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Biochemical constituents of different tomato genotypes responsible for resistance/susceptibility to South American tomato leaf miner, *Tuta absoluta* (Meyrick)

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Abstract
Studies on biochemical constituents of different tomato genotypes were estimated during *Rabi* 2016-17 and 2017-18 to identify the resistance/susceptibility to *Tuta absoluta* (Meyrick). Correlation studies of biochemical constituents with infestation of *T. absoluta* on different tomato genotypes revealed that, the phenol content in the leaves of tomato genotypes was found to be negatively associated with the *T. absoluta* infestation. Moderately resistant genotypes EC-620410, EC-620401 with 10.45, 13.21 per cent infestation on leaflets and 0.83, 1.00 larvae per compound leaf possessed high phenol content 5.27 and 5.28 per cent, respectively as compared to highly susceptible genotype EC-160885 (1.92%) which exhibited maximum infestation on leaflets (37.16%) and 3.40 larvae per compound leaf. The protein content in the leaves was found to be positively associated with the infestation of *T. absoluta* on tomato genotypes. The protein content in the leaves of moderately resistant genotype EC-620410 (1.64%) was significantly lower and in highly susceptible genotype EC160885 (8.32%) was significantly higher. The c orrelation between the reducing sugars and infestation of *T. absoluta* on leaflets, fruits and number of larvae per compound leaf was positive and significant, which indicated that increase in reducing sugar increased the infestation of *T. absoluta*. The lycopene content in fruits was found to be positive and non-significant association with the damage on fruits by *T. absoluta* in tomato genotypes.

Keywords: Biochemical constituents, *Tuta absoluta*, tomato genotypes, correlation

1. Introduction
Tomato production has been fluctuating due to many biotic and abiotic constraints. Prominent among the biotic constraints are pests and diseases which reduce yields and the quality of marketable fruits. The major insect pest complex of tomato includes fruit borer, *Helicoverpa armigera* (Hubner), tobacco caterpillar, *Spodoptera litura* (Fabricius), serpentine leaf miner, *Liriomyza trifolii* (Burgess), whitefly, *Bemisia tabaci* (Gennadius), aphids, *Aphis gossypii* (Glover), mealybugs, *Phenacoccus solenopsis* (Tinsley) and mites, *Tetranychus urticae* (Koch). Recently, South American tomato leaf miner or pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is emerging as major pest and causing extensive damage up to cent per cent yield loss in India particularly under South Indian field conditions. It has been reported from different parts of India throughout the year though the incidence level varies (Sridhar *et al.*, 2014) [28].

T. absoluta is an invasive species commonly known as South American tomato leaf miner, South American tomato pinworm, South American tomato moth and tomato borer. It is considered as one of the most devastating pests of tomato in the countries it has invaded so far. The pest is native to Peru in South America; it has spread to Argentina, Bolivia, Brazil, Chile, Columbia, Ecuador, Paraguay, Uruguay and Venezuela. Since the first detection in Spain in 2006, this pest is spreading rapidly across Southern Europe and North Africa to whole of the Mediterranean countries and in Asia, it is distributed in Bahrain, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Qatar, Saudi Arabia, Syria, Turkey, United Arab Emirates, Yemen (Desneux *et al.*, 2010) [6], India (Shasank *et al.*, 2015) [25], Bangladesh (Hossain *et al.*, 2016) [11] and Nepal (Bajracharya *et al.*, 2016) [3].

In India, *T. absoluta* was first reported during October, 2014 infesting tomato fields in Pune, Ahmednagar, Dhule, Jalgaon, Nashik and Satara districts of Maharashtra (Shashank *et al.*, 2015). Subsequently pest was recorded from Karnataka (Sridhar *et al.*, 2014, Kalleshwaraswamy *et al.*, 2015 and Ballal *et al.*, 2016) [28, 12, 4], Tamil Nadu (Shanmugam *et*

al., 2016 and Ballal *et al.,* 2016) [22, 4] Andhra Pradesh and Telangana (Anitha *et al*., 2015), New Delhi (Shashank *et al.,* 2016) [4], Gujarat (Ballal *et al.,* 2016) [4], Madhya Pradesh (Swathi *et al.,* 2017) [29], Punjab (Sandeep *et al.,* 2017) [18], Meghalaya (Sankarganesh *et al.,* 2017) [19] and Himachal Pradesh (Sharma and Gavkare, 2017) [23] causing severe damage to tomato in invaded areas in India.

