Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 1093-1096
Received: 09-05-2017
Accepted: 10-06-2017

Apexa Pareek
Research Scholar,
Plant Pathology and Tissue Culture Laboratory Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Arvind Gaur
Research Scholar,
Plant Pathology and Tissue Culture Laboratory Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Anuj Kumar
Research Scholar,
Plant Pathology and Tissue Culture Laboratory Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Payal Lodha
Associate Professor,
Plant Pathology and Tissue Culture Laboratory Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Correspondence
Apexa Pareek
Research Scholar,
Plant Pathology and Tissue Culture Laboratory Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Impacts of root knot nematode infestation on metabolites and antioxidant enzymes in *Abelmoschus esculentus* L. Moench

Apexa Pareek, Arvind Gaur, Anuj Kumar and Payal Lodha

Abstract
Root knot nematode Meloidogyne incognita is most damaging pest to all vegetable crops and cause losses in yield. Okra crop is widely suffers from root knot nematode Meloidogyne incognita. During infection, plant elicits its defence mechanisms. Defence mechanism includes various cellular activities, metabolic changes to overcome this adverse condition. In present study we study the changes in metabolites content like total protein, total carbohydrates and total protein of infected plant in response to root knot nematode and also study the activities of oxidative enzymes. The results indicated that the infestation of nematode increased the activity of peroxidase, polyphenol oxidase, and Phenylalanine ammonia lyase.

Keywords: Meloidogyne incognita, Peroxidase, Polyphenol oxidase, Okra, Protein

Introduction
Okra, *Abelmoschus esculentus* is commonly known as Bhindi in India and is very important vegetable crop. It is widely cultivated in tropical and subtropical countries [1]. India stands first rank in production of okra [2]. The okra plant is widely suffers from various pathogen such as virus, fungi, bacteria and root knot nematode. Among them Root Knot Nematode is serious pest and greatly decrease 16.9% yield of crop annually [3-6]. Root knot nematode affects the root system of plant, results in gall formation. Due to host-pathogen (nematode) interaction, Plant produces various kinds of biologically active compounds that are involved in plant defence against pest. Defence mechanism of plants involves the production of reactive oxygen species (ROS) such as singlet oxygen, superoxide ion, hydroxyl radical and hydrogen peroxide which are harmful to plant it. ROS are co-product of various metabolic pathways [7]. Under stress conditions like pathogen attack, the production of ROS is increased which react with all biomolecules like protein, lipid, Carbohydrates, DNA and RNA and damage these biomolecules [8], this condition is known as oxidative stress [9]. To control excessive production of ROS, production of oxidase enzymes is increased. Superoxide dismutase, peroxidase and polyphenol oxidase are important oxidative enzyme [10, 11]. Present study involves alternation of peroxidase, polyphenol oxidase, and PAL and TAL enzymes. We also study the total quantity of phenol, protein and carbohydrates.

Material and methods
Total Protein estimation- Estimation of total protein in normal and galled root was done according to Lowery *et al* (1951) method [12]. Absorbance was taken at 650nm wavelength and Bovine serum albumen was used as standard.

Peroxidase activity assay (EC.1.11.1.7)
Enzyme extraction-
Normal and galled root were collected freshly. Homogenized the sample in 0.1M phosphate buffer, pH-6.0 (1/10 W/V). The extract was centrifuged at 15000g for 15 min at 4 ºC. The supernatant was used as enzyme extract.

Activity assay- Peroxidase activity assay was done according to Worthington enzyme manual 1972 with some modification [13]. An amount of 2.7 ml of 0.1M Phosphate Buffer, 1mL of 1mM H_2O_2, 1mL of O-dianisidine (2mM) and.5mL of enzyme extract added to test tube and incubate it at 30ºC for 5 min. Blank was prepared in same but dis. water was added instead of H_2O_2. The activity of enzyme was expressed in change in optical density at 460nm $min^{-1}mg^{-1}$.

Polyphenol oxidase activity assay (EC.1.14.18.1)
Enzyme Extraction- Plant sample were homogenised in chilled 0.033M phosphate buffer pH 7.0 (1/10 W/V). Centrifuge the solution at 10000g for 10 min at 4ºC. The supernatant was used as enzyme crude extract.
Activity assay- 2ml enzyme extract and 3mL DL-DOPA (5mM) were taken in cuvette and measured the change in absorbance at 470 nM. The activity of enzyme expressed in change in optical density $min^{-1}mg^{-1}$.

