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Bio chemical profiling to identify resistant guava varieties against *Meloidogyne enterolobii*

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Abstract
Infestation of *M. enterolobii* alters the physiology of the host plant and reduces the yield of the crop. Thus studies were undertaken to know the biochemical changes in plants infested with *M. enterolobii* under controlled conditions. With regard to biochemical analysis, there were significant difference in total chlorophyll, total phenol, total sugar, total proline, total protein content and activity of enzymes like Peroxidase and Poly phenol oxidase in resistant and susceptible varieties. Resistant varieties like Strawberry guava, Banaras, Arka Mridula and species like *P. cattleianum*, *P. guineense* showed increased content of phenols, proline, protein, peroxidase and polyphenol oxidase activity from healthy to infected plants compared to susceptible varieties such as Lucknow- 49, Bapatla, Taiwan pink, Allahabad safeda, Luaknow-46 and Arka Amulya. There was a significant increase in the bio chemical characters except chlorophyll content due to inoculation of nematodes in both resistant and susceptible varieties. The identified variety with resistance to root knot nematodes combined with high yield and processing qualities could be exploited for future purposes.

Keywords: Total sugars, phenols, proline, protein, chlorophyll content, peroxidase

Introduction
Guava (*Psidium guajava* L.) is one of the important commercial fruits in India, known as poor man's fruit. It belongs to the family Myrtaceae, comprising large number of fruit yielding trees (Pereira and Nachitgal, 2002) [16]. It is the fourth most important fruit after mango, banana and citrus. Guava is native to South America and the West Indies. It is also grown other parts of the tropics and subtropics including India where it is considered to be important hardy crop grown in neglected soil. The cultivated area of Guava in India 261 MHa with an annual production of 3961MT with a productivity of 13.9MT/Ha. Likewise the area and production of guava in Tamilnadu is 9.78Mha and 77.41MT respectively (NHB Data Base 2017-18) [15]. Guava producing states in India are Punjab, Gujarat, Bihar, Madhya Pradesh, Tamilnadu whereas districts of Uttar pradesh has a reputation of growing the best guava in the country as well as in the World.

Guava is prone to attacked by a range of diseases from root to the crown and fruits due to wide range of variation in the climatic conditions. Number of plant pathogens including fungi, bacteria, algae and nematodes, are found to cause various types of diseases. Among them plant parasitic nematodes *Meloidogyne enterolobii* which is a polyphagous and highly adapted obligate plant pathogens and distributed worldwide, parasitizing nearly every species of higher plants (Williamson and gleason, 2003) [30].

M. enterolobii is an emerging problem in guava and has been reported from Tamil Nadu in recent years and widely spreading across the Country (Poornima *et al.*, 2016). *M. enterolobii* is a species of root knot nematode having a synonym as *Meloidogyne mayaguensis*, considered that it might have spread from other countries through saplings. Several internal factors are responsible for plant host resistance, during post infection period. In diseased conditions the sequences of biochemical changes is rather more important than the external symptoms, which are nothing but the manifestation of the internal disorders.

Biochemical changes induced by plant parasitic nematodes relating to various crops have been well reported by many authors (Mohanty *et al.*, 2001, Farooqi, *et.al*., 1988, Sundar raj and Meheta, 1991) [11, 3, 26]. In response to root-knot nematode infection, a series of biochemical and physiological reactions occur in host plants as a result of which, the plant is either overcome by the nematode or localized by the plant and disease development is limited. Detailed characterization of this biochemical analysis is essential to advance our understanding of plant-nematode interaction. Present investigation has been carried out to study the Bio chemical changes in different guava varieties by inoculation with reference to chlorophyll content, total proteins, total phenols, proline and total sugars.

Materials and methods

In order to understand the basis of nematode resistance, guava varieties namely Lalit, Banaras, Lucknow-49, Lucknow-46,Taiwan pink, Taiwan white, Allahabad safeda, Hafsi, Bapatla, Black guava, Arka Kiran, Arka Poorna, Arka Reshmi, Arka Mridula, Arka Amulya, and three species namely *Psidium chinensis, Psidium guineense* and *Psidium cattleianum var. cattleianum* were grown in earthen pot with sterilized soils in the greenhouse of Horticulture college and research institute, Periyakulam and were arranged in completely randomized design with 3 replications.

Nematode inoculum

Egg masses from single female were put into a beaker of tap water for 2-3 days. After complete hatching the nematodes were inoculated in tomato plants planted in sterile pot mixture.

Inoculation of nematodes

Egg mass from pure culture of *M. enterolobii* was kept for hatching. Population of hatched J2 were estimated by counting them under counting dish and known population of nematode was inoculated in each pots @ 1J2/g of soil.