T. absoluta attacks the tomato crop from seedling to harvesting stage. Tomato plants are damaged by feeding on leaves, stems, flower buds and both green and ripe fruits by the invasion of secondary pathogens which enters through the wounds caused by the pest (Shasank *et al.,* 2015) [25]. In early infestation, newly emerged neonates penetrate the leaf into the mesophyll layer and feed between the lower and upper surfaces of the leaf to form small and transparent mines. As a result of continuous feeding by the larvae, the irregular mines combine together and eventually form galleries. The mines were filled with black coloured fecal pellets and over time the mined areas turns brown and dry up. In fruits, the larvae tunnel inside and leave only a pinhead size hole visible from outside and make mines just below the surface. More than one hole are seen near to the calyx on fruit. It causes reduction in yield and fruit quality, known to cause 50 to 100 per cent loss under greenhouse and open field conditions. When plants from heavily infested are shaken, adult moths found flying near to ground surface (EPPO, 2005) [10].

Several chemical pesticides are used to control the pest, but none is suitably adapted for management of the tomato leaf miner because of the endophytic habit of larvae, which are protected in the leaf mesophyll or inside fruit, further foliar spray easily wash out by wind and rain (Abbes and Chermiti, 2011 and Guedes and Picanco, 2012) [1, 10]. The endophytic behavior of larvae leads to indiscriminate use of insecticides in the infested fields which results in development of insecticide resistance, pest resurgence, environmental pollution, pesticide residues in fruits, destruction of natural enemy populations and health hazards. To avoid problems caused due to indiscriminate use of insecticides, utilization of Host Plant Resistance (HPR) is an ecologically viable, alternate insect pest management strategy. The use of resistant varieties would be an alternative to chemical control. The study of the mechanisms and causes of resistance to *T. absoluta* is fundamental for the determination of the resistance factors necessary to incorporate into plant breeding programmes for insect resistance and to provide objective parameters for the crosses. It is always agreed that, pest control using resistant tomato varieties is the best and sustainable option (Oliveira *et al.,* 2009) [15]. To our knowledge, there is no longer cultivated variety resistant to *T. absoluta*. The development and cultivation of *T. absoluta* resistant tomato cultivars is very limited in India. Therefore, there is a need to identify the resistant tomato variety to *T. absoluta* and biochemical parameters responsible for resistance.

2. Materials and Methods

Biochemical constituent's *viz.*, phenols, proteins and reducing sugars in leaves and lycopene pigment in fruits of tomato genotypes were estimated for the variations in incidence of tomato leaf miner.

2.1 Biochemical Constituents

The tomato genotypes were subjected to analysis for biochemical constituents in the leaves *viz.*, phenols, proteins and reducing sugars; lycopene pigment in fruits were estimated by using standard procedures at Crop Physiology laboratory, IFT, RARS, Tirupati. The leaf and fruit samples were collected when the tomato leaf miner incidence level was at peak. Leaves and fruits were collected from three replications separately for bio-chemical analysis.

2.1.1 Estimation of phenols

Estimation of phenols content in leaves of tomato genotypes was done as per the method developed by Malick and Singh (1980) [14].

Principle

Phenols react with phosphomolybdic acid in folin-ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue).

Preparation of reagents

Ethanol 80 per cent was prepared by adding 80 ml of absolute alcohol in a beaker and made up to 100 ml by using distilled water.

Sodium carbonate 20 per cent was prepared by adding 20 g sodium carbonate in 100 ml of distilled water.

Preparation of working standards

100 mg catechol dissolved in 100 ml of distilled water and diluted 10 times for working standard, from the working standard different concentrations from 0.1 to 1.0 ml were prepared.

Procedure

From each tomato leaf sample 0.5 g was weighed and grinded in a pestle and mortar, later added 10 times volume of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was collected and residue was re-extracted with five times the volume of 80 per cent ethanol, centrifuged and the supernatants were pooled and evaporated to dryness. The dry residue was dissolved in 5 ml of distilled water and different aliquots 0.2 to 2.0 ml was pipetted to test tubes, making the volume in each tube to 3 ml by adding distilled water. Then 0.5 ml of folin-ciocalteau reagent was added. After 3 min, 1 ml of 20 per cent sodium carbonate solution was added to each tube. The material was mixed thoroughly and tubes were placed in boiling water exactly for 1 min. The tubes were cooled and the absorbance was measured at 650 nm against a reagent blank in spectrophotometer. The standard curve was prepared by using different concentrations of catechol. Catechol concentrations on Y-axis and absorbance values on X-axis were taken for standard curve preparations.

Calculation

From the standard curve, concentrations of phenols expressed in terms of per cent for different tomato genotypes.

