

Original Research Article

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Influence of Root Knot Nematode (*Meloidogyne* spp.) on Phenolic Acid Profile in Root of Tomato (*Solanum lycopersicum* L.)

Vyomesh S. Patel^{1*}, Y.M. Shukla² and J.J. Dhruve¹

¹Department of Biochemistry, BACA, Anand Agricultural University, Anand-388110, Gujarat, India

²College of Agriculture, Anand Agricultural University, Vaso-387380, Gujarat, India

*Corresponding author

ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are destructive to tomatoes. Three species of root-knot nematodes, *M. incognita*, *M. javanica* and *M. hapla* are the most widely distributed nematode pathogens of tomato. Root-knot nematodes (*Meloidogyne* spp.) found to be very fatal infective agents and cause severe yield losses. Emphasis is needed on breeding to develop root knot nematode resistant variety. This study was undertaken to find out the potential source of resistance of tomato under natural epiphytic condition. The experiment was conducted with two cultivars of tomato i.e., AT 3 (susceptible) and SL 120 (resistant) at Department of Biochemistry and Department of Nematology, B.A. College of Agriculture, Anand Agricultural University, Anand. Both tomato cultivars AT 3 (susceptible) and SL 120 (resistant) were grown under control and root knot nematode (3000 J₂ stage larvae per plant) inoculated soil and the roots of tomato were analyzed for different phenolic acid compounds during control and disease conditions. The total phenol content was accumulated more in disease condition as compared to control condition. However, significantly the highest total phenol content was recorded for SL 120 (0.36%) under disease conditions. Total twelve phenolic acids were identified and quantified by UPLC under control and disease conditions. Out of which gallic acid, protocatechuic acid, chlorogenic acid, vanillic acid, ferulic acid, sinapic acid and salicylic acid were accumulated in disease conditions as compared to control condition. Salicylic acid was the only phenolic acid found to be increased the most during disease condition as compared to control in resistant cultivar SL 120. The non-significant difference was observed for p-hydroxybenzoic acid in susceptible cultivar AT 3 upon transition from control condition to disease condition and reverse trend was recorded for ellagic acid and caffeic acid in resistant cultivar SL 120.

Keywords

Chlorogenic acid, *Meloidogyne incognita*, Phenolic acid, Root knot nematode, Sinapic acid, *Solanum lycopersicum*, UPLC.

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Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crops worldwide, owing to its high nutritive value and diversified use. Plant parasitic nematodes are important pests of tomato and cause huge economic losses (Bird and Kaloshian, 2003). India is the second largest tomato 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). Tomato is affected by various disease caused mainly by fungi, bacteria and nematodes. 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). The occurrence and metabolism of phenolic substances in plants, in response to injury or invasion by pathogens, such as fungi, bacteria and viruses, have been studied extensively (Hung and Rohde, 1973). However, Krishnappa (1985) reported from India that *M. incognita* and *M. javanica* have the widest host range infecting more than 232 and 114 genera of plants respectively. Various scientists (Patel *et al.*, 1986 and Prasad 1960) have reported that 82.5% plants of tomato, brinjal and okra infested with *M. incognita* and *M. javanica* and parasitization of the wheat roots by *M. javanica* in Gujarat. During biotic stress condition, plant under goes various molecular changes to cope up with the altered environment. One such change was in phenylpropanoid metabolism which involved in the production of phenolics and phytoalexins that prevent establishment of the pathogen (Mariutto *et al.*, 2011). Till date very limited information was available for tomato-root knot nematode infection on the biochemical and molecular changes especially phenolic acid profile of plants. This information may help us to develop new strategies for breeding of resistant line of tomato for root knot nematode infection.

