



## Research Article

# Bio-chemicals triggering host preference mechanism against tomato fruit borer, *Helicoverpa armigera* (Hübner)

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**ABSTRACT:** Mechanism of host plant resistance in tomato varieties was evaluated and compared against tomato fruit borer, *Helicoverpa armigera* (Hubner) attack in the Solan district known to be 'Tomato bowl of Himachal Pradesh'. Eight varieties utilized for the experiment included three self pollinating indeterminate varieties developed by selection (Solan Lalima, Solan Vajar, Palam Pink) and four hybrids (Naveen 2000+, Heem Sohna, Red Gold, Rakshita Yash Tomato). In order to locate the sources for resistance in tomato foliage various macro and micro- nutrients were extracted from these varieties and chemical composition of tomato fruits viz., content of total phenols, titrable acidity, reducing sugars and total sugars were also estimated to compare for varying levels of resistance to *Helicoverpa armigera*. The content of phenol and sugars in tomato fruits was found to be negatively correlated with fruit infestation with values of correlation coefficient ( $r = - 0.895$ ) and ( $r = - 0.650$ ), respectively, indicating that susceptible varieties were low in phenols as well as in sugars. Nitrogen ( $r = 0.660$ ), potassium ( $r = 0.679$ ), magnesium ( $r = 0.698$ ), iron ( $r = 0.547$ ) and manganese ( $r = 0.546$ ) content were found to be optimistically correlated with per cent fruit infestation while, phosphorous ( $r = - 0.857$ ) and zinc ( $r = - 0.801$ ) content did not favor the fruit borer attack. This observed resistance can be exploited for developing crop cultivars, which readily produce the inducible response upon mild infestation, and can act as important components of integrated pest management compatible with other approaches like biological control, cultural control as well as chemical control.

**KEY WORDS:** Correlation, *Helicoverpa armigera*, micronutrients, resistance, susceptible, tomato

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## INTRODUCTION

In horticulture, quality standards of horticultural products are very high; especially, the visual quality is of utmost importance, for fruits and vegetables that are sold fresh to the market. These stern market requirements have led to the intensive use of pesticides worldwide. Similar is the case with economically as well as nutritionally important crop, tomato. Management of fruit borer through insecticides is hazardous to human being and insecticides reduce insect predator and pollinator species in the environment. Plants must spend their energy and nutrients to grow stems, leaves, roots, and reproductive tissues. When insects attack these tissues, plants must cope up with decreased abilities to convert nutrients and energy into offspring. Some plants can remain healthy and yield well despite the damage due to evolved tolerance. These plants are able to heal wounds by growing compensatory tissues and can reproduce rapidly. Other plants have evolved traits that reduce the consumption by insects call host plant resistance. Plants respond to insect attack through various morphological, biochemical and molecular mechanisms to counter the effects

of herbivore attack. The biochemical mechanisms of defense against the herbivores are wide-ranging, highly dynamic, and are mediated both by direct and indirect defenses. The larvae affect almost all the aerial parts of the tomato plant from the early growth till the fruit maturation stage. Severe infestation causes necrosis to the leaf chlorophylls tissue, suppresses tomato flowers to bloom and makes the mature fruits unfit for consumption (Jallow *et al.*, 2001). Foss and Rieske (2003) suggested that a combination of plant chemical characteristics may be responsible for insect preference and performance, and that an optimal combination of plant components serves to maximize host suitability and are often impacted by leaf characteristics (Simmons *et al.*, 2004). The defensive compounds are either produced constitutively or in response to plant damage, and affect feeding, growth, and survival of herbivores. In addition, plants also release volatile organic compounds that attract the natural enemies of the herbivores. These strategies either act independently or in conjunction with each other. However, our understanding of these defensive mechanisms is still limited. Induced resistance could be exploited as an important tool for the pest management to

