Pakistan Journal of Nematology (2016) 34(1): 67-73

ISSN 0255-7576 (Print)
ISSN 2313-1942 (Online)
www.pjn.com.pk

http://dx.doi.org/10.18681/pjn.v34.i01.p67

Histochemical alterations of various metabolites and their localization in *Luffa cylindrica* roots infected with *Meloidogyne incognita*

R. Singh[1†] and R. Z. Sayyed[2]

[1]*Plant Nematology Research Lab, Post Graduate Department of Zoology, Bareilly College Campus, MJP Rohilkhand University, Bareilly 243005, Uttar Pradesh, India*
[2]*Department of Microbiology & Biotechnology, PSGVPM'S ASC College, Shahada-425409, Maharashtra, India*

[†]Corresponding author: singh.rajendra007@gmail.com

Abstract

Experiment was carried out to study the alterations and histochemical localization of various metabolites in normal and galled roots of sponge gourd, *Luffa cylindrica* L., infected with root-knot nematode *Meloidogyne incognita*. Histochemistry of the roots revealed an increase in protein, nucleic acids (RNA and DNA) and amylodextrin (hydrolyzed starch) concentration in the infected regions as compared with normal healthy sections. Protein localization found more pronounced at the sites of infection of *M. incognita* viz., cortical cells, giant cells, abnormal xylem, medullary region and nematode body. Active RNA synthesis in infected cortical, medullary and vascular regions was observed by positively dark stained nucleoli, nucleoplasm and cytoplasm of their cells and syncytium. High DNA amount was observed in concentrate form in infected cells of cortex, pericycle and xylem tracheid of vascular region. Hyper hydrolysis of starch induced by *M. incognita* in sponge gourd roots was tested by studying the localization of amylodextrin. A positive test was observed with nematode body and surrounding tissues when stained with toluidine blue.

Keywords: Histochemical alterations, *Meloidogyne incognita*, *Luffa cylindrica*, root galls, metabolites

A major global challenge in the coming years will be to ensure food security and to feed the increasing human population. Indian population (1.21billion in 2011) is flying at 1.58% of growth rate and it is predicted to have more than 1.53 billion people by end of 2030. FAO (2013) indicated that about 17% population of India suffering from chronic hunger. Many economic sectors depend on biodiversity and ecosystems services, including water supply, agriculture, fisheries, forestry, health, nutrition, energy, transport and tourism. It is important to study the factors which hamper the crop production and only then suitable strategy to overcome from that constraint.

Nematodes, arguably with more than one million species, are one of the most diverse groups of animals (Lambshead, 2004). About 27,000 nematode species are known in the literature, and most of them affect crops through feeding on or in plant roots, whilst a minority aerial feeders. On a global scale the distribution of nematode species varies greatly. Some are cosmopolitan such as certain species of *Meloidogyne*. Plant parasitic nematodes are major pests of agricultural crops and are responsible for yield losses upto the 50% in Pakistan (Abbasi *et al.*, 2008) and upto 43% in vegetables in India (Singh & Kumar, 2015). Nematode interaction with host cells results in altered metabolic activities (Ahmed *et al*., 2009; Hajra *et al.*, 2015). Some of the alterations have been observed in the present research work where sponge gourd, *Luffa cylindrica* L., was infected with root-knot nematode, *Meloidogyne incognita*. Present experiment was carried out to the study the alterations in biochemical metabolites in roots of sponge gourd.

Materials and Methods

Localization of different metabolites in root tissue was observed by following the methods of Johansen (1940) and modified methods of Owens & Novotny (1960).

Protein localization: Fresh transverse sections of galled and normal roots were treated with potassium ferro-cyanide solution (1 g Potassium Ferro-cyanide + 100 cc glacial acetic acid) for 1h. The sections washed in 60% alcohol and few drops of aqueous $FeCl_3$ were added. Blue colour appeared in protein rich places.

Nucleic acids localization: Paraffinized/hand sections were spread on the slides and the slides were kept in mallivine buffer of pH 4 for 3 min at 37 ºC. Sections were rinsed with distilled water and placed in stain Toluidine blue for about 5 minutes, at a temperature of 37 ºC. These sections, thereafter, dehydrated in alcohol series, cleared with xylene and finally mounted in DPX. Toluidine blue revealed differential localization of DNA and RNA. DNA stained bluish green, whereas, RNA deep violet.