Materials and Methods

The present investigation was carried out at Department of Biochemistry and Department of Nematology; B. A. College of Agriculture; Anand Agricultural University; Anand during 2015 to 2017. Two tomato cultivars AT 3 (susceptible) and SL 120 (resistant) were grown under normal (control) and disease (inoculated with J2 stage larvae) condition in pots. Tomato seedlings were infected at the stage of three true leaves with inoculum density of 3000 J₂ stage larvae/ plant at the base of each plant in small holes. 45 days

after infection, the tomato plants were carefully removed and the root systems washed free of soil and were used for biochemical analysis. The treatment details are as follows.

T₁ = control (seedlings of AT 3 grown in uninoculated sterile soil)

T₂ = Disease (seedlings AT 3 grown in soil inoculated with Root knot nematodes (3000 J₂ stage larvae / plant).

T₃= control (seedlings of SL 120 grown in uninoculated sterile soil)

T₄= Disease (seedlings of SL 120 grown in soil inoculated with Root knot nematodes (3000 J₂ stage larvae / plant).

Moisture content was estimated as per A. O. A. C. (2000) using randomly selected roots from each treatment. Total phenol was estimated by the method described by Bray and Thorpe (1954) with some modifications.

One gram of roots sample was homogenized in 80% methanol using mortar and pestle and final volume was made to 10 ml. The content was refluxed for two hour on boiling water bath at 65°C. Supernatant was collected and residue was re-extracted twice with 80% methanol. All supernatant were combined and final volume was made to 25 ml. The extract was used for the assay of total phenol.

Phenol profiling by Ultra Performance Liquid Chromatography (UPLC)

Precisely, known amount (1 g) of each sample was homogenized in 80% methanol. The extracted sample was concentrated with diethyl ether, 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. A reverse phase AccQ Tag Ultra BEH C18 column (1.7µm, 2.1 mm x 100 mm) was used with a gradient of 0.1% Formic acid (Solvent A) and 95% Methanol (Solvent B). The chromatographic separations were obtained using multi steps gradient system (Table 1) and a column and auto sampler temperature were held at 55 °C and 5 °C, respectively. PDA spectra were measured over a wavelength range of 200-400nm and detection set at 280 nm. Limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting a series of dilute solutions with known concentrations on the basis of signal to noise ratio equal to around 3 and 10, respectively. The linearity standard curves were obtained by running three injections of standards mixture at 2, 5, 10, 25 and 50 ppm (Figure 4). Empower 3 software was used for data acquisition and analysis. Peaks were identified by comparing retention times of phenolic standards and peak area was calculated automatically by integrating the peak with apex type.

Results and Discussion

Moisture plays an important role in seed germination and emergence. The analysis of seed revealed a significant (p<0.05) variation (82.06 – 90.46%) in root moisture content among all treatments (Fig. 1). Significantly the lowest moisture content was recorded

with treatment T₂, while the highest moisture content was recorded with treatment T₃. There was reduction of moisture content during transition from control to disease environment. It was also found that the rate of reduction of moisture content was higher i.e. 5.61% between treatment T₁ and T₂, while in case of the reduction rate of moisture content was found 1.0% between treatment T₃ and T₄. These results are in agreement with the results observed by Dawson and Weste (1984).