2.1.2 Estimation of proteins by Lowry's method

Estimation of protein content in leaves of tomato genotypes was done as per the method developed by Lowry *et al.* (1951) [13].

Preparation of reagents

Reagent-A: Reagent A was prepared by mixing sodium carbonate 2.0 per cent and sodium hydroxide 0.1 N with each other.

Reagent-B: Reagent B was prepared by mixing copper sulphate 0.5 per cent ($CuSO_4$ H_2O) in 1.0 per cent sodium potassium tartarate.

2N sodium hydroxide: 8 g of sodium hydroxide was taken in a beaker and made up to 100 ml with distilled water.

Reagent-C: Alkaline copper solution was prepared by mixing 50 ml of reagent A and 1 ml of reagent B.

Reagent-D: Folin-ciocalteau reagent was mixed with distilled water at a ratio of 1:1.

Preparation of working standards
Fifty milligrams of bovine serum albumin was dissolved in distilled water and the final volume of stock solution was made up to 50 ml in a volumetric flask. From this, 10 ml was taken in another standard flask and volume was made up to 50 ml. From the working standard, solutions of different concentrations of protein were prepared.

Procedure
From each leaf sample 0.5 g was weighed and grinded in pestle and mortar with 5 ml of 10 per cent trichloro acetic acid. The ground material was washed with 5 ml of cold TCA and kept in ice for 15 min. The material was centrifuged at 3500 rpm for 15 min and the supernatant was discarded and the precipitate was dissolved in 4 ml of 2N NaOH. It was allowed to stand for overnight. Then it was centrifuged and supernatant was collected and finally the aliquot was made up to 10 ml.
From this aliquot, 0.1 ml of sample extract was pipette out, to which 5 ml of reagent-C was added. The contents were mixed well and allowed to stand for 10 min. Afterwards 0.5 ml of reagent-D was added, mixed well and incubated for 30 min at room temperature in dark. The colour intensity was read at 660 nm.

Calculation
From the standard curve, concentration of protein expressed as per cent in different tomato genotypes.

2.1.3 Estimation of reducing sugars
Reducing sugar content includes some of the reducing sugars like glucose, galactose, lactose and maltose. Reducing sugar content in leaves of tomato genotypes was estimated as per the method developed by Somogyi (1952) [27].

Preparation of reagents
Reagent A was prepared by mixing 4 ml of copper sulphate solution (15 g of $CuSO_4$ dissolved in a small volume of distilled water and one drop of H_2SO_4 was added then the volume was made up to 100 ml) and 96 ml of alkaline copper tartarate reagent (2.5 g anhydrous Na_2CO_3, 2 g of Na_2HCO_3, 2.5 g of potassium sodium tartarate and 20 g of anhydrous sodium sulphate were dissolved in 80 ml water and made up to 100 ml in a volumetric flask).
Reagent B was prepared by dissolving 2.5 g of ammonium molybdate in 45 ml of distilled water adding 2.5 ml H_2SO_4. Separately 0.3 g of disodium hydrogen arsenate ($Na_2HSO_4.7H_2O$) was dissolved in 25 ml distilled water, and both solutions were mixed and placed in an incubator at 37°C for 24 to 48 hours.

Preparation of working standards
100 mg of glucose was dissolved in 100 ml of distilled water in a volumetric flask to prepare standard glucose stock. Ten ml of stock was diluted to 100 ml in a volumetric flask to prepare working standard.

Procedure
From each leaf sample 100 mg was weighed and grinded with mortar and pestle. Sugars were extracted with 5 ml of hot 80 per cent ethanol twice. The extract was centrifuged at 3500 rpm for 10 min. Supernatant was collected and the ethanol was evaporated by keeping the test tubes in a water bath at 80°C for 3 to 4 hr. Sugars collected at the base of the test tube were dissolved with 5 ml distilled water and thoroughly mixed. Aliquots of 0.5 ml of sample were pipette out in separate test tubes and the volume was made up to 1 ml with distilled water. One ml of reagent A was added to the sample and placed in boiling water bath for 10 minutes. After cooling the test tubes, 1 ml of reagent B was added and the volume was made up to 8 ml with distilled water.

Calculation
The absorbance of the solution was measured in a spectrophotometer at 620 nm. The amount of reducing sugars was estimated using a standard graph prepared with glucose and expressed in terms of per cent in different tomato genotypes.

2.1.4 Estimation of lycopene pigment
The total lycopene pigment content in fruits of tomato genotypes was estimated as per the method developed by Ranganna (1976) [16].