Amylodextrin (hydrolyzed starch) localization: The localization of amylodextrin was made by treating the root sections in iodine and potassium iodide solution for about 24 h. Red colour appeared that indicated the presence of amylodextrin.

Results and Discussion

Histochemical localization of total protein, nucleic acids (RNA and DNA) and carbohydrate (amylodextrin) in normal healthy and root-knot nematode infected roots of sponge gourd observed as follows:

Protein localization: Histochemical localization of protein was observed at the site of infection in cortical region, where hypertrophied as well as giant cells showed positive stain for protein (Fig. 1B). Nematode body stained strongly in the region of galls (Fig. 1C) due to accumulation of protein. In infected roots, the adjacent tissues including parenchyma, sieve tubes and medullary region had also been found to be rich in protein besides the giant cells and nematode bodies in comparison to the normal roots (Fig. 1A, B and D). Abnormal xylem, induced by nematode feeding due to some proteinaceous secretions also found positive for protein stain. Hypersensitive cells as well as cells undergoing necrosis in infected regions high stained strongly for protein in comparison with normal roots (Fig. 1A, B and C).

Considerable alterations were present in protein content in root-knot nematode infected roots. Hypersensitive cells exhibited positive stain for protein in the present observation possibly due to enhanced protein synthesis (Paulson & Webster, 1972). Knypl & Janas (1975) reported in carrot cv. Slendero (tolerant) and Perfekeja (sensitive) infested with *Meloidogyne hapla* showed higher protein content in tolerant variety. Similarly, Masood & Hussain (1975) also found increased protein in resistant cultivar of tomato. Several evidences from other studies had also demonstrated that qualitative and quantitative magnitude of different chemicals has various functions in plants during pathogenesis (Sarah, 1989; Mahajan *et al.*, 1992; Mantoo & Siddqui, 1996). Valette *et al.*, (1998) reported histochemical and cytochemical localization of phenolic compounds in banana roots infected with *Radopholus similis* suggesting increased phenolic synthesis as a key factor of resistance of banana to *R. similis.* Chatterjee & Sukul (1981) also measured the total galled root protein in lady's finger plant.

Nucleic acids (RNA and DNA) localization: Histochemical observations on healthy and *Meloidogyne* J_2 inoculated roots showed striking differences in the nucleic acid contents.

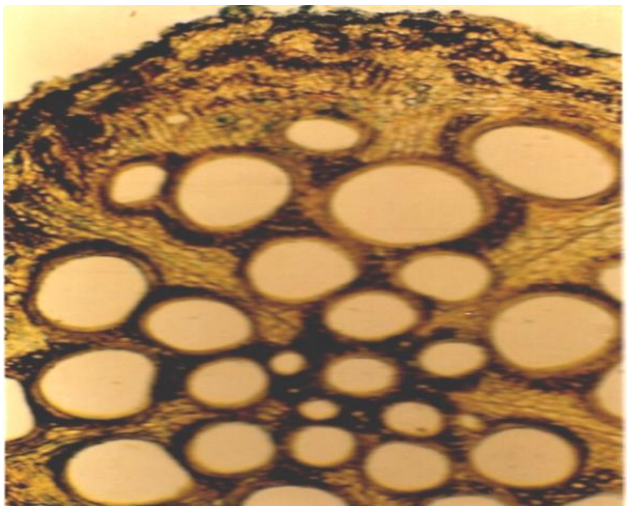

Fig. 1A. T.S. of normal root of sponge gourd showing negative stain to protein.

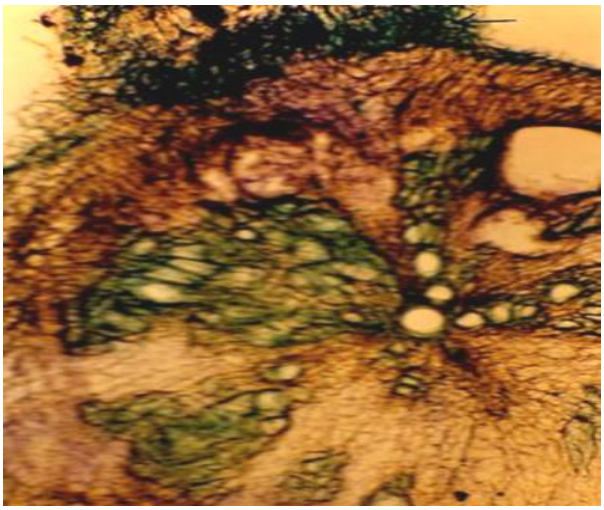

Fig. 1B. Feeding sites of nematodes in cortical and stellar regions showing positive stain for protein.