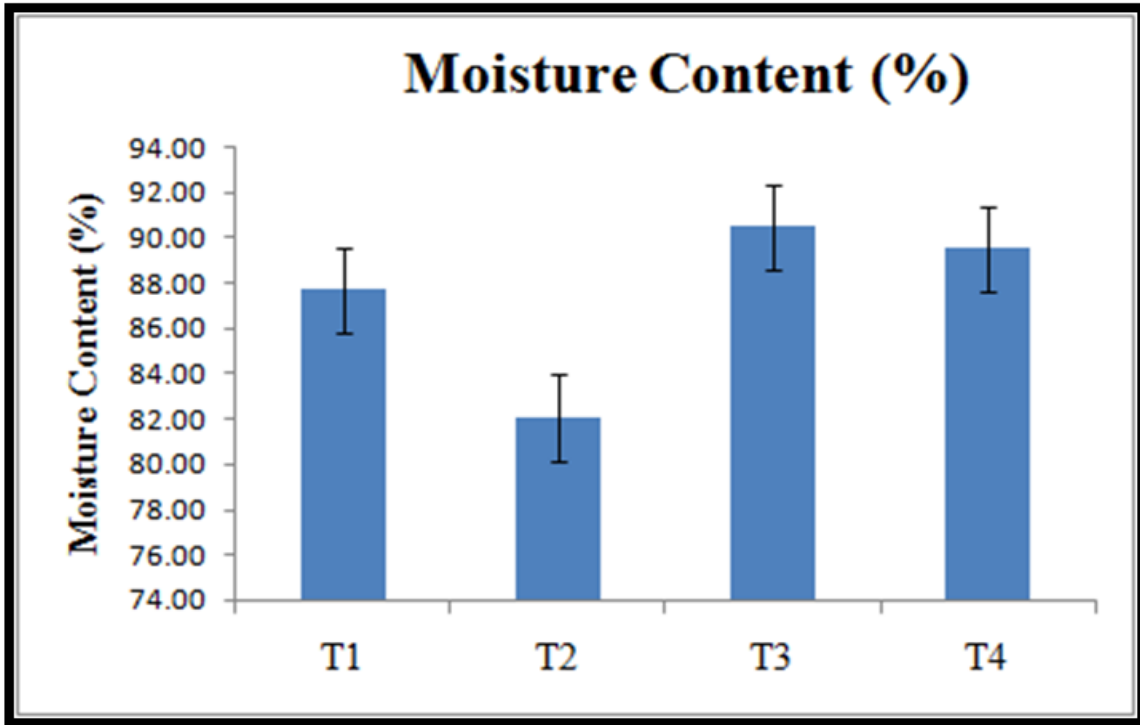
The data on phenol content of tomato roots were presented in Figure 2. The maximum and minimum content of phenol was recorded for T₁ (0.1%) and T₄ (0.36%), respectively. The total phenol content was higher in disease condition as compared to control. There was rise in total phenol content in both susceptible and resistant cultivar during transition from control to disease environment. There was a significant rise in total phenol content in susceptible cultivar AT 3 under disease condition (0.26%) i.e. T₂ as compared to the AT 3 (control) under normal condition (0.1%) i.e. T₁. The results indicate that high phenolic may provide a source of dietary anti-oxidants. The phenolic compounds may contribute directly to the antioxidant action; therefore, it is necessary to investigate total phenolic content (Syeda *et al.*, 2008). These results are in agreement with the results observed by Shreenivasa *et al.*, 2011 and Choudhary *et al.*, 2013.

Table.1 Gradient system of mobile phase used for phenol analysis

Time (min)	Flow Rate (ml/min)	%A	%B	Curve
Initial	0.5	99.0	1.0	Initial
0.50	0.5	99.0	1.0	6
3.20	0.5	75.0	25.0	6
3.50	0.5	70.0	30.0	6
4.00	0.5	65.0	35.0	6
5.00	0.5	30.0	70.0	6
5.50	0.5	30.0	70.0	6
7.00	0.5	99.0	1.0	6
7.50	0.5	99.0	1.0	6