Principle
The lycopene content in the fruit samples was extracted in acetone and then taken up in n-hexane. Lycopene has absorption maximum at 473 nm and 503 nm.

Chemicals required: Acetone 100 per cent, n-hexane
Procedure
Extraction of lycopene
From each fruit 1.0 g of sample (pericarp of ripe tomato) grinded it in pre-chilled pestle and mortar with 10 ml of acetone (100%) and centrifuged at 10,000 rpm for 5 min. Then at low temperature (4^{o}C) collected the supernatant and preserved it. Extracted the residue (pellets) with acetone (3-4 times) by centrifugation and collected the supernatant after each centrifugation until the residue (pellets) becomes colorless. Transferred all the supernatants in a volumetric flask and measured the total volume. While grinding minimum light and low temperature was maintained.

Estimation of lycopene
From each sample 10 ml of the supernatant was taken and added equal volume of n-hexane and the bottom layer of acetone. The bottom layer was discarded. OD value was measured for the upper layer extract at 503 nm wavelength using a spectrophotometer or colorimeter. Blank is prepared by mixing equal volumes of acetone and n-hexane. In a cuvette, this mixture is taken along with 0.5 ml of distilled water this serves as blank. One of the peak absorption values of lycopene was at 470 nm; this wave length is very close to the absorption maximum of carotenoids (480 nm). Therefore,

for quantitative estimation of lycopene, the OD value at 503 nm was taken into consideration.

From the standard curve, concentrations of total lycopene content expressed in terms of µg per gram fresh weight for different tomato genotypes.

Calculation

$$\text{Lycopene (µg/g fresh weight)} = \frac{3.121 \times \text{OD value at 503 nm} \times \text{Volume of smple} \times \text{Dilution factor}}{\text{Fresh weight of sample (g)}} \times 100$$

The biochemical constituents of genotypes were correlated with the South American tomato leaf miner, *T. absoluta* incidence on leaves and fruits to arrive at the relatively resistant and susceptible genotypes of tomato.

3. Results and Discussion

The results obtained on biochemical constituents *viz*., phenols, proteins, reducing sugars in leaves and lycopene content in fruits of different tomato genotypes during *Rabi* 2016-17 and 2017-18 are presented in Tables 1 to 6.

3.1 Biochemical constituents of tomato genotypes during *Rabi* 2016-17

The data on biochemical contents of different tomato genotypes during *Rabi* 2016-17 are presented in Table 1.

3.1.1 Phenol content (%)

The phenol content of all genotypes ranged 1.85 to 5.12 per cent. The highest amount of phenol content was present in EC-620433 (5.12%) followed by EC-620401 (5.04%), EC-620433 (5.02%) and EC-631369 (4.91%). Whereas, the lowest phenol content was recorded in EC-160885 (1.85%) followed by EC-620433 (2.23%), EC-620372 (2.27%) and EC-165700 (2.40%). The phenol content of other genotypes ranged from 2.79 per cent (EC-249514) to 4.91 per cent (EC-631369) (Table 1).

3.1.2 Protein content (%)

The protein content in leaves of different genotypes varied from 1.70 to 8.44 per cent. The highest protein content (8.44%) was recorded in genotype EC-160885. The protein content in other genotypes EC-620433, EC-249514, EC-620372, EC-165700 and EC-620376 were 7.94, 7.44, 7.30, 7.07 and 6.74 per cent, respectively. Whereas, the lowest protein content was recorded in genotype EC-620410 (1.70%) followed by EC-620401(1.90%), EC-620343 (1.94%) and EC-538153 (2.27%) (Table 1).

3.1.3 Reducing sugars (%)

The data on reducing sugars presented in Table 1 indicated that the genotype EC-620410 contained lowest amount of reducing sugars (2.14%) and it was followed by EC-631369 (2.22%), EC-620343 (2.24%) and EC-620401 (2.29%) while the leaves of EC-160885 had higher amounts of reducing sugars (4.52%) followed by EC-620372 (4.43%) and EC-620433 (4.38%).

3.1.4 Lycopene content (µg/g fresh weight)

The quantity of lycopene content in different genotypes varied from 116.52 to 475.43 µg/g fr.wt. The genotype EC-620372 possessed highest amount of lycopene (475.43 µg/g fr.wt**)** followed by EC-620401 (415.09 µg/g fr.wt), EC-620427 (405.73 µg/g fr.wt) and EC-620392 (398.45 µg/g fr.wt). However, the genotype EC-620394 (116.52µg/g fr.wt) showing lesser amount of lycopene content as compared to other genotypes (Table 1).

