academicJournals

Vol. 9(7), pp. 463-468, 18 February, 2015
DOI: 10.5897/AJMR2014.7356
Article Number: 969F1A050657
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Application of arbuscular mycorrhiza for managing root-knot disease in tomato (*Lycopersicon esculentum*) under glass-house conditions in Pantnagar, India

Ishwar Prakash Sharma* and A. K. Sharma

Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, 263145, U. S. Nagar, India.

Received 30 December, 2014; Accepted 9 February, 2015

Root-knot disease caused by *Meloidogyne* spp. poses a worldwide threat to agriculture as well as environment due to excess use of chemical nematicides for its management. Recently, arbuscular mycorrhizal fungi (AMF) have achieved importance as biocontrol agent for the managements of root-knot disease. In this aspect, the present study was focused on the effect of AMF (*Glomus intraradices*) against root-knot disease of tomato (*Lycopersicon esculentum*) caused by *Meloidogyne incognita*. Observations of *G. intraradices* against *M. incognita* were taken at 10, 20 and 30 days after *M. incognita* second stage juveniles (J2) inoculation on tomato roots, which were pre-colonized by *G. intraradices*. A significant effect was observed on plant growth (length and biomass) and nematode induced (root galls, egg masses, egg and adult females) parameters in nematode inoculated *G. intraradices* colonized plants as compared to non-colonized plants at all the three harvesting periods. The finding of present study indicates the potential of *G. intraradices* as a potential biocontrol tool for the management of root-knot disease and can be used in the place of environmentally hazardous chemical nematicides.

Key words: Arbuscular mycorrhiza, root-knot disease, nematicides, biocontrol, management.

INTRODUCTION

World tomato (*Lycopersicon esculentum*) production was 150513.81 million tons in the area of 4582.44 million hectares in 2010-11 (WPTC, 2012) while in India during this period the production was 16.53 million tons in area of 0.86 million hectares (NHB, 2011). It is widely grown vegetable crops of tropics and subtropics and damage caused by Root-knot nematode (*Meloidogyne* spp.) reported much higher in these regions (Taylor and Sasser, 1978). Root-knot nematode (RKN) causing more than 27% yield losses in tomato (Kaur et al., 2011). Nematicides of chemical origin are widely used for the menace of RKNs; large scale use of such chemical nematicides

*Corresponding author. E-mail: ipsharma.com@gmail.com.

Abbreviations: AMF, Arbuscular mycorrhizal fungus; **C,** control; **DAI,** days after inoculation; **J2,** second stage juvenile; **M,** mycorrhiza; **MN,** mycorrhiza nematode; **N,** nematode; **PGPR,** plant growth promoting rhizobacteria; **RKN,** root-knot nematode.

poses environmental hazards, besides being costly and uneconomical. Hence, it is imperative to search for safer and economic alternative management strategy against RKNs. Although the controlling options of RKNs are limited, the chemical nematicides and soil fumigants are being used mostly, which need to replace with alternative management strategies.

Due to the increasing demand of agriculture and environmental safety, against the chemical pesticides, AMF and PGPRs may provide a more sustainable and environmentally safer strategy against the pesticide use (Harrier and Watson, 2004; Dong and Zhang, 2006). AMF inoculation in nursery can improve the plant growth and protect the plants against soil-borne pathogens, including nematodes (Smith and Read, 1997; Elsen et al., 2008). Contributing to increase the nutrient uptake, the AMF can act as biocontrol agents by direct or indirect mechanisms, compensating the damages caused by the nematodes (Azcón-Aguillar and Barea, 1996). In this respect, present study was focused on the effect of *Glomus intraradices* on root-knot disease of tomato (*L. esculentum* var. Pant T-3).