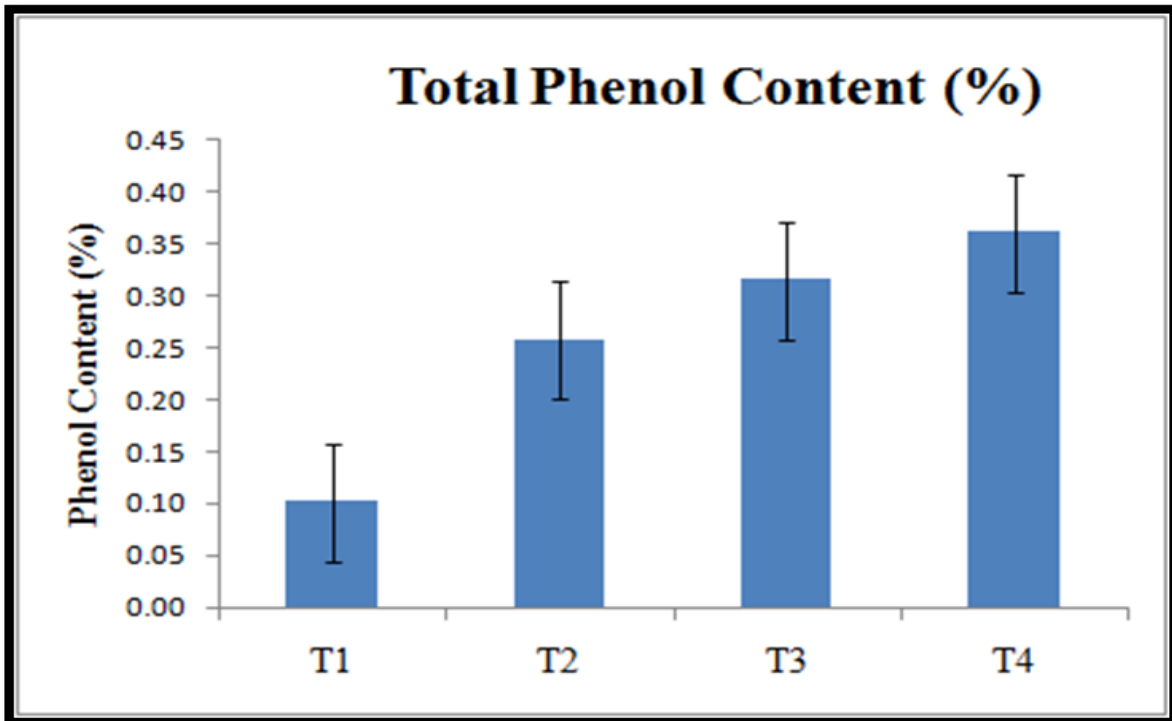
Where; A = 0.1% Formic Acid; B = 95% Methanol