Biochemical analysis of root knot nematode tolerance

Estimation of Phenol in Leaf

The phenol content was estimated following the procedure described by Bray and Thorpe 1954. Leaf sample (one g) was homogenized in 10 ml of 80 percent ethanol. The homogenate was then centrifuged at 10,000 rpm for 20 min, supernatant was saved and the residue was extracted with 5 times the volume of 80 per cent ethanol and centrifuged as above. The supernatant was saved and evaporated to dryness in a boiling water bath. The residue was dissolved in 5 ml of distilled water. An aliquot of 0.2 ml was pipetted out and made up to 3 ml with distilled water. Folin-Ciocalteau reagent (0.5 ml) was added and 2 ml of 20 per cent sodium carbonate solution was added to each tube after 3 min. This was mixed thoroughly and kept in boiling water for 1 min. The reaction mixture was cooled and absorbance was measured at 650 nm against a reagent blank. Standard curve was prepared using different concentrations of catechol. Phenol content was expressed as mg catechol equivalent of enzyme activity per g leaf tissue on fresh weight basis.

Estimation of Protein content

The protein content of the leaf was estimated by Bradford's method. One gram sample was macerated with phosphate buffer solution and the volume was made up to five ml. The extract was centrifuged at 3000 rpm for 10 minutes. After centrifugation, supernatant solution was taken and equal volume acetone was added. The supernatant was refrigerated for one hour and centrifuged again at 5000 rpm for 15 minutes. The pellet was collected and stored in 0.1 N NaOH for one day. 0.2 ml of sample was made up to one ml by using 0.8 ml of phosphate buffer and then 5 ml of Bradford's dye was added. Blue color was developed and the optical density of the clear supernatant solution was determined at 595 nm using spectrophotometer against the blank. Protein content was calculated and expressed in mg g^{-1} by using the following the formula.

$$\text{Protein content (mg g}^{-1}) = \frac{\text{OD value of test sample x 0.2 x 1}}{\text{OD value of standard x 5 x 1000}}$$

Chlorophyll content

Chlorophyll 'a', 'b' and total chlorophyll content was estimated by following the method of Yoshida *et al*. (1971). A representative sample of leaf tissue 250 mg was weighed and grounded with 10 ml of 80 per cent acetone using a pestle and mortar. The homogenized sample was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and volume was made up to 25 ml with 80 per cent acetone. The OD value of the extract was measured at 663, 652 and 480 nm using 80 per cent acetone as the blank in the spectrophotometer. The amount of pigments was calculated using the following formula and expressed in mg of pigments g^{-1} of leaf (on fresh weight basis).

Chlorophyll a = [12.7(OD at 663) – 2.69 (OD at 645)] x V/ W x 1000 mg g^{-1}

Chlorophyll b = [22.9 (OD at 645) – 4.68 (OD at 663)] x V/ W x 1000 mg g^{-1}

Total chlorophyll = [1000 (OD at 652)/34.5] x V/ W x 1000 mg g^{-1}

Where, V – Final volume of chlorophyll extract in 80 per cent acetone

W – Fresh weight of leaves extracted (g).

Estimation of Peroxidase (PO) in Leaf

Peroxidase activity was assayed by a spectrophotometric method as described by Srivastava (1987). Leaf sample of one g was homogenized in 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone was added. The homogenization was done at 4 0C using a pre-chilled mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 min at 4 0C. The supernatant was used as the enzyme extract for the assay of PO activity.

The reaction mixture consisting of one ml of 0.05 µl pyrogallol and 50 µl of enzyme extract was taken in both the reference and the sample cuvettes, mixed and kept in a spectrophotometer (systronics UV- Vis spectrophotometer 118) and the reading was adjusted to zero at 420 nm. To initiate the reaction, one ml of one percent hydrogen peroxide (H2O2) was added to the sample cuvettes and the changes in absorbance were recorded at 30 seconds interval up to 180 sec. The PO activity was expressed as changes in absorbance min-1 g-1 of tissue on fresh weight basis.

Estimation of Polyphenol Oxidase (PPO)

Peroxidase activity was determined as per the procedure given by Mayer *et al.* (1965). Leaf sample of one g was homogenized in 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 4 0C using a pre-chilled mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 min at 4 0C. The supernatant was used as the enzyme extract for the assay of PPO activity. The reaction mixture contained one ml of 0.1 M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract. The reaction was initiated after adding one ml of 0.01 M catechol. The observations were recorded in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). The change in absorbance was recorded at 495 nm at 30 seconds interval up to 180 sec. PPO activity was expressed as change in the absorbance of the reaction mixture min-1 g-1 of tissue on fresh weight basis.

