Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 1621-1629
Received: 16-07-2018
Accepted: 18-08-2018

Vyomesh Shailesh Patel
Department of Agricultural Biochemistry, Anand Agricultural University, Anand, Gujarat, India

Pitambara
Department of Agricultural Biotechnology, Anand Agricultural University, Anand, Gujarat, India

YM Shukla
Professor & Unit Officer, College of Agriculture, Vaso, Anand Agricultural University, Anand, Gujarat, India

Correspondence
Pitambara
Department of Agricultural Biotechnology, Anand Agricultural University, Anand, Gujarat, India

Biochemical characterization of root knot nematode (*Meloidogyne incognita*) infected tomato cultivar (*Solanum lycopersicum* L.)

Vyomesh Shailesh Patel, Pitambara and YM Shukla

Abstract
Root-knot nematodes (*Meloidogyne* spp.) found to be very fatal infective agents and cause severe yield loses of Tomato (*S. lycopersicum* L.). Biochemical characterization of roots of two tomato cultivars AT 3 (Susceptible) and SL 120 (Resistant) grown under sterile and root knot nematode (3000 J_2 stage larvae per plant) inoculated soil, revealed that moisture content was dropped significantly in AT 3 under disease conditions (82.06%). The total protein content of roots ranged from 2.46% - 3.56%. Highest total protein content was observed in the susceptible cultivar AT 3 under diseased condition (3.56%). Study of amino acid profile by UPLC revealed significant rise in their quantity under transition from control to diseased conditions in susceptible cultivar AT 3. Proline was increased significantly under diseased conditions in both the cultivar. The total phenol content of roots ranged from 0.1% - 0.36%. Highest total phenol content was observed in the resistant cultivar SL 120 under disease condition (0.36%). Sinapic acid content increased the most in susceptible cultivar AT 3 after infection. Chlorogenic acid (15.84 µg / g FW) and ellagic acid (7.89 µg / g FW) were also found to be increased significantly under root-knot biotic stress in susceptible cultivar AT 3 as compared to control conditions. Salicylic acid showed highest increase in resistant cultivar SL 120 (13.96 µg / g FW). Roots of susceptible cultivar (AT 3) under diseased condition showed significantly higher (19.43 mg of ascorbic acid g^{-1} FW) antioxidant activity as compared to control (8.09 mg of ascorbic acid g^{-1} FW). Peroxidase had shown the maximum activity under root knot biotic stress and was recorded highest in resistant cultivar SL 120 (111.73 ΔOD/min/ g FW). PPO activity was also found to be highest in resistant cultivar SL 120 under disease conditions (6.27 ΔOD/min/ g FW). Maximum PAL activity was found to be present in the susceptible cultivar AT 3 (11.6 µmol h^{-1} g^{-1} fw) under biotic stress condition. This information could be useful for planning better strategies in tomato breeding for resistance against root knot nematode.

Keywords: Tomato, nematode, biochemical, resistance

Introduction
Tomato (*Solanum lycopersicum* L., 2n=2x=24) belongs to the genus *Solanum* under the *solanaceae* family. Tomato is the world's largest vegetable crop after potato and sweet potato, but it tops the list of canned vegetables. The total global area under tomato is 47.30 lakh ha and the global production is 1639.60 lakh tonnes. India is the second largest tomato (*Solanum lycopersicum* L.) growing country having cultivation area of 8.8 lakh ha with production of 18.23 mmt with productivity 20.72 mt/ha during 2014-15 (NHB Database, 2016). Major tomato (*Solanum lycopersicum* L.) producing states are Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh and Assam. Gujarat is fifth largest producer of tomato (*Solanum lycopersicum* L.) after Madhya Pradesh, Andhra Pradesh, Karnataka, and Orissa. Gujarat is having cultivation area of 44,570 ha with production of 1.26 mmt with productivity 28.2 mt/ha during 2014-15 (NHB Database, 2016).

Tomato, *Solanum lycopersicum*, is an important vegetable for human use because of its vitamins and minerals content that provide the basic body nutritional requirements (Lorenz and Maynard, 1997) [7]. According to Splittstoesser (1990) [32], it is ranked 14th among sixteen common vegetables (spinach, lima beans, peas, sweet potato, carrots, cabbage, lettuce, onion, etc) based on total nutritional concentration but ranked first based on the contribution of nutrients to the diet. It is an excellent source of many nutrients and secondary metabolites that are important for human health; mineral matter, vitamins C and E, B-carotene, lycopene, flavonoids, organic acids, phenolics and chlorophyll (Giovanelli and Paradise, 2002) [15]. Tomatoes are widely consumed either raw or after processing and can provide a significant proportion of the total antioxidants in the diet (Martinez-Valvercle *et al.*, 2002) [20]. Tomatoes constitute the predominant source of lycopene and phenols.

The antioxidant activity of carotenoids is probably dependent on: (i) number of conjugated double bonds,

(ii) end groups (acyclic or cyclic), and (iii) functional groups (Stahl *et al.,* 2001) [34]. Based on these functional groups, the antioxidant potential can be rated as lycopene > α-carotene > β carotene (Anguelova and Warthesen, 2000) [3]. Hence, tomato based food products play a significant role in the protection of several forms of cancers (Garcia *et al.,* 1999; Giovanucci, 1999) [14, 16] and vascular diseases (Su *et al.,* 1998) [35].

Tomato (*Solanum lycopersicum* L.) is affected by various disease caused mainly by fungi, bacteria and nematodes. Nematodes found to be very fatal infective agents and cause severe yield loses. Root-knot nematodes (*Meloidogyne* spp.) are phytopathogenic obligate endoparasites nematodes that infect many plant species and cause serious damage to agricultural crops per year (Abad *et al.,* 2008) [1]. Management of plant parasitic nematodes has always been difficult, and the most successful strategy for many years has been the use of toxic fumigant nematicides, such as the most known methyl bromide (Oka *et al.,* 2000b) [25]. But the safe and eco-friendly approach is to use resistant variety. Even some molecular markers have to be developed for the screening of such resistant varieties.

During stress conditions, plant gene expression changes to cope up with the altered environment. Many plant enzymes are involved in defence reactions against plant pathogens (Odjakova and Hadjiivanova, 2001) [23]. A plant exposed to pathogens also activates oxidative enzymes such as peroxidase (POX) (Ryan and Jagendorf, 1995) [29]. Antioxidant enzymes inactivate active oxygen forms induced by different stresses such as H_2O_2. The enzymatic action such as catalase and peroxidase could lead to scavenge the accumulation of H_2O_2 in tissue (Tripathi, 2006) [36]. Catalase (CAT) plays an important role in the catabolism of hydrogen peroxide (H_2O_2). Phenylalanine ammonialyase (PAL) is the entry point enzyme into phenylpropanoid metabolism, involved in the production of phenolics and phytoalexins that prevent establishment of the pathogen (Mariutto *et al.,* 2011) [19].