Fig.1 Moisture content of roots of tomato cultivars



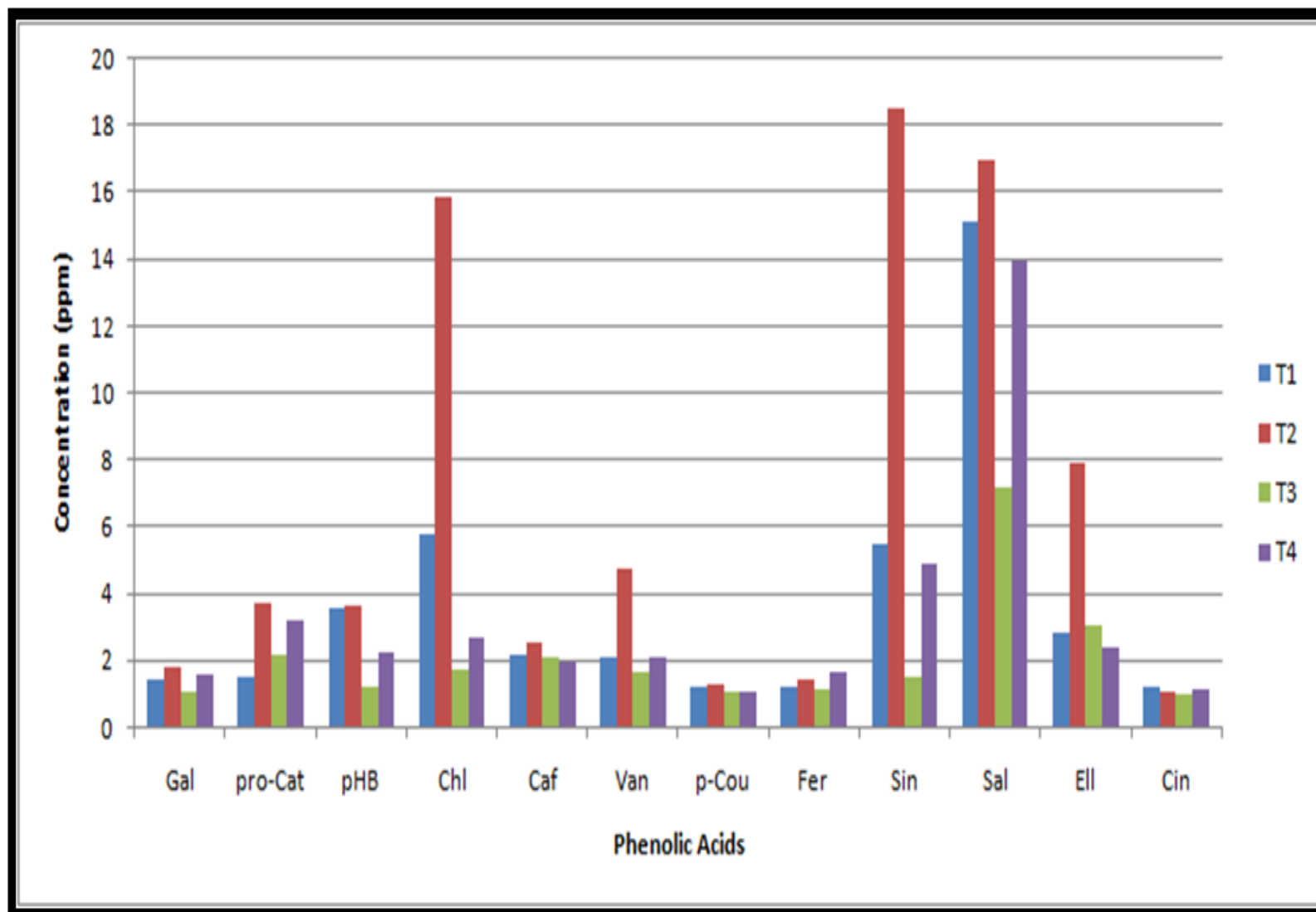
T₁: AT 3 (Control); T₂: AT 3 (Disease); T₃: SL 120 (Control); T₄: SL 120 (Disease)

Fig.2 Total phenol content of roots of tomato cultivars



T₁: AT 3 (Control); T₂: AT 3 (Disease); T₃: SL 120 (Control); T₄: SL 120 (Disease)

Fig.3 Phenolic acids profile by UPLC in roots of tomato cultivars



T₁: AT 3 (Control); T₂: AT 3 (Disease); T₃: SL 120 (Control); T₄: SL 120 (Disease)

Fig.4 UPLC overlay chromatogram of phenolic acid standard mixture

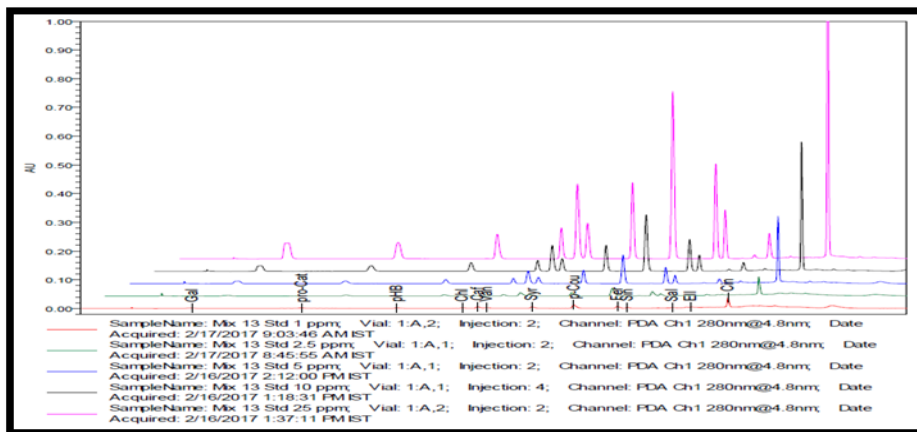


Fig.5 UPLC chromatogram of phenolic acid for T₁ i.e. AT - 3 (Control)