Estimation of Total sugars

The sugar content in both inoculated and inoculated leaves

was estimated by anthrone method (Sadasivam and Manickam, 2008) and expressed in mg/g. Leaf sample of 0.5 g was homogenized in hot 80 per cent ethanol to remove sugars and centrifuged and retained the residue. The residue was repeatedly washed with hot 80 per cent ethanol till the washing does not give colour with anthrone reagent. The residue was dried over a water bath. To that residue 5 ml of water and 6.5 ml of 52 per cent perchloric acid were added and extracted at 0ºC for 20 min. It is then centrifuged and supernatant was collected. The procedure was repeated using fresh perchloric acid and the supernatant was pooled and made up to 100 ml. Pipette out 0.1 or 0.2 ml of the supernatant and the volume was made up to one ml with water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and made up the volume to 1 ml in each tube with water. 4 ml of anthrone reagent was added to each tube and heated for eight minutes in a boiling water bath. Then it was cooled and the intensity of green to dark green colour was measured at 630 nm.

$$\text{Amount of sugars in sample (\%mg)} = \frac{\text{sugar value from graph (mg)}}{\text{Aliquot sample used}} \text{ X } \frac{\text{Total vol of extract (ml)}}{\text{wt of Sample (mg)}}$$

Estimation of Proline (mg/g):

Total proline content was determined by the ninhydrin method described by Spies (1957) [24]. Homogenize 0.5g of tissue in a pestle and mortar with 10 ml of 3% acqueous sulphosalicylic acid and filter through whatmann No.2 filter paper, by repeating the same pool the filtrates. To 2 ml of filtrate, add 2ml of glacial acetic acid and Ninhydrin and mix. Keep in hot water bath for 1 hr and then terminate reaction by placing on ice bath and add 4 ml toluene, mix vigorously for 20-30 sec. Aspirate the toluene layer and warm to room temperature. Measure the absorbance of red color at 520 nm against a reagent blank.

Calculation

$$\mu \text{ Moles of proline/g tissue} = \frac{\mu\text{g proline / ml x ml Toluene x 5}}{115.5}$$

Results

Total chlorophyll content

Chlorophyll content is the most important constituent of the plants as it manufactures the food and also useful for the growth and development of all the parts of plant. Root-knot nematodes are known to reduce the chlorophyll contents of the plants by disrupting its nutrient uptake and partitioning of the photosynthates. In the present study it was observed that the chlorophyll content reduced from1.91 to 1.68 and 1.96 to 1.44 in strawberry guava and *P. cattleianum* respectively of both inoculated and uninoculated plants. Similarly in local varieties like PKM local, Andhra local, Ayakudi local it got reduced from 2.21 to 1.12, 1.7 to 0.96 and 2.14 to 1.31 resectively. The susceptible varieties, Allahabad safeda, Lucknow-46, Bapatla, Arka Amulya shown reduction of chlorophyll content from 2.06 to 1.11,0.99 to 0.81, 1.06 to 0.87 and 2.01 to 0.74 respectively given in Table 1.

In case of chlorophyll a content reduction from healthy to infected plants is 1.25 to 1.05, 0.27 to 0.14 in strawberry guava and *P. cattleianum.* Similarly in susceptible varieties Arka Amulya, Lucknow- 46, Allahabad safeda, Lucknow-49, Taiwan pink shown reduction from 0.72 to 0.54, 1.02 to 0.25, 0.72 to 0.54, 1.15 to 0.79, 1.52 to 0.93 respectively.

In case of chlorophyll b content reduction from healthy to infected plants is 1.29 to 1.13, 1.31 to 1.05, 0.91 to 0.80 in *P. cattleianum,* Arka mridula and Arka kiran respectively. Similarly *P. guineense*, Strawberry guava, local varieties like PKM local, Ayakudi local shown reduction from 0.98 to 0.57, 0.84 to 0.71, 0.77 to 0.61 and 0.69 to 0.22 respectively.

Total Sugars

The total sugar content decreased in the susceptible varieties from healthy to infected i.e., 26.1 to 23.41, 11.09 to 11.07, 11.92 to 11.89, 11.81 to 10.32, 10.92 to 10.81 respectively. But varieties and species such as strawberry guava, Arka Mridula, Lalit, *P. cattleianum*, *P. guineense* shown progressive increase from 25.21 to 28.51, 19.64 to 19.94, 15.58 to 16.21 and 12.92 to 12.95 respectively which are shown in Table 2.