Table 1: Biochemical constituents in leaves (Phenols, proteins and reducing sugars) and fruits (lycopene) of different tomato genotypes during *Rabi* 2016-17

Genotype	Phenols (%)	Proteins (%)	Reducing sugars (%)	Lycopene (µg/g fr.wt)
EC-620410	5.02	1.70	2.14	141.49
EC-538156	3.23	6.60	3.77	184.14
EC-620395	2.94	6.24	3.27	330.83
EC-620372	2.27	7.30	4.43	475.43
EC-165700	2.40	7.07	3.85	202.87
EC-620147	4.39	6.64	3.60	267.37
EC-567305	2.86	5.04	3.14	181.02
EC-620433	2.23	7.94	4.38	308.98
EC-160885	1.85	8.44	4.52	352.67
EC-620397	3.70	5.37	3.17	381.80
EC-620406	3.26	5.87	3.32	237.20
EC-249514	2.79	7.44	3.83	303.78
EC-620394	3.74	3.34	2.61	116.52
EC-165690	3.44	3.90	2.89	203.91
EC-620376	2.94	6.74	4.14	240.32
EC-631379	4.39	2.97	2.68	255.92
EC-620401	5.04	1.90	2.29	415.09
EC-620392	3.51	4.10	2.97	398.45
EC-249508	3.80	4.27	3.03	146.69
EC-164563	3.70	5.60	3.11	155.01
EC-521067-B	3.84	5.87	3.42	177.90
EC-164577	4.56	2.64	2.61	342.27
EC-620343	5.12	1.94	2.24	337.07
EC-620382	3.47	4.00	3.18	247.60
EC-620370	4.62	3.14	2.42	379.72
EC-620396	3.78	3.14	2.63	253.84
EC-538153	3.57	2.27	2.40	277.77
EC-620422	3.55	2.44	2.31	353.71
EC-620427	3.76	3.84	2.85	405.73
EC-631369	4.91	2.30	2.22	118.60
SE(m)	0.187	0.140	0.066	10.888
CD (p=0.05)	0.530	0.399	0.188	30.903

Each value of three replications

3.2 Biochemical Constituents of Tomato Genotypes during *Rabi* 2017-18

The data on biochemical contents of different tomato genotypes during *Rabi* 2017-18 are presented in Table 2.

3.2.1 Phenol content (%)

The phenol content of all genotypes ranged 2.00 to 5.52 per cent and differences in phenol content among the genotypes were significant. The highest amount of phenol content was recorded in genotype EC-620410 (5.52%) followed by EC-620343 (5.44%), EC-620401 (5.40%) and EC-631369 (5.27%) whereas, the lowest phenol content was present in EC-160885 (2.00%) followed by EC-620372 (2.02%), EC-620433 12082 (2.48%) and EC-620376 (2.50%). The phenol content of other genotypes ranged from 2.56 per cent (EC-249514) to 4.93 per cent (EC-538153) (Table 2).

3.2.2 Protein content (%)

The difference in the protein content in leaves among the

genotypes was significant and the highest protein content (8.20%) was recorded in EC-160885 and it was on par with EC-620372 (7.67%). The protein content in other genotypes EC-620433, EC-620376, EC-249514, EC-165700 and EC-538156 were 7.47, 7.20, 6.94, 6.67 and 6.30 per cent, respectively. The lowest protein content (1.57%) was recorded in EC-620410 and was on par with EC-620343 (1.70%), EC-620401(1.74%) and EC-631369 (1.97%).The protein content of other genotypes ranged from 2.07 per cent (EC-538153) to 6.24 per cent (EC-620147) (Table 2).

3.2.3 Reducing sugars (%)

Reducing sugar content of genotypes indicated that there was significant difference among the genotypes and the genotype EC-620410 contained lower amount of reducing sugars (2.03%) and it was followed by EC-620343 (2.11%), EC-631369 (2.15%) and EC-620401 (2.18%), while the leaves of EC-160885 had higher amounts of reducing sugars (4.40%) followed by EC-620372 (4.25%) and EC-620433 (4.17%) which were on par with each other (Table 2).

3.2.4 Lycopene content (µg/g fresh weight)

The differences in the quantity of lycopene content in different genotypes were significant and the genotype EC-620372 possessed highest amount of lycopene (501.44 µg/g fr.wt**)** followed by EC-620401 (440.06 µg/g fr.wt), EC-620392 (423.42 µg/g fr.wt), EC-620427 (423.42 µg/g fr.wt) and EC-620370 (393.25 µg/g fr.wt). However, the genotype EC-620394 (99.87 µg/g fr.wt) showing lesser amount of lycopene content as compared to other genotypes (Table 2).