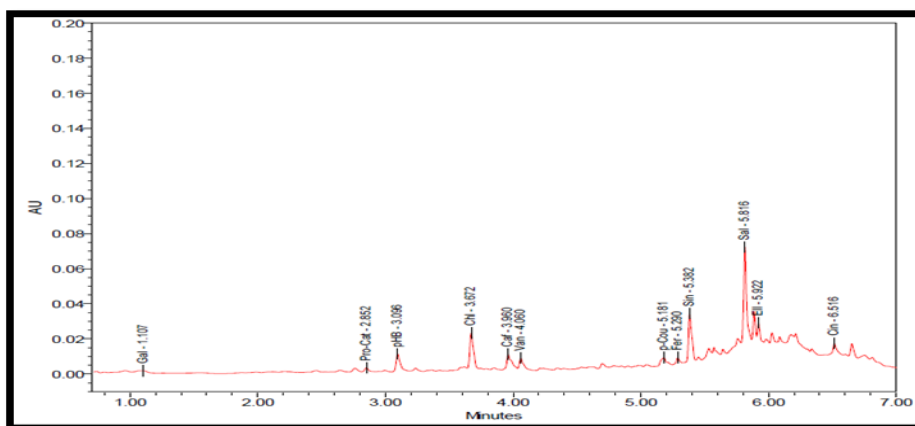


Fig.6 UPLC chromatogram of phenolic acid for T₂ i.e. AT - 3 (Disease)

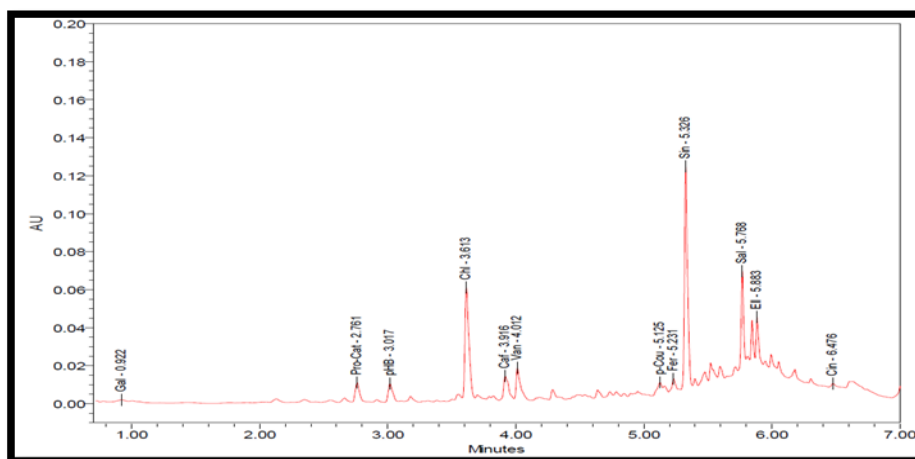


Fig.7 UPLC chromatogram of phenolic acid for T₃ i.e. SL - 120 (Control)