MATERIALS AND METHODS

Biological materials

The tomato variety Pant-T3 (*L. esculentum*) was grown under glasshouse conditions at an ambient temperature of 23 – 28°C, photo period of 16/8 h day/night cycle and relative humidity of 60%. Mother culture of AMF, *G. intraradices* obtained from Rhizosphere Biology Lab., Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. P. U. A. & T. Pantnagar was kept as a glasshouse stock culture on maize plants and applied as mycorrhizal inoculums at sowing of the test plants. The mycorrhizal inoculums consisted of rhizosphere soil from maize pot cultures containing spores, hyphae and heavily colonized root pieces. The sedentary root-knot nematode, *M. incognita* egg masses were collected from the infected tomato plant roots and cultured on tomato roots. For nematodes culture preparation, egg masses were isolated from the infected tomato roots and kept for hatching up to 48 h in single layered facial tissue paper supported by a wire mesh assembled in a Petri dish and freshly hatched second-stage juveniles (J2) were collected by using micropipette.

Experimental design

Two nurseries were prepared in pure sterilized river bad sand under glass house conditions, one with mycorrhiza and other without mycorrhizal culture. Mycorrhizal culture (containing spores, hyphae and heavily colonized root pieces) inoculates during seeds sowing in nursery, a thin layer of mycorrhizal soil from maize rhizosphere inoculate in the pure sterilized river bad sand followed by surface sterilized tomato seeds. The seeds were surface sterilized by immersion in 70% ethanol for 1 - 2 min and 0.2% (v/v) sodium hypochlorite for 5 - 10 min respectively, under aseptic conditions. For non-mycorrhizal plants, same nursery was prepared without mycorrhiza. Hoagland solution was given weekly in both the nurseries (Hoagland and Arnon, 1950). After 4 leaves stage, randomly six plants from each nursery were uprooted to determine mycorrhizal colonization by staining the roots with trypan blue (Phillips and Hayman, 1970). After confirmation of mycorrhizal colonization, plants were transplanted in ½ kg pots containing sterilized mixture of sand and soil in 1:1 ratio (pH and EC, 8.15 and 65.7 µS, respectively). Ten (10) days after transplanting, 500 freshly hatched *M. incognita* second-stage juveniles (J2) were inoculated near the rhizosphere of tomato roots. Plant growth parameters and nematode development were observed after 10, 20 and 30 days after inoculations (DAI) of nematode J2. Whole experiment was conducted in glass house condition with twenty four replications for each treatment, all the pots placed in a completely randomized design and the experiment was repeated twice.

Effect of *G. intraradices* on plant growth and root galls

Four replications of each treatment was harvested after 10, 20 and 30 DAI of nematodes J2 and washed thoroughly with tap water. After initial air drying, root/shoot length and fresh weight were recorded. Than plant samples were kept in paper bags, dried at 60°C in an electric oven for 48 h and the dry matter of plants was determined. At 10, 20 and 30 DAI of nematodes, J2 the another four replications of each treatment were harvested, washed thoroughly with tap water and the nematode root galls in the infected roots were counted in water filled clean glass Petri dishes.

Effect of *G. intraradices* on nematode egg masses, eggs and on adult females

Nematode egg masses were assessed by gently washing the roots, followed by immersing a 0.5 g root sample in 0.015% Phloxine-B for 15 min to stain the egg masses (Hooper et al., 2005). Nematodes eggs were counted by using sodium hypochlorite (NaOCl). Single egg mass from each replication kept in 2 ml vial followed by a single drop of NaOCl and four drops of tap water using 1 ml pipette tip, vortex this mixture for 30 - 40 s immediately poured into glass Petri dish and the eggs counted under microscope using pipette. Adult females were assessed by the acid fuschin staining method (Byrd et al., 1983). Root gall index (RGI) and egg mass index (EMG) measure by Talyor and Sasser (1978) method with the scale of 0 to 5. 0 = no galling or egg masses; 1 = 1 to 2 galls or egg masses; 2 = 3 to 10 galls or egg masses; 3 = 11 to 30 galls or egg masses; 4 = 31 to 100 galls or egg masses and 5 = more than 100 galls or egg masses.

Statistical analysis

The data presented are mean values ± SD using completely randomize design (CRD). Measurements were performed on four replicates for each treatment (n = 4). Plant growth parameters and nematode originated data were subjected to one factorial analysis of variance (ANOVA) using STPR-3 statistical software. The differences between the means were compared using least significant differences at $p<0.05$. Different letters denote significant differences among treatments and control.