Total protein

The amount of protein significantly increased from healthy to infected plant in varieties Strawberry guava, Banaras, Arka Mridula, *Psidim cattleianum* as 8.88 to 9.04, 3.76 to 4.56, 6.41 to 6.921 and 8.91 to 11.09. But in some susceptible varieties Lucknow-46, Lucknow-49, Arka amulya, Bapatla, Arka Poorna, Taiwan pink, Taiwan white i.e., 5.92 to 2.8, 4.32 to 2.08, 4.12 to 3.82, 1.84 to 1.33, 6.72 to 5.36 and 4.56 to 1.76 respectively, which shown decreased content of protein from healthy to infected plants compared to resistant varieties shwn in Table 3.

Total phenols

The levels of total phenols were analyzed in both resistant and susceptible varieties are presented in Table. Susceptible varieties Lucknow- 49, Allahabad safeda, Taiwan pink, Arka Amulya, Taiwan white, Lucknow-46 shown reduced content of phenols from healthy to infected plants i.e., 42.02 to 40.05, 41.6 to 40.24, 39.8 to 36.07, 47.07 to 46.21, 39.1 to 34.25, 33.9 to 31.08 respectively. Likewise the tolerant varieties Strawberry guava, Arka Mridula, *P. cattleianum*, *P. guineense* shown significant increase i.e., 85.3 to 86.54, 46.1 to 49.6, 83.11 to 84.12 and 48.09 to 49.12 respectively shown in Table 4.

Total Proline content

The progressive increase in proline was observed in varieties like Strawberry guava, Arka Mridula, Hafsi, Banaras from healthy to infected plants i.e., 2.28 to 2.08, 1.43 to 1.51, 1.45 to 1.61, and species like *P. cattleianum*, *P. guineense* shown 2.42 to 2.92, 1.71 to 1.59 respectively. There was reduction in some varieties like Lucknow-49, Allahabad safeda, Bapatla, *P.chinensis* as 0.42 to 0.35, 0.32 to 0.28, 0.56 to 0.42 and 0.91 to 0.61respectively shown in Table 5.

Enzyme analysis

Estimation of peroxidase enzyme (absorbance min-1 g-1)

The PO activity in leaf samples increased with inoculation of nematodes. The increasing PO activity from healthy to infected plants was observed in varieties like Strawberry guava, Banaras, Arka Mridula, *P. cattleianum*, *P. guineense*i.e., 0.595 to 0.645, 0.621 to 0.86, 0.702 to 0.768 respectively. But there is reduction of peroxidase activity in varieties like Lucknow – 49, Taiwan pink, Allahabad safeda, Ayakudi local as 0.314 to 0.124, 0.302 to 0.228, 0.201 to 0.129, 0.116 to 0.108 respectively shown in Table 6.

Poly phenol oxidase enzyme (absorbance min-1 g-1)

The changes in ppo activity increased from healthy to infected plants after inoculation of nematodes in varieties like Strawberry guava ArkaMridula, Banaras, *P. cattleianum*, *P. guineense i.e.,* 1.312 to 1.327, 0.821 to 1.01, 1.046 to 1.124, 1.021 to 1.042 and 0.712 to 0.845 respectively. But there also shown reduction in some varieties like Lucknow–49, Lucknow-46, Taiwan pink, Allahabadsafeda, PKM local, Bapatla and ArkaAmulya i.e., 0.132 to 0.112 0.115 to 0.113, 0.301 to 0.215, 0.143 to 0.133, 0.120 to 0.019, 0.117 to 0.112 and 0.342 to 0.339 respectively shown in Table 7.