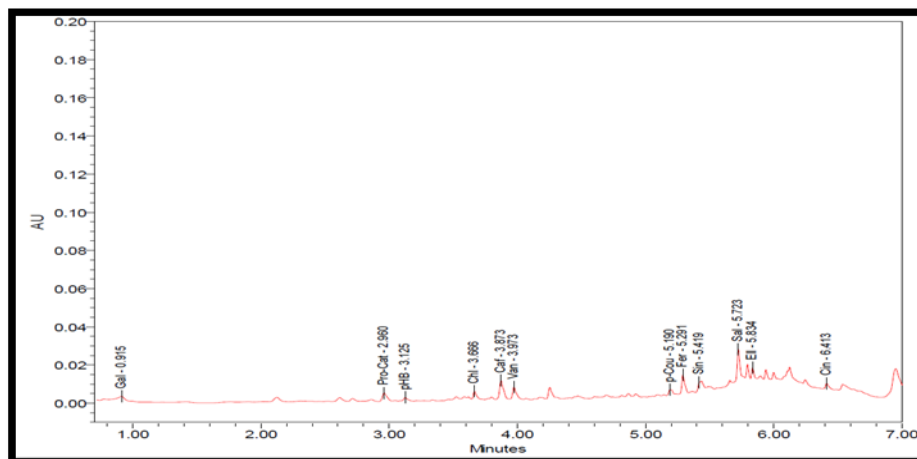
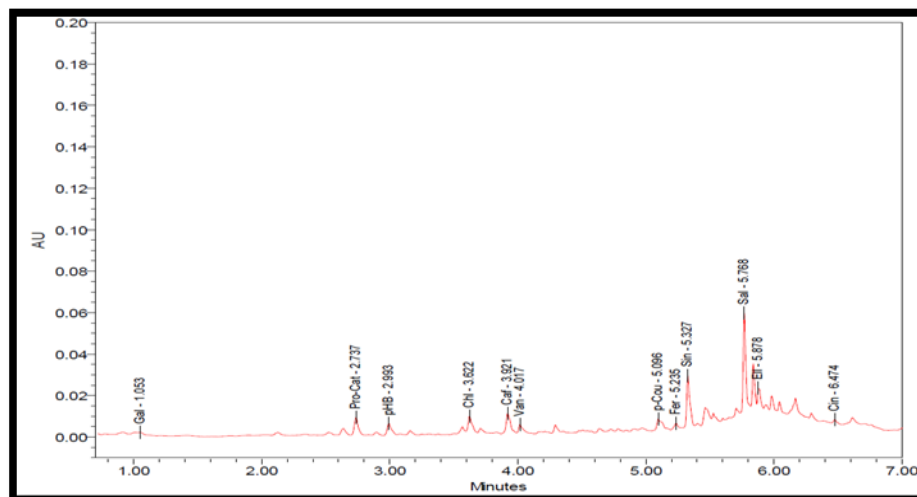


Fig.8 UPLC chromatogram of phenolic acid for T₄ i.e. SL - 120 (Disease)



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 Appendix I. The external standard method in which reference compounds were analysed under similar chromatographic conditions separately from samples was used for quantification purpose.

In the present study total twelve phenolic acids were identified and quantified in the

resistant and susceptible cultivars under control and root knot disease conditions in tomato root. 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). UPLC analysis confirmed the presence of several phenolic acids in roots of tomato with all the treatments (Fig. 5, 6, 7 & 8). Sinapic acid content was ranged from 4.89 – 18.50 ($\mu\text{g} / \text{g FW}$). It was found to be the highest (18.50 $\mu\text{g} / \text{g FW}$) in AT 3

(disease) i.e. T₂ among the all root samples and found to be the one with higher 3.4 folds change from control to disease condition (Figure 3). 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 (Fig. 3). Reverse trend was observed for ellagic acid and caffeic acid in resistant cultivar SL 120. Vanillic acid (4.69 µg / g FW) had shown significant increased under root-knot biotic stress as compared to control condition (2.05 µg / g FW) in susceptible cultivar AT 3. The non-significant difference was observed for p-hydroxybenzoic acid in susceptible cultivar AT 3 upon transition from control condition to disease condition. 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 and cinnamic acid did not differ much in their quantity between control and disease conditions in both the cultivars (Figure 3). These results were in agreement with the results obtained by Baker *et al.*, (2010).

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