RESULTS

Effect of *G. intraradices* on plant growth and root galls

Significant difference of plant growth of tomato noticed in mycorrhizal and nematode infected plants at 20 and 30

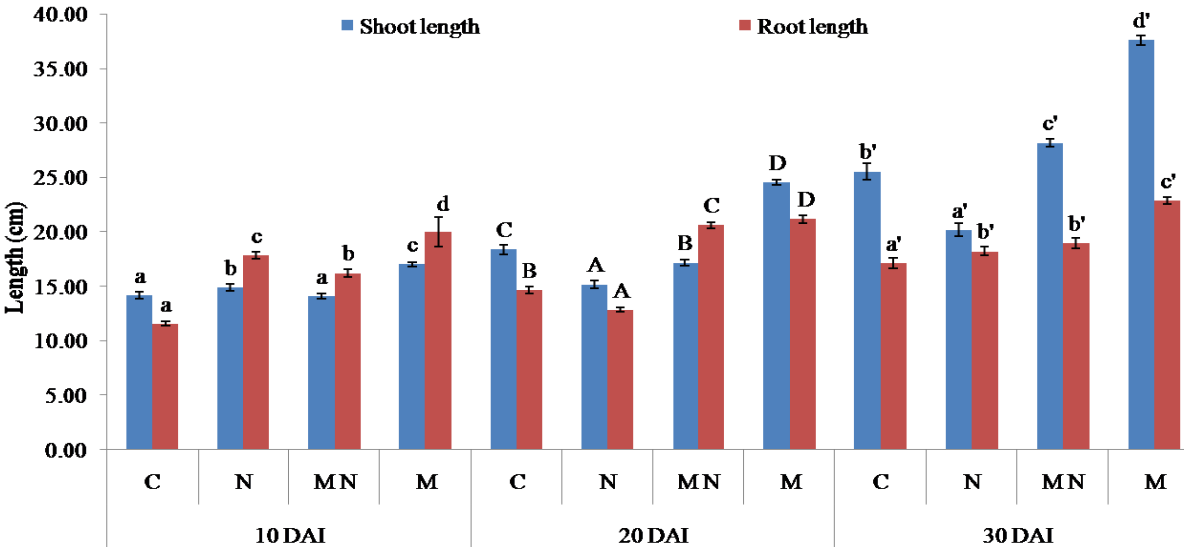

Figure 1. Effect of *Meloidogyne incognita* second-stage juveniles (J2) on the shoot and root length of tomato var. pant-T3 at 10, 20 and 30 days after inoculation (DAI). (C = control, N = nematodes, MN = mycorrhiza nematodes, M = mycorrhiza). Error bars represent standard error of three replications. Different letters indicate a significant difference (P<0.05) of treatment.