Phenylalanine ammonia lyase and Tyrosine ammonia lyase activity assay (EC.4.3.1.5)
1g plant material was homogenized with 10 ml 50 mM Tris-Hcl buffer (pH-8.8) containing 15mM of β-mercaptoethanol and centrifuged at 15000 rpm for 30 min at 4 ºC. 1mL of extraction buffer, 0.5 mL of 10mM of L-Phenylalaline, 0.4 mL Distilled water and.1mL of enzyme extract were add in test tube and incubate the reaction mixture at 30ºC for TAL and at 37 ºC for PAL enzyme for one hour. The reaction was stopped by adding 0.5mL of 6M HCL. The products of both enzymes were extracted in 10 ml Ethyl acetate, after extraction the ethyl acetate was evaporated. Remaining residue was suspended in 3mL of 0.05 M NaOH. PAL and TAL enzymes activity were measured at 290nm and 330 nm respectively. PAL enzyme activity expressed as the formation of E-Cinnemic acid $min^{-1}mg^{-1}$ and TAL enzyme activity expressed as the formation of p-coumeric acid $min^{-1}mg^{-1}$ fresh tissue.

Total Phenol estimation- Total phenolic content was estimated by Bray and Thrope 1954 using Folin ciocalteau as reagent[14]. Root tissue of normal and galled plant were homogenized with 10 ml of 80% ethanol and centrifuged at 2000 rpm for 20 min. The supernatant was collected and residue was reextracted with 80% ethanol. This process was repeated for 2-3 times and supernatant was collected every time. Take 1 ml of supernatant, 1 ml of folin ciocalteu and 2 ml of 20% sodium carbonate in a test tube and kept on waterbath for 5 min. Take absorbance at 650 nm and Catechol was used as standard.

Total soluble sugar estimation
Estimation of total soluble sugar of normal and galled root was done according to Dubois *et al* (1956) method using the phenol sulphuric acid as reagent [15]. Sample of normal and galled root were homogenised with 10 ml of 80% phenol and centrifuged at 2000 rpm for 20 minutes. Supernatant was used as extract for soluble sugar. Take 1ml of supernatant and 1ml of 5% phenol in a test tube and add 5 ml of sulphuric acid. This solution was kept in water bath for 20 minutes and take absorbance at 490 nm. Glucose solution was used as standard. The quantity of total soluble sugar was expressed as mg/g fresh weight of tissue.

Results
Total Protein- Total proteins were higher in diseased (galled) root than normal root (Fig 1).
Oxidative enzymes- Results revealed that the activity of oxidative enzymes was maximum in galled root. Peroxidase and Polyphenol oxidase activity levels increased significantly in galled root. The highest change in absorbance due to activity of peroxidase and polyphenol oxidase was recorded at 2.51±.4 units $min^{-1}mg^{-1}$ fresh tissue (Fig 2) and.368±.02 units $min^{-1}mg^{-1}$ fresh tissue (Fig 3). No significant change was observed in the activity of TAL enzyme (Fig 4) but the highest activity of PAL enzymes was observed in galled root. The recorded activity of PAL enzyme was 0.47 E -Cinnemic acid $min^{-1}mg^{-1}$ fresh tissue (Fig 5).

Total phenol and soluble sugar- Similarly total phenol and total soluble sugar were higher in galled root than normal root. The total phenol in galled root was 0.424 mg g^{-1} fresh tissue (fig 6) and total soluble sugar in root gall was 7.24 mg g^{-1} fresh tissue (fig 7).

Discussion
The plant parasitic nematode Meloidogyne species generally lives as endoparasite in a plant root and plays an important role to elicit plant defence mechanism. This results lead into activation of local and systemic defence mechanism. Induced defence mechanism increase the production of secondary metabolites or bioactive compound, Reactive oxygen species and also increase the activity of oxidative enzymes like peroxidase, Polyphenol oxidase and superoxide dismutase [16]. The major class of bioactive compound includes alkaloids, terpenois and phenolic compound.
Among all of them Phenolic compounds plays a major part in defence mechanism against various infectious agents and have studied about their occurrence and metabolism in response to various pathogens by Patil *et al* [17]. The present study in relation to total phenolic compound showed that the concentration of total phenol was increased in infected plant at the site of invasion. Similar results were observed by Bhargava *et al* (2007) [18].
During infection the reactive oxygen species production is also common [19] and a part of defence mechanism. These ROSs are injurious to plant itself so to control or destroy ROS various oxidative enzymes is produced by plant itself such as peroxidase, polyphenol oxidase, PAL and Tal etc. In the present study showed that the activity of peroxidase and poly phenol oxidase enzymes was also increased in galled root and confirmed that peroxidase and polyphenol oxidase are elicited by the infestation of nematode indicating their role in defence. It was also reported by Mahfouz *et al* 2012 for peroxidase enzyme [20].
Phenylalanine ammonia lyase activity is higher in resistant than in susceptible [21, 22]. Our present study was also observed that the activity of Phenylalanine ammonia lyase (PAL) was slightly increased by nematode infection in galled root as compared to normal root. There is no significant result was observed for TAL enzyme.