In view of the above reports, the present investigation deals with "Biochemical characterization of Root Knot Nematode (*Meloidogyne incognita)* infected tomato cultivar *(Solanum lycopersicum* L.)*"*

Materials and Methods

The present investigation on "Biochemical changes during root knot nematode *(Meloidogyne incognita)* infection in tomato *(Solanum lycopersicum)*" was carried out at Department of Biochemistry in collaboration with Department of Nematology; B. A. College of Agriculture; Anand Agricultural University; Anand; which is situated on 22º- 35' north latitude and 72º- 55' east longitudes and has an elevation of 45 meters above the mean sea level.

The seeds of tomato cultivars for the present study were procured from the Main Vegetable Research Station; Anand Agricultural University; Anand (Table 1).

Table 1: List of tomato cultivars procured from MVRS

S. No.	Tomato Cultivar	Description
1	AT 3	Root knot nematode susceptible
2	SL 120	Root knot nematode resistant

Experimental Design maintained in this study was Completely Randomized Design (CRD) with following Treatments:

1. AT 3 Control: Seedlings grown in un-inoculated sterile soil.
2. AT 3 Treated/Inoculated/Stressed: Seedlings grown in soil inoculated with Root knot nematodes (3000 J_2 stage larvae / plant).
3. SL 120 Control: Seedlings grown in un-inoculated sterile soil.
4. SL 120 Treated/Inoculated/Stressed: Seedlings grown in soil inoculated with Root knot nematodes (3000 J_2 stage larvae / plant).

Following biochemical parameters were analysed and characterised under control as well as biotic stress due to root knot nematode infection in tomato.

1. **Moisture Content:** Moisture content was estimated as per procedure developed by A.O.A.C. (2000)

$$\text{Moisture (\%)} = \frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Fresh weight}} \text{ X } 100$$

2. **Total Soluble Protein:** The total soluble protein content in roots of tomato plants under control and stressed conditions was analyzed by Lowry *et al.,* 1951 [18].
3. **Total Phenol:** Total phenol was estimated by the method described by Bray and Thorpe (1954) [7] with some modifications. Phenol content was calculated from the standard curve prepared from catechol as standard.

$$\text{Total Phenols (\%)} = \text{Graph factor x } \frac{\text{Sample reading}}{\text{weight of sample}} \text{x} \frac{\text{Total volume}}{\text{Taken volume}} \text{x } 10^{-4}$$

4. **Phenol Profiling by Ultra Performance Liquid Chromatography (UPLC):** Precisely, 1 g of each sample was crushed in 80% methanol and further concentrated with diethyl ether and filtered through 0.45 µ PVDF membrane filter and filtrate was used for further analysis. The analysis was performed using Waters system consisted of quaternary pump, photodiode array detector and an auto sampler.
5. **Amino Acid Profiling by Ultra performance Liquid Chromatography (UPLC):** Thirty mg of each sample was taken in a 4 ml capacity glass vial and 3 ml of 0.1% phenol in 6 N HCl solution was added in each vial then closed the cap and kept in oven at 110 °C for 24 hrs including blank without sample. Next day 0.7 ml of hydrolyzed sample was neutralized with 0.7 ml of 6 M sodium hydroxide and volume made to 2.0 ml with Milli Q water and filtered through 0.45 µ PVDF membrane filter. Filtrate was taken for derivatization. 10 µl aliquot of standard/sample was transferred to a clean vial and 70 µl of AccQ Tag Ultra borate buffer was added to each vial and vortexed followed by addition of 20 µl of AccQ Tag Ultra reagent and immediately vortexed for several seconds. Then vials were put in an oven at 55 °C for 10 mins and further used for UPLC analysis. The analysis was performed using Waters system consisted of quaternary pump, photodiode array detector and an autosampler.
6. **Total Antioxidant Activity:** Antioxidant activity was measured using Ferric Reducing Antioxidant Power (FRAP) method described by Arnao *et al.,* 2001 [5].
7. **Enzyme Activities:** activities of Polyphenol oxidase (EC 1.14.18.1), Peroxidase (EC 1.11.1.7) and Phenylalanine ammonia lyase (EC 4.3.1.24) was tested by assay suggested by Mayer *et al.,* 1965 [21], Reuveni, 1995 [27] and Dickerson *et al.,* 1984 [11] respectively.

Results and discussion

The present investigation was carried out at Department of Biochemistry in collaboration with Department of Nematology, B. A. College of Agriculture, Anand Agricultural University, Anand. The seeds of tomato cultivars for the present study were procured from the MVRS, Anand Agricultural University, Anand. The results obtained in the present investigation are presented and discussed under following sub-headings

1. **Moisture Content:** Moisture content of roots of both susceptible and resistant tomato cultivars under control and disease conditions ranged from 82.06% - 90.46% (Table 2). Highest moisture content was observed in the resistant cultivar SL 120 under control condition (90.46%). There was slight reduction of moisture in resistant cultivar during transition from control to disease environment while significant drop in moisture content was observed in susceptible cultivar AT 3 (Treated) under disease condition (82.06%) as compared to the AT 3 (control) under normal condition (87.67%). These results are in agreement with the results observed by Dawson and Weste (1984) [10].
2. **Total Soluble Protein:** The total protein content of roots of both susceptible and resistant tomato cultivars under control and disease conditions ranged from 2.46% - 3.56% (Table 2). Highest total protein content was observed in the susceptible cultivar AT 3 under disease condition (3.56%). There is no any significant change in protein content was found in resistant cultivar during transition from controlled to disease environment while significant rise in protein content was observed in susceptible cultivar AT 3 (Treated) under disease condition (3.56%) as compared to the AT 3 (control) under normal condition (2.46%). The results observed here are in agreement with the results obtained by Shreenivasa *et al.*, 2011 [30].
3. **Total Phenol:** The total phenol content of roots of both susceptible and resistant tomato cultivars under control and disease conditions ranged from 0.1% - 0.36% (Table 2). Highest total phenol content was observed in the resistant cultivar SL 120 under disease condition (0.36%). There was rise in total phenol content in both susceptible and resistant cultivar during transition from control to disease environment. There was a significant rise in total phenol content in susceptible cultivar AT 3 (Treated) under disease condition (0.26%) as compared to the AT 3 (control) under normal condition (0.1%). These results are in agreement with the results observed by Shreenivasa *et al.*, 2011 [30] and Choudhary *et al.*, 2013 [10].
4. **Phenol Profile by UPLC:** Phenolic profile of roots of both susceptible and resistant genotype under both disease and control conditions were analysed by UPLC, the chromatographic data are presented in Plate 1. The external standard method in which reference compounds were analysed under similar chromatographic conditions separately from samples was used for quantification purpose. The calibration curve was linear over standards concentration ranging from 1 to 25 ppm. The regression coefficients of these graphs ranged from 0.994 to 0.997. The lowest level of detection (LOD) of phenolic compound was 0.52 µg / g and the lowest level of quantification (LOQ) was at approximately 0.97 µg / g. In the present study 12 phenolic acids were identified and quantified in the roots of resistant and susceptible cultivars under control and root knot disease conditions in tomato seedlings.