minimize the amounts of insecticides used for pest control. Host plant resistance to insects, particularly, induced resistance, can also be manipulated with the use of chemical elicitors of secondary metabolites, which confer resistance to insects. By understanding the mechanisms of induced resistance, we can predict the pests that are likely to be affected by induced responses. The elicitors of induced responses can be sprayed on crop plants to build up the natural defense system against damage caused by herbivores. The induced responses can also be engineered genetically, so that the defensive compounds are constitutively produced in plants against the pests. Induced resistance can be exploited for developing crop cultivars, which readily produce the inducible response upon mild infestation, and can act as one of the components of integrated pest management for sustainable crop production.

In view of after this the study was designed to assess various chemical constituents in different varieties of tomato that can be exploited to impart resistance against fruit borer to produce hybrids with attributes of high yield as well ability to resist tomato borer attack to larger extent.

## MATERIALS AND METHODS

### Varietal screening

In a preliminary field screening of tomato varieties against *H. armigera* by Thakur *et al.* (2018) in the Solan district of Himachal Pradesh, data on number of *H. armigera* eggs and larvae was taken to know the fruit infestation caused by fruit borer in eight different varieties. These varieties included three self pollinating indeterminate varieties developed by selection (Solan Lalima, Solan Vajar, Palam Pink) and four hybrids (Naveen 2000+, Yash Tomato, Rakshita, Heem Sohna, Red Gold,) out of which, Heem Sohna was mostly favored by the farmers for its higher yield and longer shelf life. Nursery grown seedlings of these varieties were transplanted in the field with 15 plants of each entry following prevailing cultural practices to raise the tomato crop in mid hills; with the exception that no chemicals were sprayed on the crop to make the crop fully prone to insects. Plants were monitored weekly starting one week after transplanting. At each observation, the number of *H. armigera* egg and larvae per plant from five randomly-selected plants in each plot were recorded. Once tomato fruit appeared on the plants, the numbers of damaged and undamaged fruits were recorded weekly from five randomly selected plants in each plot to calculate percent of fruit infestation.

### Fruit biochemical analysis

The fruit samples for biochemical analysis of different tomato varieties were collected on the seventh day from the first appearance of tomato fruits. The fruit samples from each

variety were collected from five randomly selected plants of the three replications and assorted to obtain a representative sample. Out of these three fruit samples of each variety thus obtained were washed with distilled water and air dried for biochemical analysis. Titratable acidity was estimated by titrating 5ml of aliquot sample against 0.1N Sodium hydroxide solution using phenolphthalein as an indicator (Ranganna, 1997). For the estimation of acidity 2.5 gm of fruit sample was ground with 25 ml of water, and then filtered to obtain colorless solution. 5 ml of the colourless solution was taken and 1 drop of phenolphthalein was added as indicator. It is then titrated against 0.1 N sodium hydroxide solutions and the titre value was noted at which the solution became colorless. Reducing Sugars were determined by the method of Lane and Eynon (1923). 10 g of pulp was ground in mortar with little amount of water and then transferred to a 100 ml volumetric flask. About 25 ml water and 2 g of sodium oxalate was added followed by 4 ml acetate. Flask was shaken well and kept for 5 minutes. Volume was made up to 100 ml, centrifuged and filtered. The filtrate was taken in a burette. 5 ml of Fehling's A and 5 ml of Fehling's B solution was taken in a conical flask, boiled over a heater and sugar solution was added to it from the burette up to the brick end point using methylene blue as an indicator. Total sugars were estimated by adding 5g of citric acid to 50 ml of calibrated sample (prepared for reducing sugars) and heated for 10 minutes. For complete inversion of sugar samples, NaOH was added. The final volume was made 250 ml using distilled water. Total phenols content was determined by Folin-Ciocalteu procedure described by Mahadevan and Sridhar (1982) at 650 nm by using spectrophotometer. Phenols with phosphomolybdic acid in Folin-Ciocalteu reagent and in alkaline medium produce a highly dark blue coloured complex (molybdenum blue). The intensity of this colour was measured at 650 nm.

