion

In addition to physical plant characters, chemistry of host plant also exerts profound effects on insect pest by making host plant less attractive and unsuitable for insect attack. Significant variation was found in the response of tested genotypes Present study revealed that none of the tested genotypes were free from the attack of *H. armigera.* However, the genotypes genotype R 165, GS 5575 and Super classic were found to be most the susceptible while the genotype chinar, Sourabh and sultan were declared as most resistant genotype. Some earlier researchers Sajjad *et al.*, [15] and Ashfaq *et al*. [5] had screened tomato genotypes for resistance against *H.armigera* and found none of the genotypes were completely free from *H. armigera* attack. It was found that higher phenol content in tomato fruits than leaves. Genotype Chinar being resistant on the basis of lower larval population plant^{-1} contain higher phenol content than susceptible genotypes (GS 5575). According to Selvanarayanan and Narayanasamy [16] high phenol content could contribute to the development of resistant variety. Annadurai *et al.* [2] also reported that high concentration of phenols and other secondary metabolites led to resistance against *H. armigera.* Similarly Banerjee and Kallo [6] also found that tomato varieties with high phenol content were resistant to *Heliothis*. In the present study, correlation analysis showed negative correlation of phenols content with both larval population and fruit infestation. Similar results had been reported in previous studies Kashyap and Verma [13], Selvanarayanan and Narayanasamy [16]. Genotype Chinar with higher acidity is considered comparatively more resistant than genotypes with lower acidity. Srivastava and Srivastava [22] also reported that least acidity in leaf is associated with the susceptibility against *H. armigera*. Similarly Selvanarayanan and Narayanasamy [17] reported high acidy content in resistant tomato genotypes than susceptible check. Significantly negative correlation of acidity with both larval population and fruit infestation, was in accordance with the findings of Kashyap and Verma [13] as well as Selvanarayanan and Narayanasamy [17]. The ascorbic acid content was low in tomato leaves than in fruits. The genotype R 165 and GS 5575 were found to be susceptible and genotype Chinar, Sourabh

and Sultan were found to be comparatively resistant to *H. armigera*. High ascorbic acid was found in resistant genotype than susceptible genotype. The present findings are in agreement with Sharma *et al.,* [21] that ascorbic acid, acidity and phenols were negatively correlated with fruit infestation. Moisture percentage in the leaves of all tested tomato genotypes varied significantly and ranged from 65.28 to 68.33% while in tomato fruits, no significant variation was found in moisture content where it ranged from 77.07 to 83.36% in green tomato. Suarez *et al.* [23] also reported non significant results regarding moisture content in tomato genotypes (93.8 to 94.1%) in ripe tomato. The results further indicated that moisture content had no significant effect on larval population and fruit infestation. Ash content was highest in tomato fruits than leaves. The susceptible tomato genotypes GS 5575, R 165 and Super classic contained high ash content than resistant genotypes and had positive correlation with larval population and fruit infestation. Some contradictory results have been reported by Wakil *et al.* [24] that ash content had negative correlation with larval population and pod damage by *H. armigera* in chickpea. The contradictory results may be due to the host plant chemistry other than tomato.

Multivariate regression analysis showed the overall contribution of 92.8 % towards the larval population and 94.6 % towards fruit infestation of *H. armigera* were obtained by the combination of all the biochemical factors characters including ascorbic acid, pH, titratible acidity, phenols, ash and moisture. From the results it was observed that ascorbic acid was the most contributing factor toward resistance against *H. armigera* in tomato crop. Similar studies were conducted by Ashfaq *et al*. [5] but the present findings cannot be compared with the finding of Ashfaq *et al*. [5] because of differences in tomato genotypes and biochemical factors studied. Similar findings have also been reported by Afzal *et al*, [1] that the role of biochemical factors was increased step wise as other factors were added and 100 R^2 value was reached to maximum when all the biochemical factors were studied together.

5. Conclusion

It was concluded that none of the tested genotypes were free from *H. armigera* infestation. However, based on the percent fruit infestation genotype Chinar, Sourabh and Sultan were found to be most resistant while R 165, GS 5575 and Super Classic were declared as the most susceptible and the genotype Mission 102, Bambino and Roma VF were declared as intermediate resistant. Correlation analysis revealed that ascorbic acid, acidity and phenol contents showed negative correlation while pH and ash content showed positive correlation with both larval population and fruit infestation. The non significant negative correlation of moisture content was found with larval population as well as fruit infestation. Multiple regression models showed that ascorbic acid played major role in contribution resistance followed by phenols, acidity while moisture had no contribution towards resistance against *H. armigera* in tomato. Further study is needed to explore the influence of physical plant characters of tested genotypes in relation to resistance against *H. armigera.*

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