Table 1: Chlorophyll content of Health and infected plants

Variety	Chlorophyll a			Chlorophyll b			Total Chlorophyll		
	Healthy	Infected	% decrease	Healthy	Infected	% decrease	Healthy	Infected	% decrease
Lalit	1.02	0.88	13.73	0.39	0.31	20.51	1.38	1.22	11.59
Banaras	1.24	0.39	22.56	0.69	0.61	11.59	1.9	0.79	28.42
Taiwan pink	1.15	0.79	31.30	0.52	0.5	13.85	1.73	0.92	46.82
Taiwan white	0.83	0.65	21.69	0.37	0.28	24.32	1.04	0.87	56.32
Lucknow -49	1.6	0.57	26.48	0.82	0.66	19.51	2.35	1.13	51.91
Lucknow-46	0.72	0.54	25.00	0.66	0.52	21.21	0.99	0.81	38.88
Allahabad Safeda	1.71	0.63	33.16	0.91	0.34	32.54	2.06	1.11	46.12
Hafsi	1.43	0.87	39.16	0.61	0.48	21.31	2.08	1.39	33.17
Bapatla	1.02	0.25	52.39	0.52	0.23	55.77	1.06	0.87	57.21
strawberry guava	0.27	0.14	18.15	0.98	0.57	18.97	2.98	1.78	10.00
P. cattleianum	1.25	1.05	16.00	1.29	1.13	12.40	1.96	1.41	11.06
P. guineense	0.65	0.32	20.77	0.34	0.21	38.24	1.01	0.61	19.63
p.chinensis	1.17	0.69	41.03	0.75	0.81	19.63	2.15	1.41	34.42
Arka Kiran	1.11	1.02	19.00	0.91	0.8	12.09	1.39	1.29	13.12
Arka Poorna	0.83	0.65	21.69	0.42	0.32	23.81	1.31	1.03	21.37
Arka Amulya	0.73	0.21	47.22	0.38	0.39	18.75	2.01	0.74	53.16
Arka Reshmi	0.87	0.63	27.59	0.74	0.58	21.62	1.22	0.87	28.69
Arka Mridula	1.24	0.3	29.63	1.31	1.05	19.85	2.24	.0.91	32.51
Andhra local	0.96	0.59	38.54	0.67	0.44	34.33	1.7	0.96	29.64
Ayakudi local	1.34	0.91	32.09	0.69	0.22	68.12	2.14	1.31	38.79
PKM local	1.52	0.93	38.82	0.77	0.61	27.31	2.21	1.12	49.32

Table 2: Total sugars in Healthy and infected plants of guava varieties

Variety	Healthy	Infected	t value	T0S
Lalit	19.64	19.94	2.07	S
Banaras	23.41	26.1	2.04	S
Taiwan pink	10.81	10.44	1.01	NS
Taiwan white	9.48	9.45	0.97	NS
KG guava	13.9	13.6	1.54	S
Lucknow-49	11.09	11.07	0.94	NS
Lucknow-46	12.21	12.12	1.62	NS
Allahabad Safeda	11.92	11.89	1.71	NS
Hafsi	12.95	12.81	0.98	NS
Bapatla	11.58	11.46	1.11	NS
Black guava	25.21	28.51	2.12	S
Psidium cattleianum	24.42	30.85	2.1	S
Psidium guineense	12.92	12.95	2.01	S
Psidium chinensis	11.81	10.32	1.91	NS
ArkaKiran	10.92	10.81	2.08	NS
ArkaPoorna	9.85	9.92	1.33	S
ArkaAmulya	9.25	8.64	1.13	NS
ArkaReshmi	9.83	9.89	2.03	S
ArkaMridula	15.58	16.21	1.87	S
Andhra local	15.61	16.53	2.06	S
Ayakudi local	14.52	15.61	2.05	S
PKM local	15.25	16.82	1.94	S

P (T<=t) one-tail, TOS=Test of significance, S- significant NS- Non significant

Table 3: Total Protein in Healthy and infected plants of guava varieties

Variety	Healthy	Infected	t value	TOS
Lucknow-49	4.32	2.08	1.78	NS
Banaras	3.76	4.56	2.04	S
Taiwan pink	6.72	5.36	1.34	NS
Taiwan white	4.56	1.76	1.23	NS
KG guava	1.76	5.92	1.92	S
Lalit	6.32	11.05	2.01	S
Lucknow-46	5.92	2.8	1.44	NS

Allahabad safeda	4.96	1.44	1.12	NS
Hafsi	5.04	6.48	1.26	S
Bapatla	1.84	1.33	1.18	NS
Strawberry guava	8.88	9.04	2.02	S
Psidium Cattleianum	8.91	11.09	2.08	S
Psidium guineense	6.71	6.79	1.28	S
Psidium chinensis	5.82	6.02	1.68	S
Arka Kiran	4.65	4.89	1.52	NS
Arka Poorna	4.39	4.78	1.11	S
Arka Amulya	4.12	3.82	1.07	NS
Arka Reshmi	6.85	6.12	1.28	S
Arka Mridula	6.41	6.92	1.86	S
Andhra local	7.52	7.12	2.03	S
Ayakudi local	6.44	6.67	1.98	NS
PKM local	7.34	7.66	1.84	S