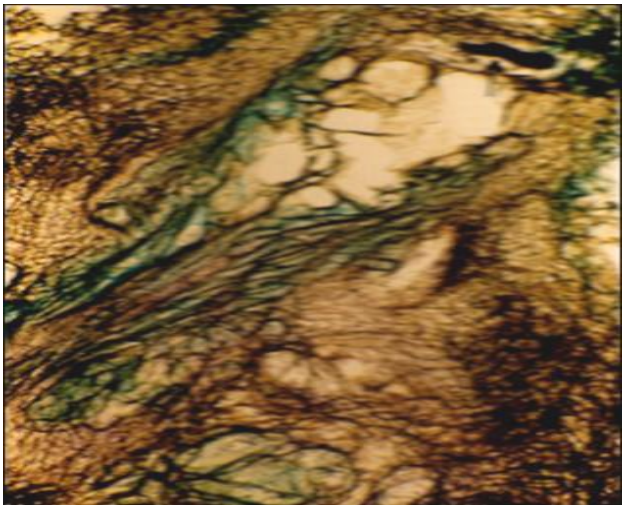

Fig. 1C. Showing infected zone of medullary rays with increased concentration of proteins.

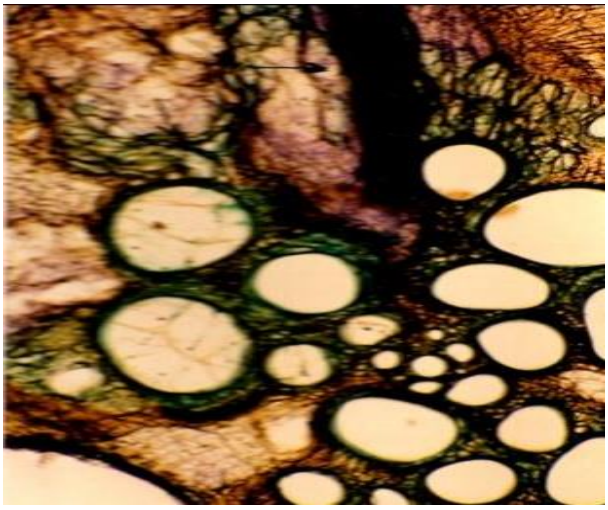

Fig. 1D. Showing positively stained nematode body and xylem elements in roots.

Ribo nucleic acid (RNA): Stimulation of RNA synthesis in diseased root tissues recorded by positively stained regions in cortex, medullary rays and vascular bundles. Nucleoli, nucleoplasm and cytoplasm of gall cells and syncytium had been stained strongly for RNA when compared with normal root section (Fig. 2A and B). In stellar region abnormal xylem and abnormal phloem parenchyma had also been found to be positive for RNA content (Fig. 2B).

De-oxyribo nucleic acid (DNA): DNA concentration was more in the feeding sites of *Meloidogyne incognita* in sponge gourd roots. Hypersensitive and giant cells in cortical region, pericycle and xylem vessels in vascular zone showed greater concentration of DNA than other tissues. Infected region in medullary rays also exhibited more concentrated localization of DNA over normal healthy counter parts (Fig. 2C). Granular cytoplasm of syncytium indicated the presence of enhanced amount of DNA (Fig. 2D).