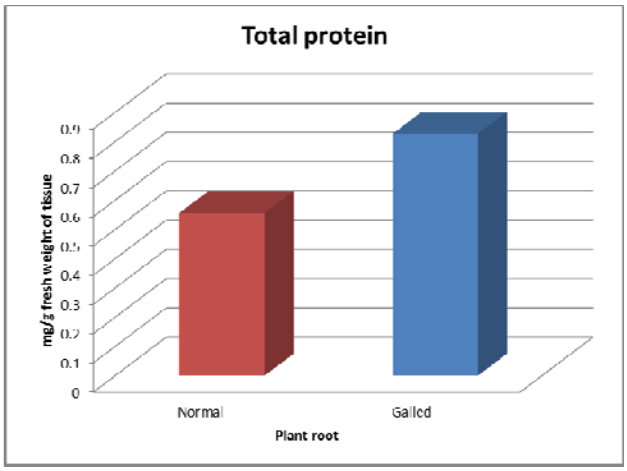

Fig 1: Shows impact of *Meloidogyne incognita* on total protein estimation in normal and galled root of *Abelmoschus esculentus*.

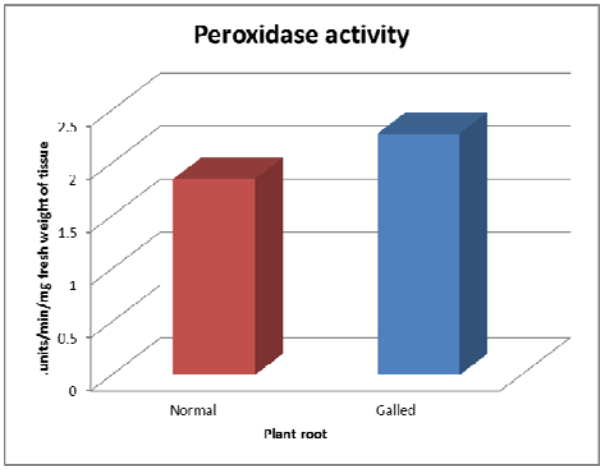

Fig 2: Shows impact of *Meloidogyne incognita* on activity of peroxidase enzyme in normal and galled root of *Abelmoschus esculentus*.

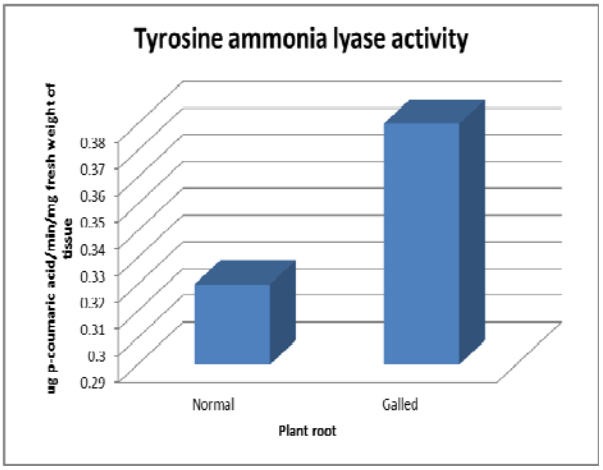

Fig 5: Shows impact of *Meloidogyne incognita* on activity of tyrosine ammonia lyase enzyme in normal and galled root of *Abelmoschus esculentus.*