   The contents of phenolic compounds as quantified by UPLC were presented in the table 3. The results showed that the content and type of phenolic compounds varied depending on the condition (control and root knot disease) and type of cultivar (AT 3 and SL 120). Sinapic acid content was ranged from 4.89 – 18.50 (µg / g FW). It was found to be the highest (18.50 µg / g FW) in AT 3 (Treated) among the all root samples and found to be the one with highest 3.4 folds change from control to disease condition. While chlorogenic acid (15.84 µg / g FW) and ellagic acid (7.89 µg / g FW) were the other phenolic acids after sinapic acid which were found to be increased significantly under root-knot biotic stress in susceptible cultivar AT 3 as compared to control condition. Vanillic acid (4.69 µg / g FW) was another phenolic acid which had shown significant increased under root-knot biotic stress as compared to control condition (2.05 µg / g FW) in susceptible cultivar AT 3 where as the quantity of vanillic acid had shown no any significant difference between control and disease conditions in resistant cultivar SL 120. Most of the phenolic acids did not differ much under stress and control conditions in resistant cultivar. Salicylic acid was the only phenolic acid found to be increased the most (1.95 folds) during disease condition as compared to control in resistant cultivar SL 120 (13.96 µg / g FW). p - Coumaric acid, gallic acid, caffeic acid, ferulic acid and cinnamic acid did not differ much in their quantity between control and disease conditions in both the cultivars. These results were in agreement with the results obtained by Baker *et al.*, (2010) [6].
5. **Amino Acid Profile by UPLC:** There are various proteins such as pathogenesis related proteins as described in previous sections which show differential response to various stresses. Although the specific proteins synthesized by a plant are determined by its genetic makeup, but the rates at which the individual proteins are synthesized are influenced by the kind of stress prevailed. Similarly there exist various stress responsive amino acids as well. There are some amino acids which showed good positive correlation with various stresses. This study was carried out to check any amino acids which could have any relation with root knot nematode infection under susceptible and resistant cultivars.

   Different 18 amino acids were studied by UPLC in the roots of two tomato cultivars under root knot biotic stress and control conditions. Out of 18 amino acids studied thirteen amino acids were found to be present in detectible quantities in the roots of tomato cultivars. All the 13 amino acids were significantly higher in susceptible cultivar AT 3 under disease condition as compared to control (Table 4). Plate 2 shows the chromatograms of amino acid profiling in roots of tomato cultivars under disease and control conditions.

   Out of 13 amino acids, cysteine, glutamic acid, aspartic acid, alanine, proline and tyrosine were found in high amounts, which cover almost 17%, 14%, 11%, 10%, 7.63 and 7.31% respectively of total amino acid content. Glutamic acid (113.88 mg/100 g) and cysteine (113.62 mg/100 g) were found to be highest in quantity in AT 3 (Treated) amongst all the other samples. They showed 153% and 92.48 % increase under root knot biotic stress

against control condition in susceptible cultivar AT 3. Quantity of most of the amino acids do not changed significantly under the transition from control to root knot biotic stress conditions in resistant cultivar. Apart from glutamic acid and cysteine as described earlier alanine (99.57 mg/100 g), aspartic acid (85.82 mg/100 g), tyrosine (63.97 mg/100 g), leucine (53.41 mg/100 g) and serine (44.89 mg/100 g) had also shown significant rise in their quantity under transition from control to disease conditions in susceptible cultivar AT 3. Proline is the only amino acid which had shown significant increment in its quantity under disease condition in both the cultivar. It found to be in higher amount in resistant cultivar as compared to susceptible cultivar under both control and disease conditions.

6. **Total Antioxidant Activity:** Antioxidants are an atom donor of an electron to a free radical. If a molecule has one or more unpaired electron it works as a free radical. Any antioxidants delays or prevents the process of oxidation. During oxidation free radicals are produced which can damage the cells. Antioxidants are responsible for terminating the chain reaction by removing free radicals. A number of substances act like antioxidants, viz., carotenoids, vitamin A, C and E, phenols and several other non-nutrients (Singh *et al*. 2012) [31]. According to Oderonke (2012) [22], who had proposed hypothesis in respect to these increases; it considers the responses of plants to stressful environments such as attacks from insects, pests, pathogens and weeds. More the stress conditions (Biotic or Abiotic) prevailed; more the free radicals produced and hence more the production of secondary metabolites, such as carotenoids and antioxidants to compensate and scavenge the free radicals. As shown in table 5 biotic stress had increased the antioxidant activity of tomato cultivars over control condition. Disease condition showed significantly higher (19.43 mg of ascorbic acid g^{-1} FW) antioxidant activity as compared to control (8.09 mg of ascorbic acid g^{-1} FW) in roots of AT 3 cultivar. Among all samples AT 3 (Control) showed minimum (8.09 mg of ascorbic acid g^{-1} FW) antioxidant activity. Antioxidant activity was raised in resistant cultivar SL 120 in transition from control (13.40 mg of ascorbic acid g^{-1} FW) to disease (15.54 mg of ascorbic acid g^{-1} FW) condition. Resistant cultivar SL 120 (13.40 mg of ascorbic acid g^{-1} FW) had shown higher antioxidant activity as compared to susceptible cultivar AT 3 (8.09 mg of ascorbic acid g^{-1} FW) under control condition. The higher value of antioxidant activity was associated with higher value of phenolic acids which are involved in defence mechanism. El-beltagi *et al.* (2012) [12] demonstrated that the root knot nematode infected plants contained 8 to 20% higher antioxidant activity compared to roots of their control healthy plants.

7. **Enzyme Activities**

1. **Polyphenol Oxidase:** As shown in the table 6; among all the samples, significantly higher PPO activity was found in resistant cultivar SL 120 under disease condition (6.27 ΔOD/min/ g FW). However, the lower PPO activity was observed in susceptible cultivar AT 3 (3.3 ΔOD/min/ g FW and 4.4 ΔOD/min/ g FW) as compared to resistant cultivar SL 120 (4.12 ΔOD/min/ g FW and 6.27 ΔOD/min/ g FW) under both control and disease conditions respectively. These results are in accordance with the results obtained by Afifi *et al.,* (2014) [14], Shreenivasa *et al.,* (2011) [30] and Rani *et al.,* (2008) [26].
2. **Peroxidase:** Peroxidase might generate free radicals which are highly toxic to many organisms by cross-linking hydroxyproline rich glycoproteins and by lignifying plant cell walls. Such mechanism might be operating in the root knot resistant tomato seedlings to impart disease resistance to host. In both conditions, resistant cultivar recorded higher peroxidase activity than the susceptible one under root knot biotic stress, maximum peroxidase activity was recorded in resistant cultivar SL 120 (111.73 ΔOD/min/ g FW). Resistant cultivar SL 120 had shown higher peroxidase activity (102.33 ΔOD/min/ g FW and 111.73 ΔOD/min/ g FW) as compared to susceptible cultivar AT 3 (65.78 ΔOD/min/ g FW and 88.12 ΔOD/min/ g FW) under control and stressed conditions respectively (Table 6). Both cultivars showed rise in peroxidase activity upon transition from control condition to disease condition. These results are in harmony with the results acquired by Afifi *et al.,* (2014) [14] and Rivero *et al.,* (2001) [28]. A positive role of POX in activation of resistant responses had seen as reported by (Sreedhara, 1995) [33].
3. **Phenylalanine ammonia liase:** PAL activity was studied in roots of tomato seedlings. Maximum PAL activity was found to be present in the cultivar AT 3 (11.6 µmol h^{-1} g^{-1} fw) under biotic stress condition amongst the all samples (Table 6). PAL activity was found to be increased significantly upon transition from control environment (6.7 µmol h^{-1} g^{-1} fw and 7.8 µmol h^{-1} g^{-1} fw) to disease condition (11.6 µmol h^{-1} g^{-1} fw and 10.4 µmol h^{-1} g^{-1} fw) in both the cultivars AT 3 (susceptible) and SL 120 (resistant) respectively. These results supported the facts reported by Gao *et al.,* (2008) [13], Chandra *et al.,* (2007) [8] and Rivero *et al.,* (2001) [28].