P (T<=t) one-tail, TOS=Test of significance, S- significant NS- Non significan

Table 4: Total Phenols in Healthy and infected plants of guava varieties

Variety	Healthy	Infected	t-vaue	TOS
L-49	42.2	40.5	2.01	NS
Banaras	45.1	46.05	2.04	S
Taiwan Pink	39.8	36.7	1.67	NS
Taiwan white	39.1	34.25	1.96	NS
KG guava	40.8	41.02	2.06	S
Lalit	46.1	49.6	2.08	S
L-46	33.9	31.08	1.86	S
Allahabad Safeda	41.6	40.24	1.91	NS
Hafsi	49.7	44.8	2.00	S
Bapatla	37.9	36.21	1.77	NS
Strawberry guava	85.3	86.54	2.16	S
Psidium cattleianum	83.1	84.12	2.13	S
Psidium guineense	48.09	49.12	1.55	S
Psidium chinensis	50.02	50.11	1.97	NS
ArkaKiran	51.04	51.2	1.79	S
ArkaPoorna	45.30	45.91	1.69	S
ArkaAmulya	47.05	46.21	1.59	NS
ArkaReshmi	41.21	40.05	1.66	S
ArkaMridula	50.09	50.56	2.05	S
Andhra local	61.58	61.76	2.07	S
Ayakudi local	59.24	59.81	1.99	S
PKM local	52.03	52.53	1.82	S

P(T<=t) one-tail, TOS=Test of significance, S- significant NS- Non significant

Table 5: Total Proline in Healthy and infected plants of guava varieties

Variety	Healthy	Infected	t value	T0S
Lalit	1.02	1.62	1.19	NS
Taiwan Pink	0.41	0.81	1.25	NS
Taiwan White	0.36	0.42	1.07	NS
Banaras	0.74	0.86	2.02	S
KG guava	0.51	0.64	1.65	S
Allahabad Safeda	0.32	0.28	1.52	NS
Lucknow-46	0.39	0.48	1.34	NS
Lucknow-49	0.42	0.35	1.23	NS
Bapatla	0.56	0.42	1.09	NS
Hafsi	1.45	1.61	1.12	NS
Strawberry guava	2.08	2.28	2.06	S
Psidium cattleianum	2.42	2.92	2.08	S
Psidium guineense	1.59	1.71	1.87	S
Psidium chinensis	0.61	0.91	1.82	NS
ArkaKiran	0.75	0.82	1.21	NS
ArkaPoorna	0.58	0.66	1.34	S
ArkaReshmi	0.87	0.72	1.28	S
ArkaMridula	1.43	1.51	1.39	S
ArkaAmulya	0.31	0.39	1.19	NS
Andhra local	0.89	0.91	2.01	S
Ayukudi local	0.38	0.44	1.97	S
PKM local	0.63	0.77	1.92	S

P (T<=t) one-tail, TOS=Test of significance, S- significant NS- Non significant

Table 6: Total Peroxidase (absorbance min-1 g-1) in Healthy and infected plants of guava varieties

Variety	Healthy	Infected
Psidium.Cattleianum	0.621	0.846
Psidium.guineense	0.707	0.768
Psidium.chinensis	0.258	0.345
ArkaKiran	0.151	0.155
ArkaPoorna	0.291	0.326
ArkaReshmi	0.328	0.371
ArkaMridula	0.315	0.321
ArkaAmulya	0.215	0.232
Andhra local	0.482	0.531
Ayukudi local	0.116	0.106
PKM local	0.412	0.448
Lalit	0.505	0.618
Banaras	0.492	0.512
Taiwan pink	0.201	0.129
Taiwan white	0.212	0.119
KG guava	0.218	0.313
Lucknow -49	0.314	0.124
Lucknow-46	0.219	0.214
Allahabad safeda	0.302	0.228
Hafsi	0.191	0.182
Bapatla	0.110	0.021
Strawberry guava	0.645	0.734

Table 7: Total polyphenol oxidase (absorbance min-1 g-1 in Healthy and infected plants of guava varieties

Variety	Healthy	Infected
P. cattleianum	1.021	1.042
P. guineense	0.712	0.845
P.chinensis	0.425	0.453
Arka Kiran	0.387	0.512
Arka Poorna	0.191	0.202
Arka Reshmi	0.405	0.482
Arka Mridula	0.821	1.01
Arka Amulya	0.342	0.239
Andhra local	0.328	0.518
Ayukudi local	0.414	0.448
PKM local	0.516	0.621
Lalit	1.012	1.119
Banaras	1.046	1.124
Taiwan pink	0.301	0.215
Taiwan white	0.212	0.124
KG guava	0.49	0.521
Lucknow-49	0.132	0.112
Lucknow-46	0.115	0.104
Allahabad safeda	0.143	0.133
Hafsi	0.121	0.109
Bapatla	0.117	0.112
Strawberry guava	1.312	1.327