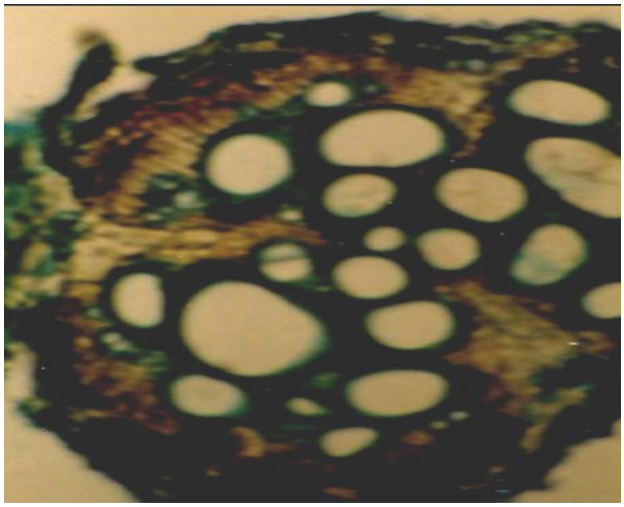

Fig. 2A. T.S. of normal root of sponge gourd showing presence of nucleic acids in green colour.

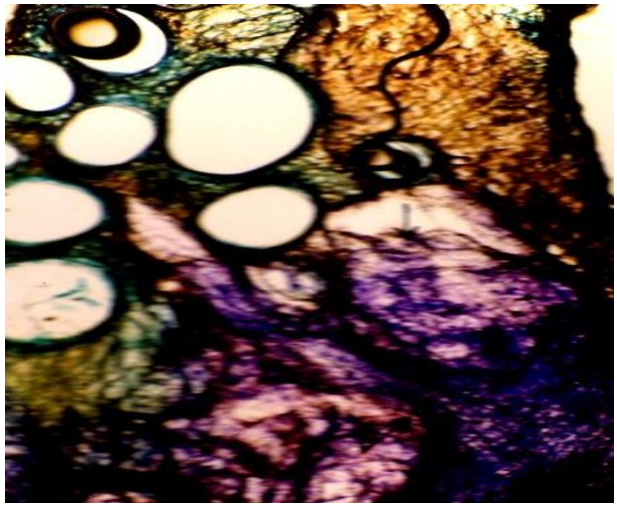

Fig. 2B. Showing syncytial cytoplasm stained strongly purple for RNA.

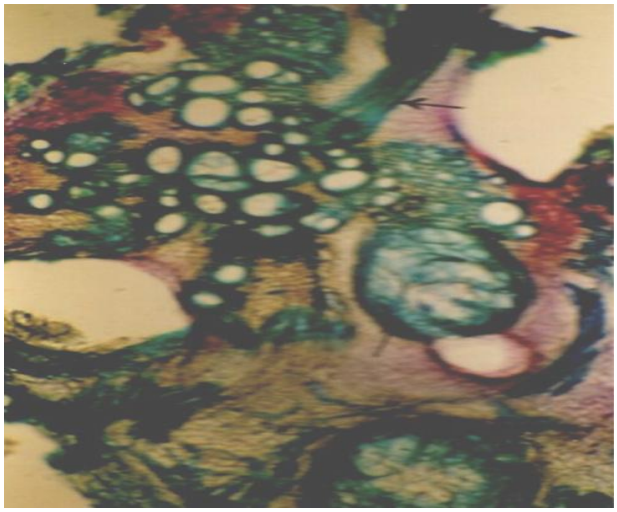

Fig. 2C. Showing heavily deposition of DNA (green colour) in nematode infected stellar and medullary region

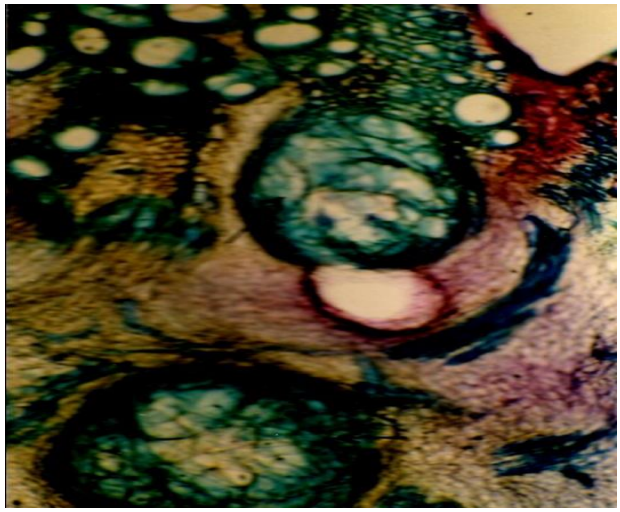

Fig. 2D. Showing infected abnormal xylem sieves rich in DNA (green colour) content.