The titratable acidity was expressed as per cent citric acid as:-

$$\text{Titratable acidity \%} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Volume made up} \times \text{Eq. weight of Dominating acid} \times 100}{\text{Weight of sample for estimation} \times \text{Volume of aliquot} \times 1000}$$

Per cent reducing sugars and total sugars were calculated as:-

$$\text{Reducing sugars \%} = \frac{\text{*Factor} \times \text{Dilution} \times 100}{\text{Titre value} \times \text{Weight / Volume of sample}}$$

$$\text{Total sugars \%} = \frac{\text{*Factor} \times \text{Dilution} \times 100}{\text{Titre value} \times \text{Weight / Volume of sample}}$$

$$\text{*Factor} = 0.05$$

### Leaf nutrient analysis

Leaf samples for analysis were collected from the

growing shoots of 30-day-old plants as recommended by Selvanarayanan and Narayanasamy (2006). The samples from each variety per replication were collected from more than one plant and mixed to obtain a representative sample for analysis. Cleaning, drying, grinding and storage of samples were carried out under laboratory condition according to the procedure laid down by Chapman (1964). The digestion of the leaf samples for various nutrient elements was done in diacid mixture (nitric acid: perchloric acid 4:1). For nitrogen estimation, a separate digestion was carried out using concentrated H<sub>2</sub>SO<sub>4</sub> and digestion mixture (Jackson, 1967). The methods used to determine the percentage of different minerals in the leaves were: Kjeldhal method for Nitrogen, U V-vis-Spectrophotometer for per cent phosphorous, Flame photometer was used for per cent potassium while, for estimation of per cent copper, manganese, zinc, calcium and magnesium Atomic Absorption Spectrophotometer was used.

### Statistical analysis

Data on chemical characteristics of different varieties of tomato to determine the significance of differences were analyzed by using Randomized Block Design (RBD) - one way Analysis of Variance (ANOVA) as suggested by Gomez and Gomez (1984). In addition to show the interrelationships between tomatoes fruit borer infestation and mean values of each studied bio-chemical character statistical analysis program (SPSS) was used.

## RESULTS AND DISCUSSION

### Varietal infestation screening

There were momentous differences among the eight tomato varieties relating to *H. armigera* egg and larval population per plants such that damage by tomato fruit borer resulted in lower yield. The highest larval count (1.45 larvae/plant) was recorded on 'Heem Sohna' while the lowest larval was recorded on variety 'Solan Lalima' (0.17 larvae/plant) (Fig. 1). The per cent tomato fruit borer infestation varied significantly among the different tomato varieties and ranged from 15.57% (Solan Lalima) to 45.30% (Red Gold). The mean fruit yield/plant of each variety which was evaluated by combining their individual yield obtained after each picking ranged from 3.52 to 0.79 kg/plant. Both characteristics are similarly expressed across the eight varieties (Fig. 2). A rating system for fruit damage developed by Kashyap and Verma (1987) was followed for estimating resistance and susceptibility of selected tomato varieties. None of the screened varieties were completely immune to the attack of *H. armigera*. 'Red Gold' was found to be highly susceptible having infestation more than 40% and 'Palam Pink', 'Yash' and 'Heem Sohna' were found to susceptible with 30.1 to 40%

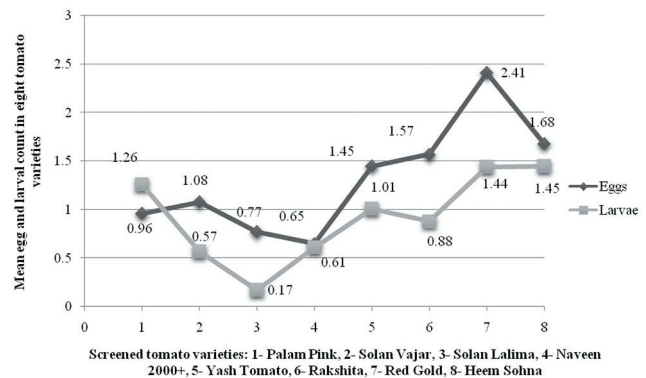


Fig. 1. *Helicoverpa armigera* and larval population in eight varieties at weekly interval of 3rd, 4th, 5th week of May till 1st and 2nd week of June 2017.