Table 2: Biochemical constituents in leaves (Phenols, proteins and reducing sugars) and fruits (lycopene) of different tomato genotypes during *Rabi* 2017-18

Genotype	Phenols (%)	Proteins (%)	Reducing sugars (%)	Lycopene (µg/g fr. wt)
EC-620410	5.52	1.57	2.03	133.16
EC-538156	3.09	6.30	3.63	169.57
EC-620395	3.15	5.97	3.45	351.63
EC-620372	2.02	7.67	4.25	501.44
EC-165700	2.73	6.67	3.74	190.38
EC-620147	4.89	6.24	3.42	282.97
EC-567305	3.82	4.67	3.06	171.66
EC-620433	2.48	7.47	4.17	331.87
EC-160885	2.00	8.20	4.40	331.87
EC-620397	3.44	5.77	3.35	410.93
EC-620406	3.68	5.37	3.14	223.67
EC-249514	2.56	6.94	3.74	291.29
EC-620394	4.03	3.17	2.74	99.87
EC-165690	3.93	3.60	2.78	184.14
EC-620376	2.50	7.20	3.96	223.67
EC-631379	4.68	2.77	2.54	265.29
EC-620401	5.40	1.74	2.18	440.06
EC-620392	3.93	3.77	2.82	423.42
EC-249508	3.89	4.10	2.89	137.32
EC-164563	3.82	5.17	3.24	138.36
EC-521067-B	3.51	5.50	3.29	161.25
EC-164577	4.89	2.44	2.42	328.75
EC-620343	5.44	1.70	2.11	318.34
EC-620382	3.84	4.50	3.00	265.29
EC-620370	4.75	2.60	2.50	393.25
EC-620396	4.14	2.97	2.46	242.40
EC-538153	4.93	2.07	2.29	268.41
EC-620422	3.97	2.20	2.43	326.66
EC-620427	3.97	3.47	2.71	423.42
EC-631369	5.27	1.97	2.15	105.07
SE(m)	0.316	0.129	0.049	10.129
CD (p=0.05)	0.896	0.367	0.139	28.749

Each value of three replications

3.3 Biochemical Constituents of Tomato Genotypes during *Rabi* 2016-17 and 2017-18 (Pooled Data)

The pooled data on biochemical constituents of different tomato genotypes for two consecutive years of *Rabi* 2016-17 and 2017-18 are presented in Table 3.

3.3.1 Phenol content (%)

The pooled data presented in Table 3 revealed that the phenol content of different genotypes ranged 1.92 to 5.28 per cent. The highest amount of phenol content (5.28%) was recorded in genotype EC-620343 followed by EC-620410 (5.27%), EC-620401 (5.22%) and EC-631369 (5.09%) while, the lowest phenol content was recorded in EC-160885 (1.92%) followed by EC-620372 (2.14%), EC-620433 (2.35%) and EC-165700 (2.56%). The phenol content of other genotypes ranged from 2.68 per cent (EC-249514) to 4.73 per cent (EC-164577).

3.3.2 Protein content (%)

The perusal pooled data on protein content of different genotypes showed that significantly lowest protein content (1.64%) was observed in genotype EC-620410 whereas, the highest protein content was recorded in genotype EC-160885 (8.32%) followed by EC-620433 (7.70%), EC-620372 (7.49%), EC-249514 (7.14%) and EC-620376 (6.97%). The protein content of other genotypes ranged from 1.82 per cent (EC-620343 and EC-620401) to 6.87 per cent (EC-165700) (Table 3).

3.3.3 Reducing sugars (%)

The results of pooled data on reducing sugar content in genotypes presented in Table 3 indicated that the genotype EC-620410 contained lower amount of reducing sugars (2.08%) followed by EC-620343 (2.18%), EC-631369 (2.19%) and EC-620401 (2.24%), while the genotype EC-160885 had higher amounts of reducing sugars (4.46%) followed by EC-620372 (4.34%), EC-620433 (4.27%) and EC-620376 (4.05%).

3.3.4 Lycopene content (µg/g fresh weight)

Lycopene content in different genotypes revealed that the genotype EC-620372 possessed highest amount of lycopene (488.44 µg/g fr.wt**)** followed by EC-620401 (427.58 µg/g fr.wt), EC-620427 (414.57 µg/g fr.wt) and EC-620392 (410.93 µg/g fr.wt). However, the genotype EC-620394 (108.19 µg/g fr.wt) found lowest amount of lycopene content followed by EC-631369 (111.84 µg/g fr.wt), EC-620410 (137.32 µg/g fr.wt) and EC-249508 (142.01 µg/g fr.wt) (Table 3).