Table 2: Biochemical parameters from roots of tomato cultivars

Samples	Moisture Content (%)	Total Protein Content (%)	Total Phenol Content (%)
AT 3 (Control)	87.67	2.46	0.10
AT 3 (Treated)	82.06	3.56	0.26
SL 120 (Control)	90.46	2.88	0.31
SL 120 (Treated)	89.46	3.00	0.36
S. Em.	0.27	0.07	0.01
C. D.	0.87	0.24	0.01

Table 5: Total anti-oxidant activity from roots of tomato cultivars

Samples	Total Anti-oxidant Activity (µg of Ascorbic Acid/ g FW)
AT 3 (Control)	8.09
AT 3 (Treated)	19.43
SL 120 (Control)	13.40
SL 120 (Treated)	15.54
S. Em.	0.18
C. D.	0.57
C. V. %	2.14

Table 6: Enzyme activity from roots of tomato cultivars

Samples	Enzyme Activity		
	PPO*	POX^	PAL#
AT 3 (Control)	3.30	65.78	6.7
AT 3 (Treated)	4.40	88.12	11.6
SL 120 (Control)	4.12	102.33	7.8
SL 120 (Treated)	6.27	111.73	10.4
S. Em.	0.30	4.85	0.127
C. D.	0.99	15.81	0.415
C. V. %	11.61	9.13	2.42

Note: *PPO activity is measured as Change in OD (at 490 nm)/ min/ g FW ^POX activity is measured as Change in OD (at 460 nm)/ min/ g FW #PAL activity is measured as µmol h^{-1} g^{-1} f

Table 3: Phenolic acid profile by UPLC in roots of tomato cultivars.

Samples	Phenolic Acids (µg / g FW)											
	Gal	pro-Cat	pHB	Chl	Caf	Van	p-Cou	Fer	Sin	Sal	Ell	Cin
AT-3 (Control)	1.41	1.46	3.55	5.78	2.12	2.04	1.17	1.20	5.44	15.10	2.79	1.20
AT-3 (Treated)	1.80	3.66	3.60	15.84	2.53	4.69	1.24	1.43	18.50	16.99	7.89	1.01
Sl-120 (Control)	1.04	2.12	1.17	1.69	2.04	1.65	1.06	1.14	1.49	7.15	2.99	0.97
Sl-120 (Treated)	1.58	3.17	2.23	2.68	1.93	2.04	1.02	1.63	4.89	13.96	2.39	1.07
S. Em.	0.05	0.04	0.03	0.09	0.01	0.03	0.01	0.01	0.07	0.49	0.25	0.06
C. D.	0.17	0.13	0.11	0.29	0.03	0.08	0.02	0.04	0.22	1.61	0.83	NS
C. V. %	6.20	2.70	2.13	2.34	0.77	1.65	0.84	0.49	1.52	6.43	10.93	9.77

Table 4: Amino acid profile by UPLC in roots of tomato cultivars

Samples	Amino Acids (mg / 100 g)												
	THR	MET	ILE	LEU	PHE	SER	GLY	ASP	GLU	ALA	PRO	CYS	TYR
AT-3 (Control)	15.53	8.47	14.01	24.72	14.53	21.35	17.37	39.36	44.96	32.68	25.24	59.03	24.40
AT-3 (Treated)	32.55	27.37	29.16	53.41	30.28	44.89	40.83	85.82	113.88	99.57	50.39	113.62	63.97
Sl-120 (Control)	20.37	17.15	17.88	34.04	18.95	26.57	21.97	53.92	77.25	44.16	35.21	91.24	34.97
Sl-120 (Treated)	23.65	25.56	20.30	38.31	22.20	30.36	24.40	58.08	73.37	46.93	52.83	98.02	37.83
RSD	0.11	0.21	0.12	0.12	0.12	0.07	0.79	0.12	0.15	0.13	0.10	2.50	0.45