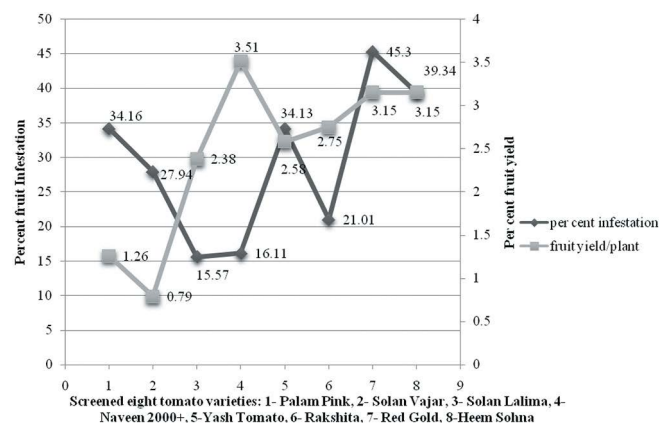


Fig. 2. Per cent fruit borer infestation and tomato fruit yield in eight tomato varieties

fruit damage while 'Solan Lalima', 'Solan Vajar', 'Naveen 2000+' and 'Rakshita' were categorized as moderately or comparatively less susceptible with infestation rate falling between 20.1 to 30.0%.

### Influence of plant and fruit chemical constituents on fruit infestation

#### Fruit chemical analysis

Estimation of various biochemical analyses like titrable acidity, reducing sugars, total sugars and total phenols content were conducted in the eight tomato varieties. Fruit chemical analysis Data on acidity in different tomato varieties have been presented in Table 1 revealed that there was a significant difference among varieties in relation to titratable acidity. Fruits of 'Solan Vajar' (moderately susceptible variety) had the highest citric acid content (0.50%) while the lowest citric acid content (0.24%) was recorded in 'Red Gold' (highly susceptible variety) which was statistically at par with 'Yash' (0.26%) and 'Rakshita' (0.28%). The highest reducing sugars of 2.86% was observed in 'Heem Sohna' (susceptible variety) while the lowest reducing sugars of 1.79% was obtained

in ‘Palam Pink’ and it was at par with ‘Yash’ (1.83%). The maximum total sugars content was recorded in ‘Naveen 2000+’ (4.29%) and minimum in ‘Red Gold’ (3.22) which was highly susceptible, to be at par with ‘Palam Pink’ (3.57%). Total phenols were measured at 650 nm using spectrophotometer.

The data presented in (Table 1) revealed that the total phenols content in different varieties ranged from 0.32 mg/100g in ‘Red Gold’ to 0.85 mg/100g in ‘Solan Lalima’ the later was significant with ‘Naveen 2000+’ (0.84 mg/100g) and ‘Solan Vajar’ (0.82mg/100g). The acid content data when subjected to correlation analysis revealed a non-significant negative correlation ( $r = - 0.302$ ) (Table 2). Our results found support to the results of Singh *et al.* (1982) who also reported non-significant correlation between these two parameters. The correlation between reducing sugars and fruit infestation was found to be negative ( $r = - 0.305$ ) but non-significant (Table 2).