Stimulated RNA synthesis in lateral roots of carrot, infected with *Meloidogyne hapla* was reported by Chylinska & Knypl (1975). Paulson & Webster (1972) also observed such increase in RNA content in the cytoplasm of hypersensitive cells of tomato roots, infected with *M. incognita*. However, similar results observed with *M. incognita* inoculated sponge gourd roots in the current study. Paulson & Webster (1970) investigated lobed syncytial nuclei in galled tissues and correlated it with intense nucleic acid synthesis induced by nematode parasite.

The present observations confirmed the close relationship of greater DNA content and increasing nematode infestation reported by Rubinstein & Owens (1964) in roots of tomato. Premchandran & Dasgupta (1983) investigated an increase in nucleic acid content due to hyperactivity of ribonuclease enzyme in *M. incognita* infected roots of tomato. Arya & Tiagi (1985) reported more concentrated localization

of RNA and DNA in nucleoli, nucleoplasm and in other cytoplasmic bodies in galled roots. These findings are also parallel to the present study. Ravichandra (2014) described that syncytia and giant cells contain higher level of total proteins, amino acids, lipids, DNA and sugars (Abawi & Chen, 1998), which was expected to beneficial in fungal association, but the relationship remain unproven.

Carbohydrate (amylodextrin) localization: Among carbohydrates, the test was made for the localization of amylodextrin (hydrolyzed starch) only and observed that the nematode body, eggs and associated sites possessed more amylodextrin than normal healthy root section (Fig. 3A, B, C and D). Increased amount of amylodextrin in infected sites was possibly due to the hyperactivity of amylase enzyme. This observation suggested that amylase enzyme, secreted by the nematode, hydrolyzed the starch into easily assimilable simple components including amylodextrin. Whereas, Roy (1979) reported that localization of invertase was in the oesophagus and intestine of the nematode parasite and its secretion thus, resulting into changed carbohydrate metabolism during the course of host parasite interaction. Increased amylase was suggested for lowering of the starch content (Tayal & Agrawal, 1982).

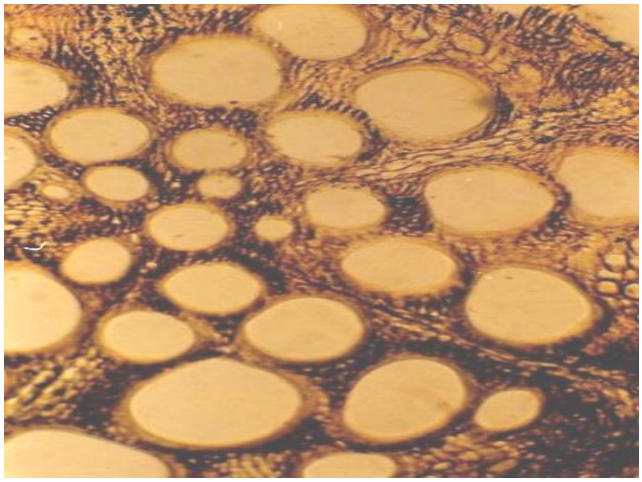

Fig. 3A. T.S. of normal root of sponge gourd with normal tissues.

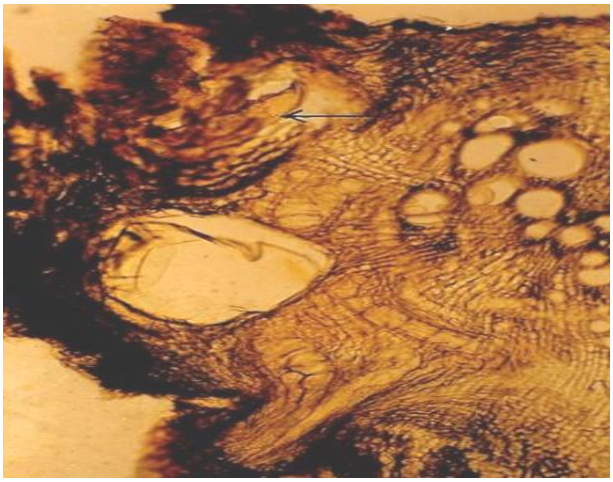

Fig. 3B. Damaged cortical zone of infected roots showing positive stain with nematode body.