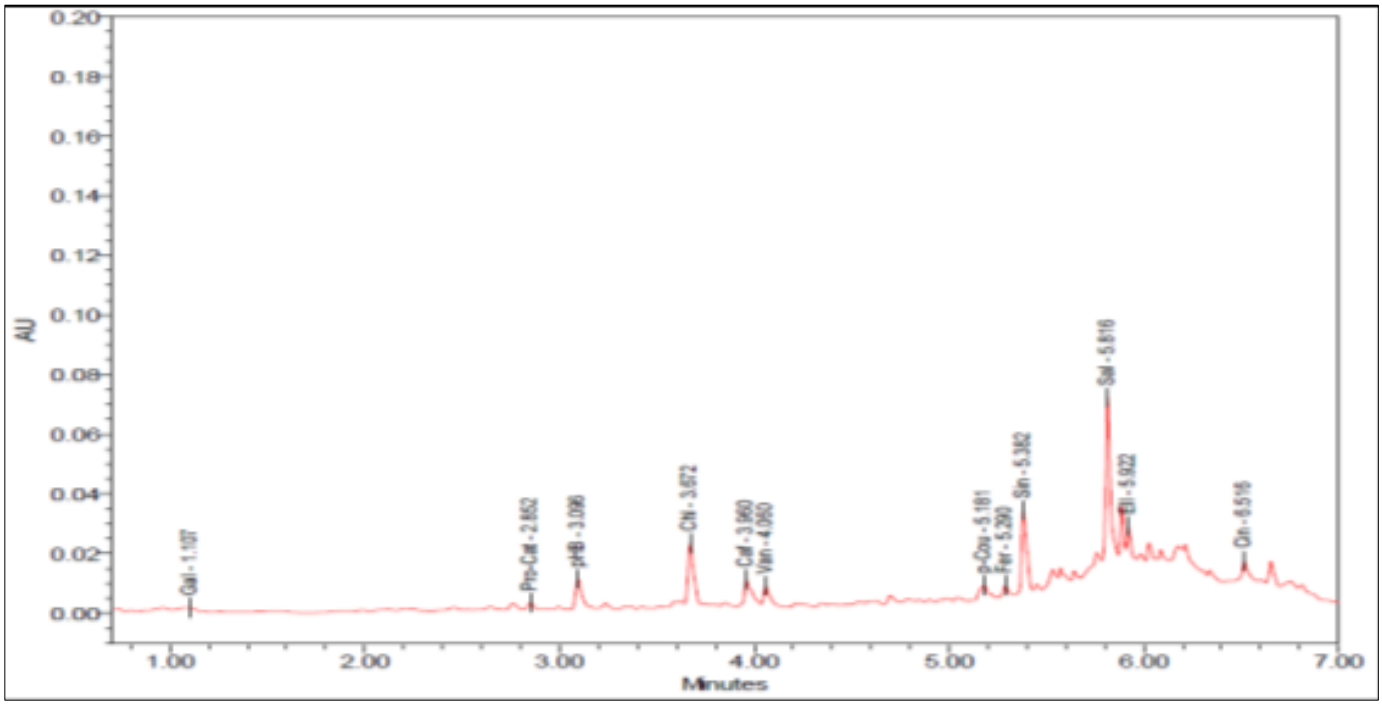

Plate 1.1: UPLC chromatogram of phenolic acid for AT - 3 (Control)

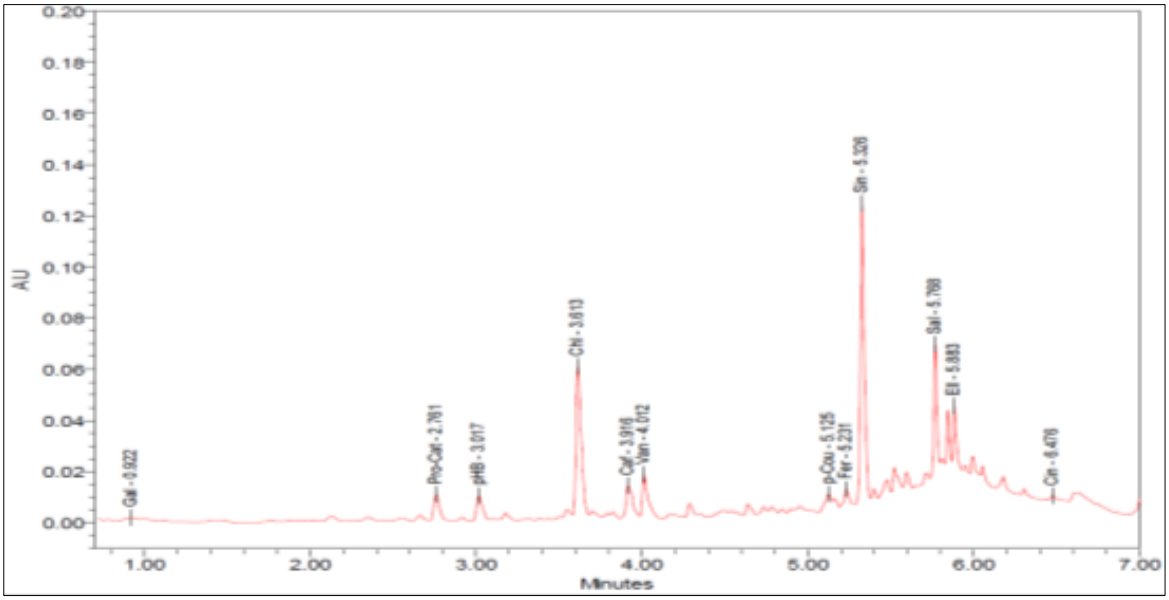

Plate 1.2: UPLC chromatogram of phenolic acid for AT - 3 (Treated)

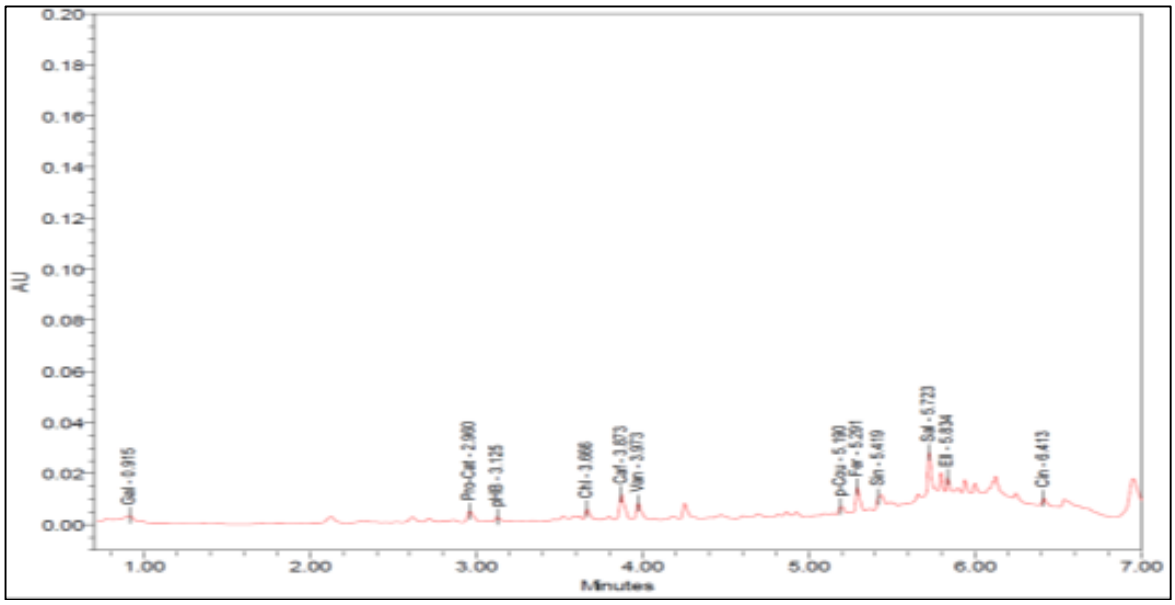

Plate 1.3: UPLC chromatogram of phenolic acid for Sl - 120 (Control)