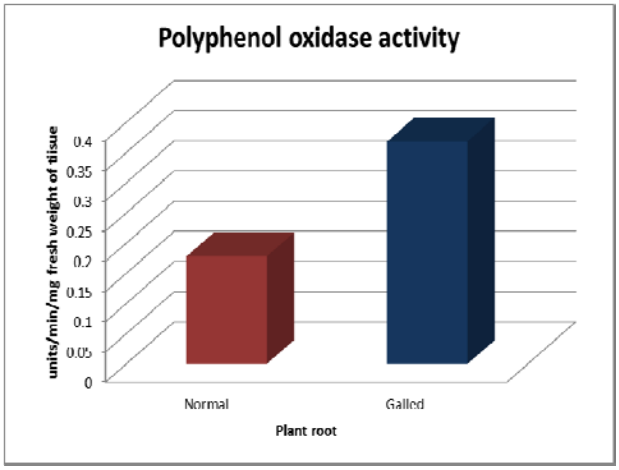

Fig 3: Shows impact of *Meloidogyne incognita* on activity of Polyphenol oxidase enzyme in normal and galled root of *Abelmoschus esculentus*.

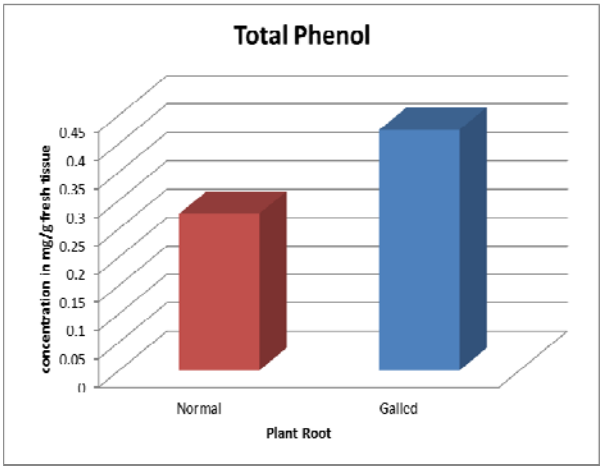

Fig 6: Shows impact of *Meloidogyne incognita* on total phenol estimation in normal and galled root of *Abelmoschus esculentus*.

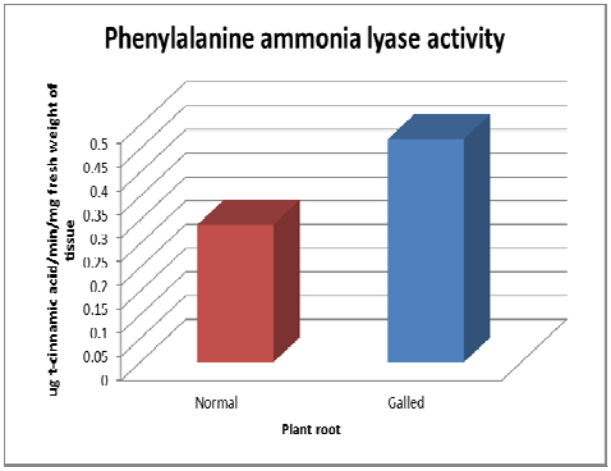

Fig 4: Shows impact of *Meloidogyne incognita* on activity of Phenylalanine ammonia lyase enzyme in normal and galled root of *Abelmoschus esculentus.*

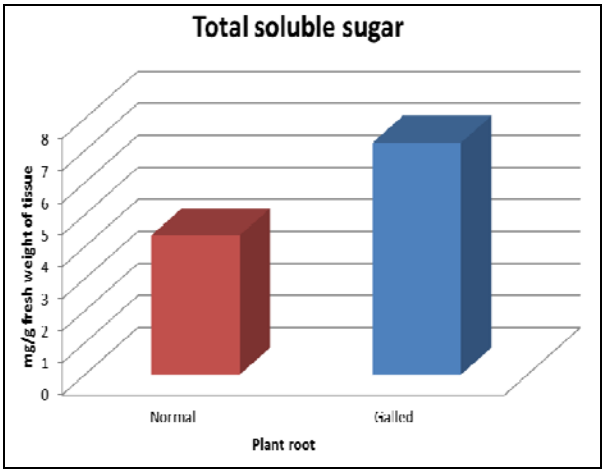

Fig 7: Shows impact of *Meloidogyne incognita* on total soluble sugar estimation in normal and galled root of *Abelmoschus esculentus*.

Conclusion

Increased activity of enzymes in young gall was showed defence nature of plant against pathogen to reduce further infection. Increased metabolites in galled tissue were showed that the pathogen recreates or influences on the host metabolism so that it can get nutrition for its further development and reproduce successfully.

Acknowledgement

We are thankful to DRS for providing necessary chemical to this research work.