Table 3: Biochemical constituents in leaves (Phenols, proteins and reducing sugars) and fruits (lycopene) of different tomato genotypes during *Rabi* 2016-17 and 2017-18 (Pooled Data)

Genotype	Phenols (%)	Proteins (%)	Reducing sugars (%)	Lycopene (µg/g fr. wt)
EC-620410	5.27	1.64	2.08	137.32
EC-538156	3.16	6.45	3.70	176.86
EC-620395	3.05	6.10	3.36	341.23
EC-620372	2.14	7.49	4.34	488.44
EC-165700	2.56	6.87	3.79	196.62
EC-620147	4.64	6.44	3.51	275.17
EC-567305	3.34	4.85	3.10	176.34
EC-620433	2.35	7.70	4.27	320.42
EC-160885	1.92	8.32	4.46	342.27
EC-620397	3.57	5.57	3.26	396.37
EC-620406	3.47	5.62	3.23	230.43
EC-249514	2.68	7.19	3.79	297.54
EC-620394	3.89	3.25	2.68	108.19
EC-165690	3.69	3.75	2.83	194.02
EC-620376	2.72	6.97	4.05	231.99
EC-631379	4.54	2.87	2.61	260.60
EC-620401	5.22	1.82	2.24	427.58
EC-620392	3.72	3.94	2.90	410.93
EC-249508	3.84	4.19	2.96	142.01
EC-164563	3.76	5.39	3.18	146.69
EC-521067-B	3.68	5.69	3.36	169.57
EC-164577	4.73	2.54	2.52	335.51
EC-620343	5.28	1.82	2.18	327.71
EC-620382	3.65	4.25	3.09	256.44
EC-620370	4.68	2.87	2.46	386.48
EC-620396	3.96	3.05	2.54	248.12
EC-538153	4.25	2.17	2.35	273.09
EC-620422	3.76	2.32	2.37	340.19
EC-620427	3.86	3.65	2.78	414.57
EC-631369	5.09	2.14	2.19	111.84
SE(m)	0.222	0.095	0.047	8.939
CD (p=0.05)	0.629	0.270	0.133	25.369

Each value of three replications

3.4 Correlation of Biochemical Constituents of Different Tomato Genotypes against Infestation of *T. absoluta* during *Rabi* 2016-17 and 2017-18

Correlation studies of biochemical constituents with infestation of *T. absoluta* on different tomato genotypes are presented hereunder (Tables 4 to 6).

The phenol content in the leaves of tomato genotypes found to be negatively associated with the *T. absoluta* infestation. Moderately resistant genotypes, EC-620410, EC-620401 with 10.45, 13.21 per cent infestation on leaflets and 0.83, 1.00 larvae per compound leaf possessed high phenol content 5.27 and 5.28 per cent, respectively as compared to highly susceptible genotype EC-160885 (1.92%) which exhibited maximum infestation on leaflets (37.16 %) and 3.40 larvae per compound leaf.

The protein content in the leaves exhibited positively associated with the infestation of *T. absoluta* on tomato genotypes. The protein content in the leaves of moderately resistant genotype EC-620410 (1.64%) was significantly lower and in highly susceptible genotype EC-160885 (8.32%) was significantly higher.

The correlation between the reducing sugars and per cent infestation on leaflets, fruits and number of larvae per compound leaf was positive and significant, which indicated that increase in reducing sugar and increased infestation of *T. absoluta*.

The reducing sugar content in the leaves was found to be positively associated with the infestation of *T. absoluta* on tomato genotypes. The reducing content in the leaves of moderately resistant genotypes EC-620410 (2.08%) and EC-620343 (2.18%) were significantly lower whereas in highly susceptible genotypes EC-160885 (4.46%) and EC-620372 (4.34%) significantly higher.

The lycopene content in fruits was non-significant and positively correlated with the damage on fruits by *T. absoluta* in tomato genotypes.