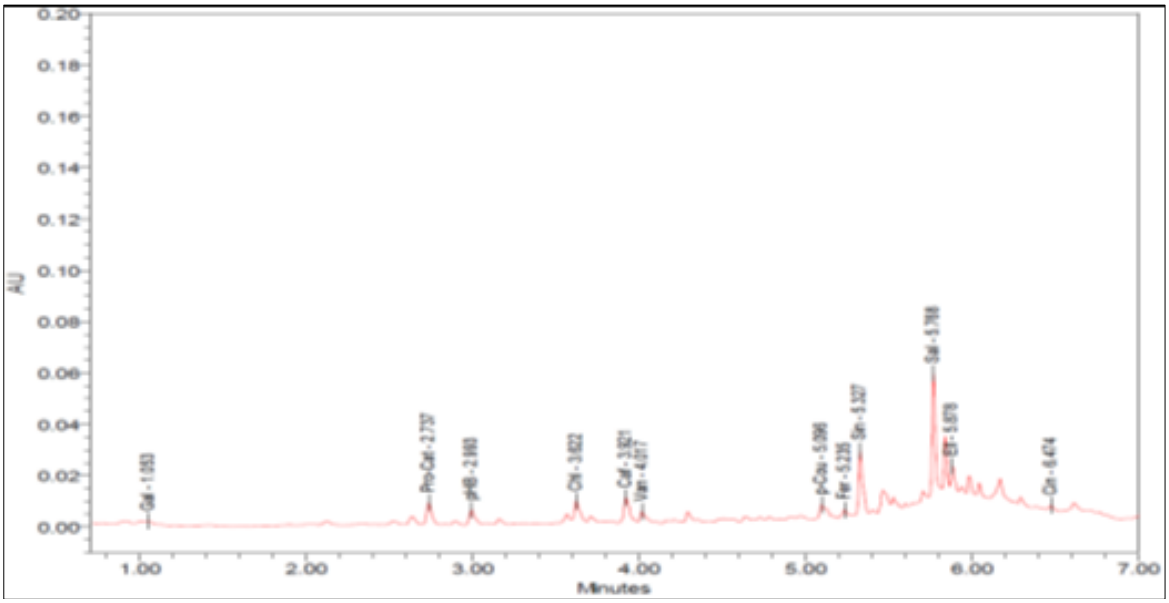

Plate 1.4: UPLC chromatogram of phenolic acid for Sl - 120 (Treated)

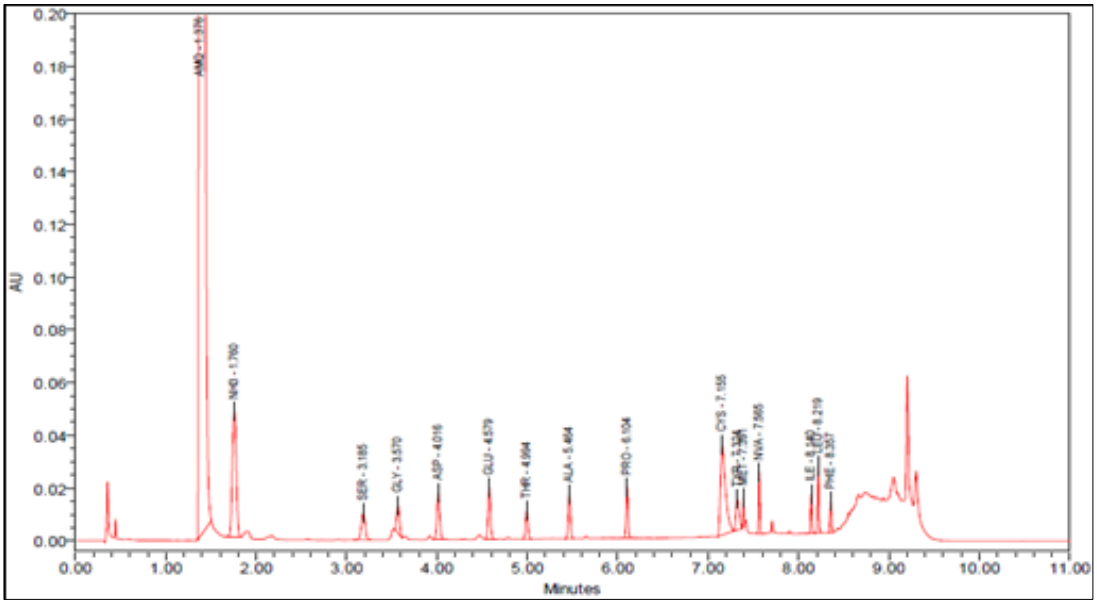

Plate 2.1: UPLC chromatogram of amino acids for AT - 3 (Control)

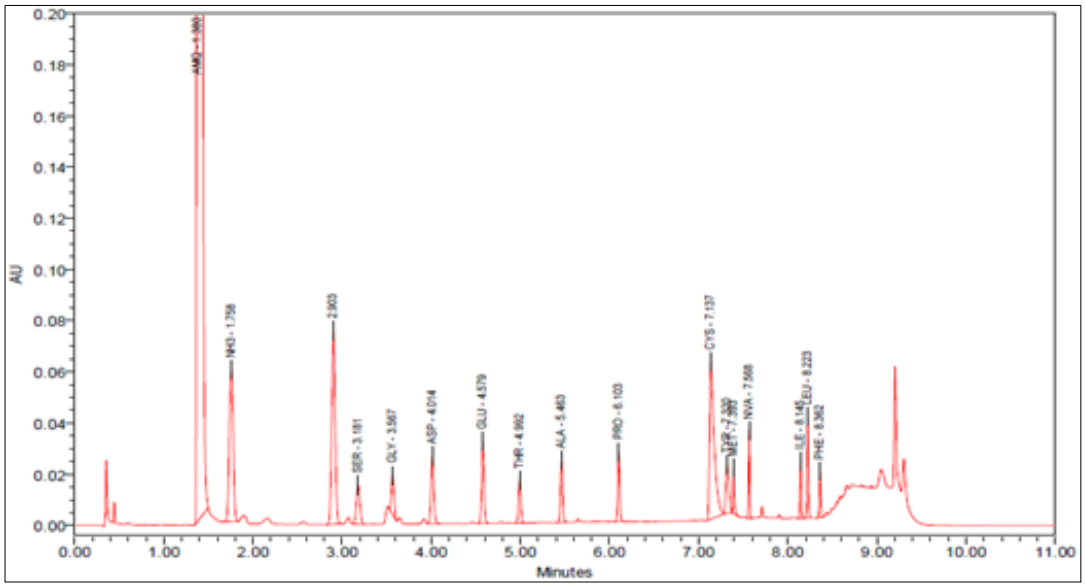

Plate 2.2: UPLC chromatogram of amino acids for Sl - 120 (Treated)