Discussion

Total Chlorophyll content

Similar findings of Nayak *et al*., 2016 reported that changes in chlorophyll content reduced from 2.702 mg/g to 2.010 mg/g due to nematode infection in case of PusaKranti variety. Similarly in the varieties Kantabaigan and Pusa purple long chlorophyll ‘a’ reduced from 2.542 to 1.736 and 2.687 to 1.920 mg/g respectively. Chlorophyll ‘b’ content reduction from healthy to infected plants recorded were 1.114 to 1.020, 0.986 to 0.820 and 1.082 to 0.414 mg/g in case of varieties PusaKranti, Kantabaigan and Pusa Purple long respectively. Chlorophyll ‘b’ content reduction from healthy to infected plants recorded were 1.114 to 1.020, 0.986 to 0.820 and 1.082 to 0.414 mg/g in case of varieties PusaKranti, Kantabaigan and Pusa Purple long respectively.

Likewise the findings were observed in greengram by Ritupandey, 2005 were similar to the present study as which Chlorophyll ‘a’ content reduced from 1.33 mg/g to 0.08 mg/g and 1.38 mg/g to 0.66 mg/g due to nematode infection in case of 28 PM 10-12 and 29 PUSA 0672 variety. Similarly in the varieties 24 ML 233,7GGG 10-14, 17 IPM 9901-6 and 8 GM 04-02 chlorophyll ‘a’ reduced from 0.82 to0.88, 1.10 to 0.04, 0.06 to 1.36 and 1.41 to 1.43 mg/g respectively. chlorophyll ‘b’ content reduction from healthy to infected plants recorded were 0.53 to 0.36,0.55 to 0.21,0.33 to 0.34,0.45 to 0.21,0.30 to 0.53, and0.60 to 0.54 mg/g respectively in case of varieties 28 PM 10-12, 29 PUSA 0672,24ML-233,7 GGG 10-14,17 IPM 9901-6 and 8 GM 04-02. the total chlorophyll content was decreased from 1.86 to 1.25 mg/g in 28 PM 10-12, 1.93 to 0.87 mg/g in 29 PUSA 0672, 1.55 to 0.71 mg/g in 7 GGG 10-14 and 2.01 to 2.00 mg/g in 8 GM 04-02, but increased

from1.16 to 1.23 mg/g in 24ML-233 and 1.02 to 1.90 mg/g in 17 IPM 9901-6.

Total sugars

The observations recorded in the present study were similar to the findings of Nayak *et al*., 2016. The amount of total sugars contained in the healthy plants was 1.445, 1.198 and 1.628 per cent in PusaKranti, Kantabaigan and Pusa Purple Long respectively. In the infected plants of these varieties sugar contents were 2.568, 2.102 and 3.565 per cent respectively. Similar findings given by Robert lepcha, 2013 [19], decrease in the total sugar was observed in *M. incognita* infested roots of susceptible "K8" coleus cultivar (0.98 mg/ g) compared to resistant "MV7" coleus cultivar. Similar findings of Gautham and Adithi, 2014, in bittergourd shown that there is gradual increase from 7.62%in the 3rd week and being maximum (50.33 %) in the 4th week of inoculation of nematode. The amount of sugar present in the portion of the inoculated plants were recorded as 0.132, 0.081, 0.095, 0.050, 0.048 and 0.077 percent of the blackgram varieties PU 0-36, mu-44, VBG 11-031, VBG 11-016, KUG-715, and NUL-205 respectively by the similar findings of present study given by Robert lepcha, 2013 [19]. In reference to Kananan, 1968 [7], the sugar values of the leaves of the uninfected plant were two and half times greater than in those of the infected plant. Increased sugar content in infected samples might be due to movement of various metabolites towards the infection site from other parts of plants.

Protein Content

Similar findings were given by Robert lepcha, 2013 [19], in Black gram the protein content increases in infected cultivars due to infection of root-knot nematode**,** as 0.103, 0.231, 0.351, 0.239, 0.151 and 0.202 mg/100g in varieties PU 0-36,mu-44, VBG 11-031, VBG 11-016, KUG-715, and NUL-205 compared to 0.069, 0.106, 0.330, 0.178, 0.092 and 0.141 in varieties PU 0-36, mu-44, VBG 11-031, VBG 11-016,KUG-715, and NUL-205. Similar observations were seen in Bitter gourd by Gautham and Adithi, 2013, Increase in total protein content was maximum (105.26%) in the 1st week of infection compared from control. Some enzymatic proteins are produced in the penetrated cells by pathogens themselves. Thus, qualitative and quantitative changes in proteins occur in the penetrated plant cells, the noninfected tissue adjacent to the infected tissue shows changes in metabolism that involve in the stimulation of synthesis and degradation of specific proteins reported by Uritani, 1979 [29]. Similarly a significant increase of root protein in nematode infested plants. The increase of protein in roots of tomato cultivated under polyhouse infested with *M. incognita* was 40.27 per cent during fruiting stage followed by 38.57per cent during vegetative stage over the healthy roots of tomato reported by Jancy rani, 2013 [6].