**Influence of leaf nutrient status**

In order to locate the sources for resistance in tomato against *H. armigera* various macro and micronutrients in the foliage of eight tomato varieties were evaluated. Meticulous methods were used to determine the per cent of minerals

**Table 1. Fruit chemical constituents and tomato fruit borer infestation of different tomato varieties tolerant and susceptible to tomato fruit borer infestation**

Average fruit chemical constituents and tomato fruit borer infestation					
Varieties	Per cent citric acid	Per cent reducing sugars	Per cent total sugars	Total phenols (mg/100g)	Per cent fruit infestation
Palam Pink	0.35	1.79	3.57	0.53	34.16 (35.45)
Solan Vajar	0.50	2.49	4.16	0.82	27.94 (30.74)
Solan Lalima	0.34	2.46	4.16	0.85	15.57 (22.76)
Naveen 2000+	0.34	2.52	4.29	0.84	16.11 (23.03)
Yash	0.26	1.83	3.67	0.36	34.13 (34.89)
Rakshita	0.28	2.26	3.80	0.71	21.01 (26.28)
Red Gold	0.24	1.97	3.22	0.32	45.30 (42.20)
Heem Sohna	0.34	2.86	4.24	0.49	39.34 (38.43)
CD (p = 0.05%)	0.04	0.11	0.37	0.02	9.27

\*Figures in parentheses are angular transformed values

**Table 2. Coefficient of correlation between tomato fruit chemical constituents and tomato fruit borer infestation**

Tomato fruit borer infestation	Per cent citric acid	Per cent reducing sugars	Per cent total sugars	Total phenols (mg/100g)
	- 0.302	- 0.350	- 0.650*	- 0.895*

\*  $P \leq 0.05$

in the leaves. The data presented in (Table 3) revealed that leaf nitrogen content in eight tomato varieties varied from 3.78 to 4.24% which is high as compared to the results of Ashfaq *et al.* (2012) who reported nitrogen content ranging from 2.52 to 2.73%. The highest leaf nitrogen (4.24%) content was recorded in ‘Palam Pink’ (susceptible variety) and the least leaf nitrogen (3.78%) content was recorded in ‘Naveen 2000+’ (3.78%) (Moderately susceptible variety). Higher nitrogen content in plants results in more susceptibility towards pest (Minkenber and Ottenheim, 1990). The phosphorous content in the foliage of tomato varieties ranged between 0.37 to 0.46%. These results are similar to Ashfaq *et al.* (2012) who reported phosphorous content in tomato foliage ranged from 0.36 to 0.43%. The highest phosphorus content (0.46%) was recorded in Naveen 2000+ (moderately susceptible variety) which was statistically at par with ‘Solan Vajar’ (0.43%), ‘Solan Lalima’ (0.44%) and ‘Rakshita’ (0.42%) while the lowest leaf phosphorous was obtained in ‘Yash’ (susceptible variety) which was at par with ‘Heem Sohna’ (0.40%), ‘Palam Pink’ (0.39%) and ‘Red Gold’ (0.39%). Potassium content varied from 2.86 to 2.01% with maximum content in ‘Palam Pink’ at par with Red Gold (2.51%), ‘Yash’ (2.46%) and ‘Heem Sohna’ (2.58%). However, the lowest leaf potassium was observed in ‘Naveen 2000 +’ which was statistically at par with ‘Solan Vajar’ (2.17%), ‘Solan Lalima’ (2.32%) and ‘Rakshita’ (2.34%). The highest calcium (0.59%) content was recorded in ‘Rakshita’ (0.59%) (moderately susceptible variety) while the lowest in ‘Heem Sohna’ (0.42%) (susceptible variety). ‘Red Gold’ (highly susceptible variety) recorded the highest magnesium content of 0.87% and ‘Solan Lalima’ and ‘Solan vajar’ had minimum content of 0.70%. The iron content in foliage of tomato varieties ranged from 288.10 to 174.71 ppm while, the content of copper varied from 11.40 to 16.73 ppm. The manganese content in the foliage of tomato varieties varied from 32.96 to 41.72 ppm which is in close proximity to the results of Nicholas (1946) and Sankhyan and Verma (1997) who reported the range of manganese content in tomato foliage to be 10-60 ppm and 31.75-91.30 ppm. The highest leaf zinc content was recorded in ‘Solan Lalima’ (41.73 ppm) while the lowest leaf Zn content was in ‘Yash’ (32.96 ppm). The coefficient of correlation between nitrogen content in foliage and mean fruit infestation was positive and statistically significant ( $r = 0.660$ ) (Table 4). In case of