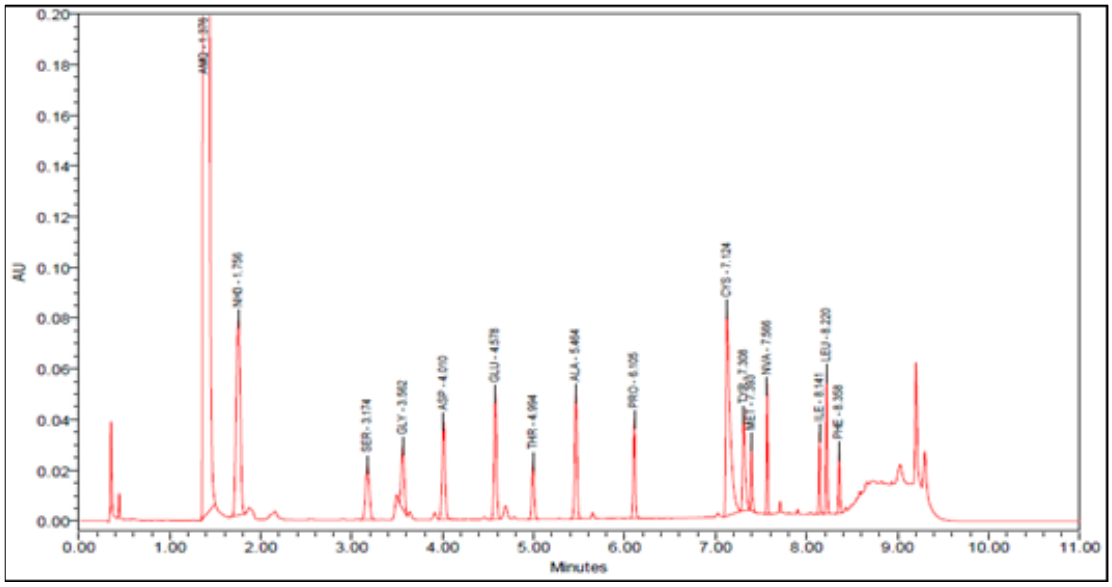

Plate 2.3: UPLC chromatogram of amino acids for AT - 3 (Treated)

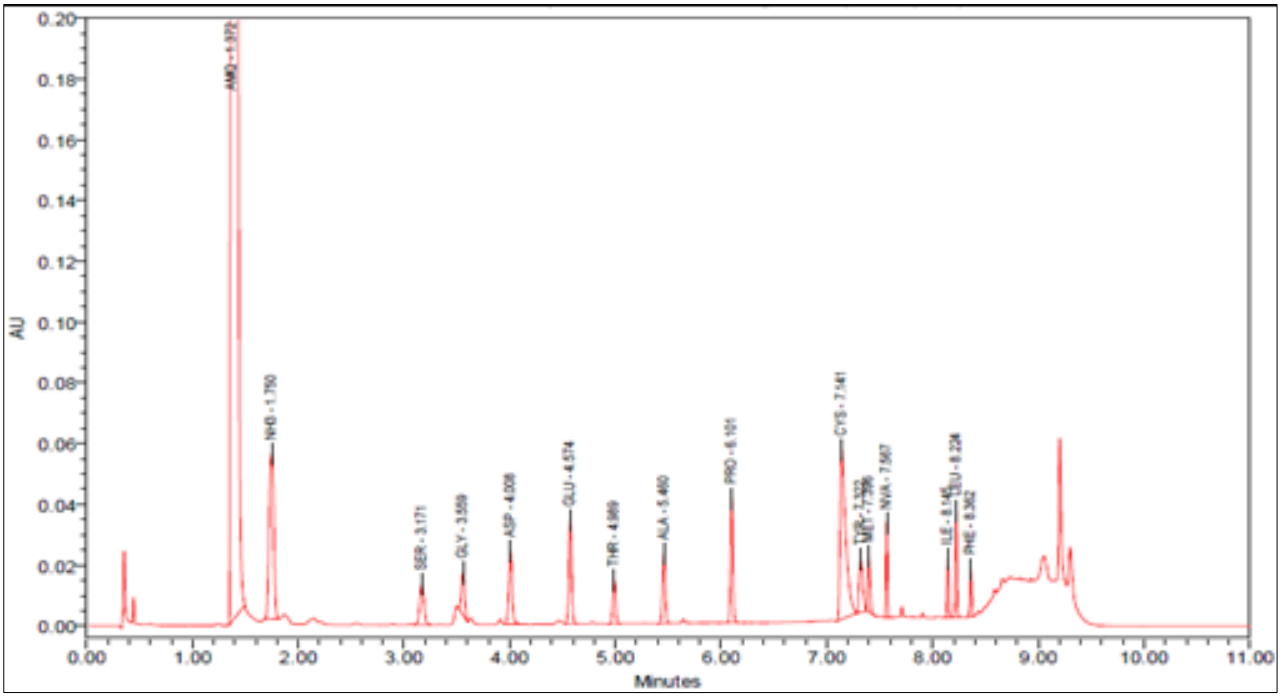

Plate 2.4: UPLC chromatogram of amino acids for Sl - 120 (Control)

Conclusion

The present investigation entitled "Biochemical changes during root knot nematode *(Meloidogyne incognita)* infection in tomato *(Solanum lycopersicum* L.*)*" was undertaken to enhance understanding regarding the biochemical changes that takes place in the tomato seedlings under root knot biotic stress. Better understanding about the exact mechanism of plant response towards such biotic stress can help to improve screening strategies for the selection of resistant cultivars. Biochemical characterization of tomato cultivars under control and disease conditions revealed the following results like moisture content of roots of both susceptible and resistant tomato cultivars under control and disease conditions ranged from 82.06% - 90.46%. Highest moisture content was observed in the resistant cultivar SL 120 under control condition (90.46%) while significant drop in moisture content was observed in susceptible AT 3 (Treated) under disease condition (82.06%). The total protein content of roots of both susceptible and resistant tomato cultivars under control and disease conditions ranged from 2.46% - 3.56%. Highest total protein content was observed in the susceptible cultivar AT 3 under disease condition (3.56%). The total phenol content of roots of both susceptible and resistant tomato cultivars under control and disease conditions ranged from 0.1% - 0.36%. Highest total phenol content was observed in the resistant cultivar SL 120 under disease condition (0.36%).

Amino acid profile by UPLC revealed that glutamic acid (113.88 mg/100 g), cysteine (113.62 mg/100 g), alanine (99.57 mg/100 g), aspartic acid (85.82 mg/100 g), tyrosine (63.97 mg/100 g), leucine (53.41 mg/100 g) and serine (44.89 mg/100 g) showed significant rise in their quantity under transition from control to disease conditions in susceptible cultivar AT 3. Proline had shown significant increment in its quantity under disease condition in both the cultivar making it a good stress responsive amino acid.

During oxidation free radicals are produced which can damage the cells. Antioxidants are responsible for terminating the chain reaction by removing free radicals. Disease condition showed significantly higher (19.43 mg of ascorbic acid g^{-1} FW) antioxidant activity as compared to control (8.09 mg of ascorbic acid g^{-1} FW) in roots of AT 3 cultivar.

Enzyme activity of polyphenol oxidase (PPO), peroxidase (POX) and phenylalanine ammonia liase (PAL) were analysed and results revealed that under root knot biotic stress, peroxidase had shown the maximum activity amongst the three enzymes and was recorded highest in resistant cultivar SL 120 (111.73 ΔOD/min/ g FW). PPO activity was also found to be highest in resistant cultivar SL 120 under disease condition (6.27 ΔOD/min/ g FW). Maximum PAL activity was found to be present in the cultivar AT 3 (11.6 µmol h^{-1} g^{-1} fw) under biotic stress condition.