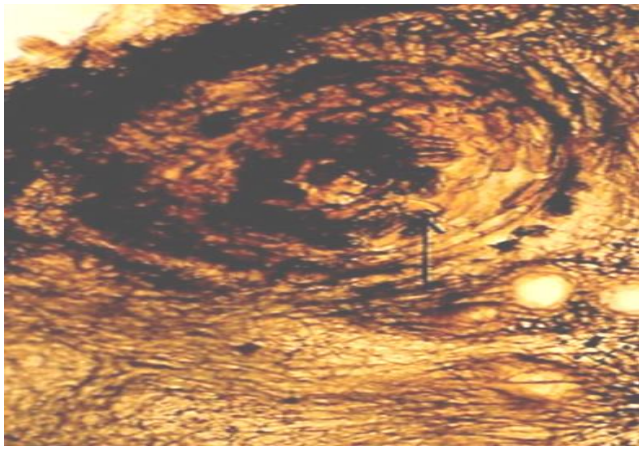

Fig. 3C. Egg-masses showing positive stain for amylodextrin in cortical region of sponge gourd roots.

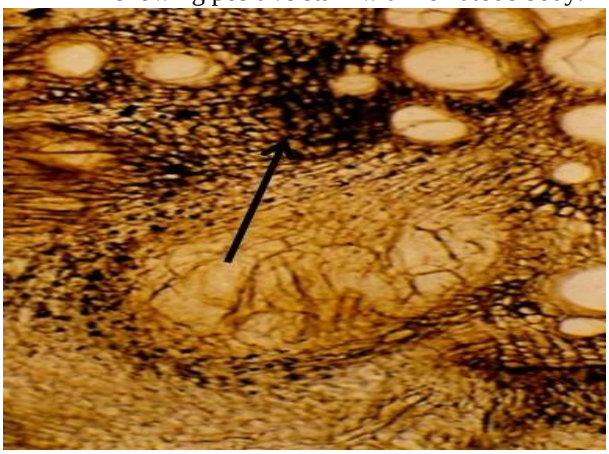

Fig. 3D. Section showing high concentrations of amylodextrin in infected area of medullary region.

Conclusion

Based on the observations of the present study, this can be concluded that feeding forms of *M. incognita* induce the chemical alterations in tissues of the plants which results in yellowing, necrosis and stunting growth of host plant. Studies of such alterations, interaction and behaviour of host and parasite relationship will help in formulations of unique management strategy.

Acknowledgments

All the experiments of this work were conducted in greenhouse of botanical garden of Bareilly College Campus of MJP Rohilkhand University, Bareilly. The authors wish to thanks Principal and associated staff. These findings are the part of a major research project (F. No. 41-498/2012 (SR) funded by University Grant Commission (UGC) of Government of India. The authors are grateful to chairman and sanctioning body of UGC.

References

Abbasi, W. M., Ahmed, N., Zaki, M. J. & Shaukat, S. S. (2008). Effect of *Barleria acanthoides* Vahl. on root-knot nematode infection and growth of infected okra and brinjal plants. *Pakistan Journal of Botany*, 40, 2193-2198.

Abawi, G. S. & Chen, J. (1998). *Concomitant pathogen and pest interactions*. In: Barker, K. R., Pederson, G. A. & Windham, G. L. (Eds.). *Plant and nematode interactions*. Agronomy Monograph 36. American Society of Agronomy, Madison, WI, 135-158 pp.

Ahmed, N., Waseem, M., Abbasi, S., Shaukat, S. S. & Zaki, M. J. (2009). Physiological changes in leaves of mungbean plants infected with *Meloidogyne javanica*. *Phytopathologia Mediterranea*, 48, 262-268.

Arya, M. & Tiagi, B. (1985). Nucleic acid changes in carrot cultivars infected with *M. incognita*. *Indian Journal of Nematology*, 15, 21-25.

Chatterjee, A. & Sukul, N. C. (1981). Total protein content of galled roots as an index of root-knot nematode infestation in lady's finger plats. *Phytopathology*, 71, 372-374.

Chylinska, K. M. & Knypl, J. S. (1975). Decreased phenylalanine ammonia-lyase and ribonuclease activity in side roots of carrot infected with northern root-knot nematode. *Nematologia*, 21, 129-133.