Phosphorous it was found to be negative and statistically significant ( $r = -0.857$ ). The relationship between potassium content in tomato foliage and mean fruit infestation was significantly positive ( $r = 0.679$ ). The correlation between magnesium content in foliage and mean fruit infestation was found to be positive and significant ( $r = 0.698$ ). In the present study, the iron content among different varieties was found to be positive ( $r = 0.547$ ). The coefficient of correlation between foliage manganese content and mean fruit infestation was found to be positive ( $r = 0.546$ ). For calcium and copper correlation was not significant. The correlation between foliage zinc content and fruit infestation by *H. armigera* was found to be negative and highly significant ( $r = -0.801$ ).

There was noticeable variation in the infestation level of *H. armigera* on the screened tomato varieties which is in line with results of various workers. Ashfaq *et al.* (2012) recorded 1.50 larvae/plant as the highest larval population on tomato hybrids Roma VFN and NARC-1 in Pakistan. Usman and Khan (2012) recorded minimum number of larvae/plant on tomato genotypes ‘Chinar’ (1.52 larvae) and 2.10 larvae on ‘R165’ that had significantly the highest larval population/plant. Discrepancy in fruit damage in our studies and the studies carried out by these workers might be due to the differences in genetic potential or biochemical constituents of tomato varieties that would be the source for HPR. Singh and Narang (1990) found 51.2% fruit damage by *H. armigera* in unsprayed tomato plants in Punjab, while,

Sahu *et al.* (2005) reported 16.29 to 34.77% fruit damage in the different tomato genotypes. These discoveries are in line with our results where infestation level varied from 27.94% to 21.01%.

Less susceptible tomato varieties (Solan Lalima, Solan Vajar, Naveen 2000 + and Rakshita) were found to contain high reducing sugars as compared to susceptible varieties (Red Gold, Palam Pink, Yash) except ‘Heem Sohna’. While, the effect of reducing sugars in tomato cultivars on the infestation of tomato fruit borer has been reported to be positively correlated with per cent fruit borer infestation by various workers (Kashyap and Verma, 1987; Sharma *et al.*, 2008); the reducing sugars in our studies do not seem to play any important role in imparting resistance. The correlation coefficient of total sugars with fruit infestation indicated lower total sugars in susceptible varieties as compared to moderately susceptible. This is in contrary to Kaloo (1986) and Kashyap and Verma (1987) who recorded high concentrations of sugars in the susceptible cultivars against *H. armigera*. These variations might be due to the difference in the time of picking fruits in different varieties. Total phenol contents revealed that *H. armigera* preferred tomato varieties, *viz.*, ‘Red Gold’, ‘Heem Sohna’, ‘Palam Pink’ and ‘Yash’ contain low amounts of total phenols in the fruits as compared to less preferred ones *i.e.*, ‘Solan Vajar’, ‘Solan Lalima’, ‘Naveen 2000+’ and ‘Rakshita’ which had comparatively high amount of total phenols. These results

**Table 3. Leaf nutrient contents and tomato fruit borer infestation of different tomato varieties tolerant and susceptible to tomato fruit borer infestation**