References

1. Abad P, Gouzy J, Aury JM, Castagnone-Sereno P, Danchin EG, Deleury E *et al*. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature biotechnology. 2008; 26:909-915.
2. Afifi AMR, Al-Sayed AA, Mahfoud NAM, Farahat AA. Enzymatic and non-enzymatic oxidants and antioxidants involved in defense mechanisms against root-knot, reniform and citrus nematodes in their hosts. Egypt. J Agronematol. 2014; 13(1):172-188.
3. Anguelova T, Warthesen J. Lycopene stability in tomato powders. Food Chem. Toxicol. 2000; 65:67-70.
4. OAC. Association of Official Analytical chemists, 17th Ed. Official Methods of Analysis, Washington, DC, USA, 2000.
5. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry. 2001; 73:239-244.
6. Baker CJ, Owens RA, Whitaker BD, Mock NM, Roberts DP, Deahl KL *et al*. Effect of viroid infection on the dynamics of phenolic metabolites in the apoplast of tomato leaves. Physiol. Mol. Plant Pathol. 2010; 74:214-220. Doi: 10.1016/j.pmpp.2010.02.001.
7. Bray HG, Thorpe WV. Meth. Biochem. Anal. 1954; 1:27-52.
8. Chandra A, Saxena R, Dubey A, Saxena P. Changes in phenylalanine ammonia lyase activity and isozyme

patterns of polyphenol oxidase and peroxidase by salicylic acid leading to enhanced resistance in cowpea against *Rhizoctonia solani*. Acta Physiologiae Plantarum. 2007; 29:361-367.
9. Choudhary K, Chawla N, Kaur S, Jindal S. Analysis of biochemical parameters in tomato fruits before and after inoculation with root knot nematode *(Meloydogyne incognita)*. Vegetable Science. 2013; 40(2):178-181.
10. Dawson P, Weste G. Impact of root infection by *Phytophthora cinnamomi* on the water relations of two Eucalyptus species that differ in susceptibility. Phytopathology. 1984; 74:486-490.
11. Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL. Phenylalanine ammonia-lyase and hydroxyl cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiol. Plant Pathol. 1984; 25:111-123.
12. El-Beltagi HS, Farahat AA, Alsayed AA, Mahfoud NA. Response of antioxidant substances and enzymes activities as a defense mechanism against root-knot nematode infection. Not. Bot. Hort. Agrobot. Cluj. 2012; 40(1):132-142.
13. Gao S, Ouyang C, Wang S, Xu Y, Tang L, Chen F. Effects of salt stress on growth, antioxidant enzyme phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. Plant Soil Environ. 2008; 54:374-381.
14. Garcia R, Gonzalez CA, Agudo A, Riboldi E. High intake of specific carotenoids and flavonoides does not reduce the risk of bladder cancer. Nutr. Cancer. 1999; 35:212-14.
15. Giovanelli G, Paradise A. Stability of dried and intermediate moisture tomato pulp during storage. Journal of Agriculture and Food Chemistry. 2002; 50:7277-7281.
16. Giovanucci E. Tomatoes, tomato-based products, lycopene and cancer: Review of the epidemiologic literature. J Nat. Cancer Inst. 1999; 91:317-31.
17. Lorenz OA, Maynard DN. Knott's Handbook for Vegetable Growers. John Wiley and sons. New York. 1997; 3(23-38):341-342.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein mesurement with the Folin phenol reagent. J biol. chem. 1951; 193:265-275.
19. Mariutto M, Duby F, Adam A, Bureau C, Fauconnier ML, Ongena M *et al*. The elicitation of a systemic resistance by *Pseudomonas putida* BTP1 in tomato involves the stimulation of two lipoxygenase isoforms. BMC Plant Biology. 2011; 11:29.
20. Martinez-Valvercle I, Periage MJ, Provan G, Chesson A. Phenolic compounds, Lycopene and antioxidant activities in commercial varieties of tomato (*lycopersicon esculentum*). Journal of the Science of Food and Agriculture. 2002; 82:323-330.
21. Mayer AM, Harel E, Shaul RB. Assay of catechol oxidase a critical comparison of methods. Phytochemistry. 1965; 5:783-789.
22. Oderonke I. Comparison of the quality aspects of organic and conventional green beans (*Phaseolus vulgaris* L.). An M. Sc. thesis submitted to the University of Guelph, Ontario, Canada, 2012.
23. Odjakova M, Hadjiivanova C. The complexity of pathogen defense in plants. Bulgarian Journal of Plant Physiology. 2001; 27:101-109.
24. Oka Y, Koltai H, Bar-Eyal M, Mor M, Sharon E, Chet I *et al*. New strategies for the control of plant-parasitic nematodes. Pest Management Science. 2000a; 56:983-988.
25. Oka Y, Nacar S, Putievsky E, Ravid U, Yaniv Z, Spiegel Y. Nematicidal activity of essential oils and their components against the root-knot nematode. Journal of Phytopathology. 2000b; 90:710-715.
26. Rani IC, Veeraragavathatham, Sanjutha S. Analysis on biochemical basis of root knot nematode *(Meloidogyne incognita*) resistance in tomato (*Lycopersicon esculentum* Mill.). Res J Agric Biol Sci. 2008; 4(6):866-70.
27. Reuveni R. Biochemical markers as tools for screening resistance against plant pathogens, In: Reuveni, R. (Eds.), Novel Approaches to Integrated Pest Management, CRC Press, Boca Raton, FL, 1995, 21-45.
28. Rivero RM, Ruiz JM, Garcia PC, Lopez-Lefebre LR, Sanchez E, Romero L. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. Plant Sci. 2001; 160:315-321.
29. Ryan CA, Jagendorf A. Self defense by plants. Proceedings of the National Academy of Sciences of the United States of America. 1995; 92:4075.
30. Shreenivasa KR, Krishnappa K, Rekha D. Interaction effect of arbuscular mycorrhizal fungus, *Glomus fasciculatum* and root knot nematode *meloidogyne incognita* on biochemical parameters in tomato. I. J. S. N. 2011; 2(3):534-537.
31. Singh S, Gupta AS, Kaur N. Influence of drought and sowing time on protein composition, antinutrients and mineral contents of wheat. The Scientific World Journal. 2012. DOI:10.1100/2012/485751.
32. Splittstoesser WE. Vegetable Growing Handbook: Organic and Traditional Methods. *Vannostrand Reinbold*, New York. 1990; 3:167-171.
33. Sreedhara HS, Nandini BA, Shetty SA, Shetty HS. Peroxidase activities in the pathogenesis of *Sclerospora graminicola* in pearl millet seedlings. Int. J tropical plant diseases. 1995; 13:19-32.
34. Stahl W, Ulrike H, Sheila W, Olaf E, Helmut S. Dietary tomato paste protects against ultraviolet light- induced erythema in humans. J Nut. 2001; 131:1449-1451.
35. Su CJM, Bui A, Kardinaal J, Gomez-Aracena J, Martin-Moreno B, Martin M *et al*. Differences between plasma and adipose tissue biomarkers of carotenoids and tocopherols. *Cancer epidemiol.* Biomarkers Prev. 1998; 7:1056.
36. Tripathi RD. Plant response to environmental stress. International Book Distributing Company, 2006.