References

1. Singh D. Genetic control of aluminium tolerance in okra (*Abelmoschus esculentus* (L.) Moench). Sci. Hortic & Amsterdam, 2012; 138:134-137.
2. FAOSTAT. (Food and Agricultural Organization of the United Nations). On-line and multilingual database currently covering international statistics, 2008. http://faostat.fao.org/foastat/.
3. Hussain MA, Mukhtar T, Kayani MZ. Assessment of the damage caused by Meloidogyne incognita on okra (*Abelmoschus esculentus*). J Anim. Plant Sci. 2011; 21:857-861
4. Bhatti DS, Jain RK. Estimation of losses in okra, tomato and eggplant yield due to Meloidogyne incognita. Indian J. Nematol. 1977; 7:37-41.
5. Sasser JN. Economic importance of Meloidogyne in tropical countries. In: Lamberti, F., Taylor, C.E. (Eds.), Root-knot Nematodes (*Meloidogyne* spp.): Systematics, Biology and Control. Academic Press, New York, 1979, 359-374
6. Agrios GN. Plant Pathology, Ed 5, Elsevier Academic Press, 2005.
7. Reddy AM, Kumar SG, Jyothsnakumari G, Thimmanaik S, Sudhakar C. Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and Bengal gram (*Cicer arietinum* L.). Chemosphere. 2005; 60:97-104.
8. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, Ed 3, Oxford University Press Oxford, 1999.
9. Sreedhar M, Chaturvedi A, Aparna M, Kumar PD, Singhal RK, Venu BP. Influence of γ-radiation stress on scavenging enzyme activity and cell ultra-structure in groundnut (*Arachis hypogea* L.). Adv. Appl Sci Res. 2013; 4:35.
10. Gout E, Boisson AM, Aubert S, Douc R, Bligny R. Origin of the cytoplasmic ph changes during anaerobic stress in higher plant cell carbon-13 and phosphorous-31 nuclear magnectic resonance studies. Plant Physiol. 2001; 125:912.
11. Mehlhorn H, Lelandais M, Korth HG, Foyer CH. Ascorbate is the natural substrate for plant peroxidases. FEBS Letters. 1996; 378:203.
12. Lowry O, Rosebrough NH, Farr AL, Randall RJ. Protein measurement with folic phenol reagent. Journal of Botnical Chemistry. 1951; 193:225-272.
13. Worthington-Enzyme Manual. Enzymes, Enzyme reagents, related biochemical and Worthington Corporation Freehold, New Jersey, U.S.A., 1972, 216.
14. Bray HC, Thorpe WV. Analysis of phenolic compound of interest in metabolism. Meth Biochem Analysis. 1954; 1:27-52.
15. Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. A colorimeter method for the determination of sugars. *Nature*. 1956, 168-167.
16. Zacheo G, Orlando C, Bleve Zacheo T. Characterization of anionic peroxidase in tomato isolines infected by Meloidogyne incognita. J Nematol. 1993; 25:249-256.
17. Patil SS, Powelson RL, Young RR. Relation of chlorogenic acid and free phenols in potato roots to infection by *Verticillium albo-atrum.* Phytopathology. 1964; 54:531-535
18. Bhargava S, Sharma MK, Dashora PK. Histopathological and Biochemical changes induced by Root Knot Nematode *Meloidogyne incognita* of resistance and susceptible varieties of cowpea. J. Mycol Plant Pathol. 2007; 37:112-117.
19. Montes MJ, Lopez-Brana I, Delibes A. Root enzyme activities associated with resistance to *Heterodera avenae* coferred by gene Cre7 in wheat, *Aegilops triuncialis* introgression line. J Plant Physiol. 2004; 161:493-495.
20. Mahfouz MM, Abd-Elgawad, Marie-Claire K, Sergio M, Abd-El-Kareem F, Sanaa SA *et al*. Histopathological changes and enzymatic activities induced by *Meloidogyne incognita* on resistance and susceptible potato. *Plant Pathol.* 2012; 1:62.
21. Gerald DB, Nichole RO, Deborah AS. Alfalfa (*Medicago sativa* L.) resistance to the root-lesion nematode, P*ratylenchus penetrans*: defense response gene mRNA and isoflavonoid phytoalexin levels in roots. *Plant Mol Biol*. 1998; 38:999-1010.
22. Wuyts N, De Waele D, Swennen R. Activity of phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase in roots of banana (*Musa acuminate* AAA, CVs 'Grande Naine' and 'Yangambikm 5' before and after infection with *Radopholus similis*). J Nematol. 2006; 38:201-209.