FAO, (2013). Food and Agriculture Organization of the United Nations, Rome, 2013. Available at: http://www.fao.org/docrep/018/i3434e/i3434e00.htm

Hajra, N., Shahina, F. & Firoza, K. (2015). Damage induced by root-knot nematodes and its alleviation by vesicular arbuscular mycorrhizal fungi in roots of *Luffa cylindrica*. *Pakistan Journal of Nematology*, 33, 71-78.

Johansen, D. A. (1940). *Plant Microtechnique*. New York: McGraw-Hill Book Company, New York, 523 pp.

Knypl, J. S. & Janas, K. M. (1975). Synthesis of RNA and protein with ribonuclease activity in carrot root infested with *Meloidogyne hapla* Chitw. *Physiological Plant Pathology*, 7, 213-220.
DOI: 10.1016/0048-4059(75)90026-0

Lambshead, P. J. D. (2004). Marine nematode biodiversity. In: Chen, Z. X., Chen, S. Y. & Dickson, D. W. (Eds.). *Nematology: Advances and Perspectives, Vol. 1*. CABI Publishing, Wallingford, UK, 438-468 pp.

Mahajan, R., Kaur, D. J. & Bajaj, K. L. (1992). Nematicidal activity of phenolic compounds against *M. incognita*. *Nematologia Mediterranea*, 20, 217-219.

Mantoo, M. A. & Siddiqui, M. N. (1996). Reaction of chickpea varieties to root-knot nematode and their effect on peroxidase activity and protein content. *Indian Journal of Plant Pathology*, 14, 41-45.

Masood, A. & Hussain, S. I. (1975). Amino acid and protein changes and their role in the resistance and susceptibility of three tomato varieties. *Geobios*, 2, 16-17.

Owens, R. G. & Novotny, H. M. (1960). Physiological and biochemical studies on nematode galls. *Phytopathology*, 50, 650.

Paulson, R. E. & Webster, J. M. (1970). Giant cell formation in tomato roots caused by *M. incognita* and *M. hapla* (Nematoda) infection. A light and electron microscopic study. *Canadian Journal of Botany*, 48, 271-276.

DOI: 10.1139/B70-041

Paulson, R. E. & Webster, J. M. (1972). Ultrastructure of the hypersensitive reaction in roots of tomato, *Lycopersicon esculentum* L., due to infection by the root-knot nematode, *Meloidogyne incognita*. *Physiological Plant Pathology*, 2, 227-234.

Premchandran, D. & Dasgupta, D. R. (1983). Changes in nucleic acids and ribonuclease in tomato plants during infection by the root-knot nematode, *M. incognita*. *Revue de Nematologie*, 6, 33-52.

Ravichandra, N. G. (2014). *Horticultural Nematology*, Springer New Delhi, India. DOI: 10.1007/978-81-322-1841-8

Roy, T. K. (1979). Histochemical studies of hydrolytic enzymes in *M. incognita* and infected host *Lycopersicon esculentum* and their role in host-parasite relationship. *Indian Journal of Experimental Biology*, 17, 1357-1362.

Rubinstein, J. H. & Owens, R. G. (1964). Thymidine and Uniodine incorporation in relation to ontogeny of root-knot syncytia. *Contributions Boyce Thompson Institute*, 22, 491-502.

Sarah, J. L. (1989). Banana nematodes and their control in Africa. *Nematropica*, 19, 199-216.

Singh, R. & Kumar, U. (2015). Assessment of nematode distribution and yield losses in vegetable crops of Western Uttar Pradesh in India. *International Journal of Science and Research*, 4, 2812-2816.

Tayal, M. S. & Agrawal, M. L. (1982). Biochemical alterations in galls induced by *Meloidogyne incognita*: Some hydrolyzing enzymes and related chemical metabolites. *Indian Journal of Nematology*, 12, 379-494.

Valette, C., Mountport, D., Nicole, M., Sarah, J. L. & Baujard, P. (1998). Scanning electron microscopy study of two African populations of *Radopholus similis* (Nematoda: Pratylenchidae) and proposal of *R. citrophilus* as a junior synonym of *R. similis*. *Fundamental and Applied Nematology*, 21, 139-146.

(Accepted: June 5, 2015)