The present investigations are in close agreement with the findings of Benerjee and Kalloo (1989) [5], Selvanarayanan

(2000) [21], Dhakshinamoorthy (2002) [7], Selvanarayanan and Narayanasamy (2006) [22] and Gopalakrishnan (2006) and Usman *et al.* (2015) [30] who reported as high phenol content in tomato leaves imparted resistance to fruit borer *H. armigera*. Rath and Nayak (2007) [17] found that less protein content was responsible for the low susceptibility of tomato varieties to fruit borer especially in genotype BT 10 and BT 12. Similar results obtained by Sharma *et al.* (2008) [24] who reported as reducing sugars showed positive correlation while total phenol exhibited negative correlation with fruit infestation of *H. armigera*. Dias *et al.* (2013) [8] and Firdaus *et al.* (2013) [9] found that tomato genotypes with high contents of acyl sugars were more effective in reducing the damage caused by the tomato pinworm, *T. absoluta*.

The present investigation clearly suggested that tomato genotypes with more phenols and less proteins and reducing sugars in leaves less damage on leaflets and fruits by *T. absoluta*. Therefore, these biochemical leaf traits can be used as marker to identify the resistance sources of tomato pinworm with different mechanism of resistance against *T. absoluta*. This finding can be used very effectively in *T. absoluta* resistant breeding programmes.

Table 4: Influence of phenol content in leaves of different tomato genotypes with infestation of South American tomato leaf miner, *T. absoluta* on leaflets, fruits and number of larvae per compound leaf during *Rabi* 2016-17 and 2017-18

S. No.	Variable	Correlation Coefficient	Regression equation	R^2 Value
a.	Phenols (x) Vs per cent infestation on leaflets (y)	-0.83**	Y= 40.26-5.47 x	0.69
b.	Phenols (x) Vs number of larvae/compound leaf (y)	-0.88**	Y= 4-0.60 x	0.78
c.	Phenols (x) Vs per cent damage on fruits (y)	-0.87**	Y= 57.66-7.24 x	0.76

** Significant at 0.01 level

Table 5: Effect of protein content in leaves of different tomato genotypes with infestation of South American tomato leaf miner, *T. absoluta* on leaflets, fruits and number of larvae per compound leaf during *rabi* 2016-17 and 2017-18

S. No.	Variable	Correlation Coefficient	Regression equation	R^2 Value
a.	Proteins (x) Vs per cent infestation on leaflets (y)	0.85**	Y= 8.08 + 2.55 x	0.72
b.	Proteins (x) Vs number of larvae/compound leaf (y)	0.93**	Y= 0.43 + 0.28 x	0.87
c.	Proteins (x) Vs per cent damage on fruits (y)	0.96**	Y= 13.95 + 3.62 x	0.92

** Significant at 0.01 level

Table 6: Influence of reducing sugars in leaves and lycopene content in fruits of different tomato genotypes with infestation of South American tomato leaf miner, *T. absoluta* on leaflets, fruits and number of larvae per compound leaf during *rabi* 2016-17 and 2017-18

S. No.	Variable	Correlation coefficient	Regression equation	R^2 value
a.	Reducing sugars (x) Vs per cent infestation on leaflets (y)	0.90**	Y= -4.84 + 8.00 x	0.81
b.	Reducing sugars (x) Vs number of larvae/compound leaf (y)	0.96**	Y= -0.95 + 0.88 x	0.92
c.	Reducing sugars (x) Vs per cent damage on fruits (y)	0.94**	Y= -2.20 + 10.64 x	0.89
d.	Lycopene (x) Vs per cent damage on fruits	0.08 NS	Y= 28.85 + 0.00 x	0.00

NS Non Significant

** Significant at 0.01 level

4. Conclusions

Correlation studies of biochemical constituents with infestation of *T. absoluta* on different tomato genotypes revealed that, the phenol content in the leaves of tomato genotypes was found to be negatively associated with the *T. absoluta* infestation. Moderately resistant genotypes EC-620410, EC-620401 with 10.45, 13.21 per cent infestation on leaflets and 0.83, 1.00 larvae per compound leaf possessed high phenol content 5.27 and 5.28 per cent, respectively as compared to highly susceptible genotype EC-160885 (1.92%) which exhibited maximum infestation on leaflets (37.16%) and 3.40 larvae per compound leaf. The protein content in the leaves was found to be positively associated with the infestation of *T. absoluta* on tomato genotypes. The protein content in the leaves of moderately resistant genotype EC-620410 (1.64%) was significantly lower and in highly susceptible genotype EC-160885 (8.32%) was significantly higher.

The correlation between the reducing sugars and infestation of *T. absoluta* on leaflets, fruits and number of larvae per compound leaf was positive and significant, which indicated that increase in reducing sugar increased the infestation of *T. absoluta*. The lycopene content in fruits was found to be positive and non-significant association with the damage on fruits by *T. absoluta* in tomato genotypes.

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