Average leaf nutrient contents and tomato fruit borer infestation										
Varieties	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)	Per cent fruit infestation
Palam Pink	4.24	0.39	2.89	0.54	0.82	248.10	14.96	46.83	33.93	34.16 (35.45)
Solan Vajar	3.87	0.43	2.17	0.47	0.70	222.91	12.90	48.63	38.33	27.94 (30.74)
Solan Lalima	3.88	0.44	2.32	0.43	0.70	216.70	11.40	38.70	41.73	15.57 (22.76)
Naveen 2000+	3.78	0.46	2.01	0.54	0.73	199.80	14.40	39.00	37.43	16.11 (23.03)
Yash	3.93	0.37	2.46	0.49	0.74	234.61	16.73	40.23	32.96	34.13 (34.89)
Rakshita	3.87	0.42	2.34	0.59	0.81	174.71	13.80	47.03	37.86	21.01 (26.28)
Red Gold	4.01	0.39	2.51	0.51	0.87	238.15	12.00	47.40	34.63	45.30 (42.20)
Heem Sohna	4.04	0.40	2.58	0.42	0.78	218.73	15.26	43.03	34.93	39.34 (38.43)
CD ( $P = 0.05\%$ )	NS	0.05	0.44	NS	0.09	NS	NS	NS	NS	9.27

\*Figures in parentheses are Angular transformed values.

**Table 4. Coefficient of correlation between tomato leaf nutrient contents and tomato fruit borer infestation**

Tomato fruit borer infestation	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
	0.660*	-0.857*	0.679*	-0.063	0.698*	0.547*	0.296	0.546*	-0.801*

\*  $P \leq 0.05$

are in agreement with Isman and Duffey (1982) who found inhibition in larval growth of *H. zea* when semi purified extracts of phenolics from tomato foliage were added in the artificial diet of tomato fruit borer and with Banerjee and Kalloo (1989) who reported that *Lycopersicon hirsutum* f. *glabratum* B 6013, a resistant wild species of tomato, had high total phenol (134.22 µg/g) compared to the fruit borer susceptible varieties (24.42 µg/g). Johnson *et al.* (2009) also reported that phenol limits the entry of pests by increasing the leaf toughness that reduces the feeding by herbivores.

The coefficient Johnson *et al.* (2009) of correlation between nitrogen content in foliage and mean fruit infestation was positively significant (Table 4). This is in agreement with the results reported by Ashfaq *et al.* (2012) who reported positive relationship between the two ( $r = 0.65$ ). In case of Phosphorous it was found to be negative and statistically significant which are in line with that of Ashfaq *et al.* (2012) who also reported the per cent fruit infestation to be negatively correlated with phosphorous concentration ( $r = - 0.43$ ). The relationship between potassium content in tomato foliage and mean fruit infestation was affirmative. The results are in disagreement with those of Adam (1986) and Kashyap and Verma (1987) who reported a negative correlation between these two parameters. These variations might be due to high potassium content in our selected varieties which resulted in more number of fruits thus might provided *H. armigera* larvae with wider range of oviposition site and good quality sufficient food leading to higher infestation. The present findings are in agreement with the studies carried out by Sankhyan and Verma (1997) who reported sure relation between the fruit damage and manganese content in tomato foliage. The peak infestation of *H. armigera* in the study area was recorded in the last two weeks of May month and usually its lifecycle occur during the months of March end to may sometimes extend up to June. Alternating the days of planting can be useful to avoid *H. armigera* peak infestation period to some extent, but as tomato is the most economic crop of this area and people prefer to raise 2 crops, i.e., in summer as well as monsoon season, delaying in planting times might cost them one season plus there are losses from diseases in monsoon as well. Also people in hills have small land holdings so skipping or delaying in planting for longer period would result in high losses to the farmers. In that case resistant as well as tolerant varieties can play major role definitely providing for high cost benefit ratio.

The potential utility of host plant resistance in an IPM system is based partly on the assumption that it is compatible with other control tactics. Therefore, observed resistance can be exploited for developing cultivars which could produce the inducible response upon mild infestation, and can be compatible with other approaches like biological control,

cultural control as well as chemical control.

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