Total phenols

Oxidized forms of phenolic compounds occurring in high concentration in roots of resistant tomato plants and it contributes to the *M. incognita* resistance by creating a toxic environment for nematode penetration and multiplication reported by Bajaj and Mahajan, 1977 [1]. Giebel, 1974 [4] considered the phenolic substances were the best known factors in susceptible and resistant response of plants towards the nematode infection. Infested roots of *Solanum melongena* recorded increased phenol by 7-14 per cent than the healthy roots reported by Singh *et al*. (1979) [22]. Similarly, the findings of Ravichandra *et al*., 1988 [17] shown that the root-knot nematode highly susceptible brinjal variety "Erengere" recorded maximum quantity of total phenols (0.53 mg/g) than a resistant variety "Gulla" (0.48 mg/g). Likewise Mohanta and Mohanty, 2012 [10] reported increased phenolic substances by 36.92 per cent during post infection period in inoculated sample over healthy check due to nematode infection and The significant increase of phenolic compounds were observed in resistant varieties compared with the susceptible green gram variety, 29PUSA0672, the increase of phenolic compound in the resistant varieties was remarkable in this study by Ritu pandey, 2015 [18]. Similarly, Narayana Shankaranavar, 2014 showed that Susceptible "K8"cultivar had total phenol of 0.38 mg/g per root sample and resistant "MV7" cultivar had total phenol of 1.05 mg/g per sample. A decrease in total phenol was observed in roots of susceptible "K8" cultivar of coleus infested by *M. incognita* which accounted for 63.81 percent decrease over resistant cultivar.

Proline Content

Similar observations were noticed by Singh *et al*., 1979 [22] that quantities of total protein, free amino acids and proline in infested roots exceeded compared to healthy roots. Similar observations were given by Robert lepacha, 2013 [19], in blackgram, the amount of proline in the uninoculated plants were recorded as 19, 35, 45, 49,52 and 43 µg/g on fresh weight basis on varieties PU 0 36, mu-44, VBG 11-031, VBG11-016, KUG-715, and NUL-205.while this amount was increased in inoculated infected plants as 24, 47, 52, 58, 64 and 47 respectively. Likewise the total phenol content in roots of tomato indicates the degree of resistance to root knot nematode, Phenol and orthodihydroxy phenol contents were found to be more in the parent 120 given by Indu rani, 2008 [5].

Peroxidase and poly phenol oxidase activity

The increased peroxidase activity was observed in nematode inoculated samples of both susceptible and resistant brinjal varieties but higher enzymatic activity was recorded in resistant varieties and registered 45.18, 32.09 and 11.30 per cent increase over control Nayak *et al*., 2016. Highest PO, PPO and PAL content was observed in the variety IISR Mahima (9.02 changes in absorbance min-1 g-1, 0.94 changes in absorbance min-1 g-1 and 17.78 changes in cinnamic acid min-1 g-1 respectively) there by exhibit more defence mechanism against nematode and lowest PO, PPO and PAL activity was seen in variety Karthika (6.92 changes in absorbance min-1 g-1, 0.70 changes in absorbance min-1 g-1 and 14.59 changes in cinnamic acid min-1 g-1 respectively). Similarly, Shukla and Chakraborthy, 1988 [21] also reported that the *M. incognita* infected resistant varieties of tomato showed significantly higher peroxidase enzyme activity than the susceptible varieties. Studies on the changes in the peroxidase and polyphenol oxidase isozymes clearly indicated that there was enhancement of activities of these enzymes in the CLN 2026C x SL 120 given by Indu rani, 2008 [5]. Increase in peroxidase activity in response to *M. phaseolina* infection over control in resistant var., BGD-98 was 45.24%. The increase in peroxidase activity ranged between 28.88% to 37.43%, in tolerant varieties whereas in susceptible varieties it ranged from 13.51% to 25.54% similar to the present study given by Ashraf *et al*., 2005. Likewise higher peroxidase activity resulted in an increase in the phenolic contents of plant, which plays an important role in the resistance of cultivar given by Mahmood & Saxena, 1986 [8].

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