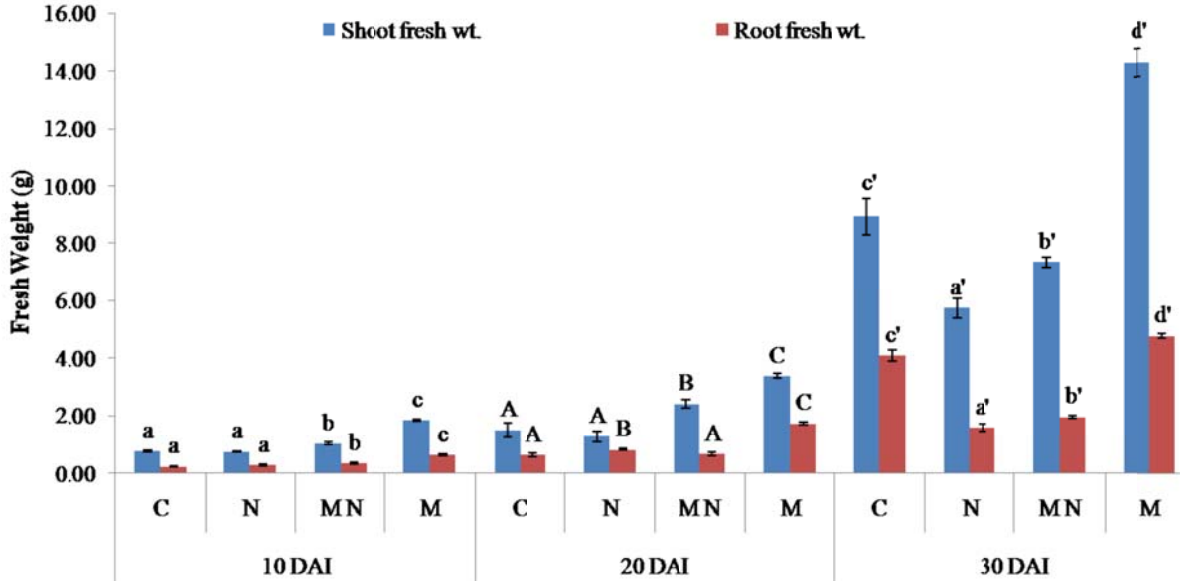

Figure 2. Effect of *Meloidogyne incognita* second-stage juveniles (J2) on the shoot and root fresh weight of tomato var. pant-T3 at 10, 20 and 30 days after inoculation (DAI). (C = control, N = nematodes, MN = mycorrhiza nematodes, M = mycorrhiza). Error bars represent standard error of three replications. Different letters indicate a significant difference (P<0.05) of treatment.

DAI. Significant reduction in shoot and root length was observed in nematode inoculated plants at 20 DAI and 30 DAI (Figure 1). The root and shoot fresh weight was significantly lower in nematodes (N) inoculated plants as compared to mycorrhizal (M) plants. Significant differences were recorded at 30 DAI harvesting, where 59.76% reduction in shoot fresh weight and 66.95% in root fresh weight were observed in nematode (N) inoculated plants (Figure 2). For dry matter observation, harvesting at 30 DAI in nematodes (N) inoculated plants, 61.40% lower shoot dry weight and 68.08% lower root dry weight were recorded (Figure 3).

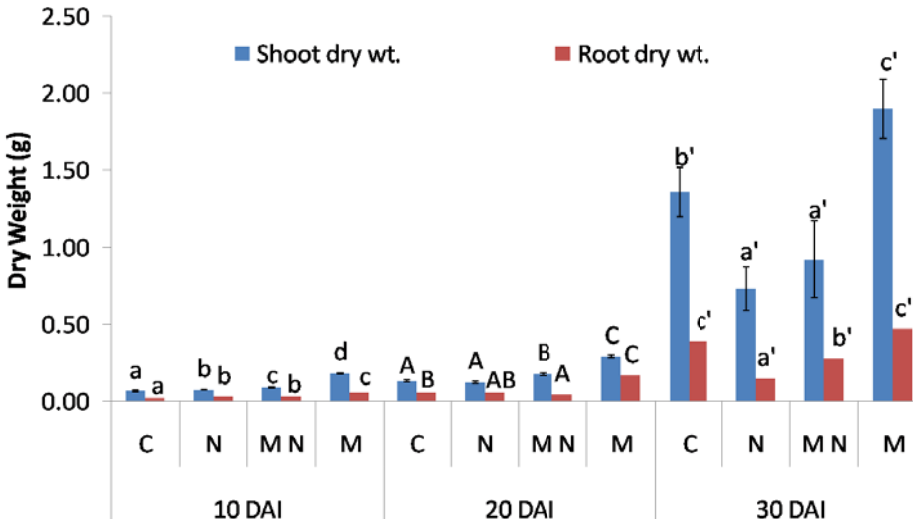

Figure 3. Effect of *Meloidogyne incognita* second-stage juveniles (J2) on the shoot and root dry matters of tomato var. pant-T3 at 10, 20 and 30 days after inoculation (DAI). (C = control, N = nematodes, MN = mycorrhiza nematodes, M = mycorrhiza). Error bars represent standard error of three replications. Different letters indicate a significant difference (P<0.05) of treatment.

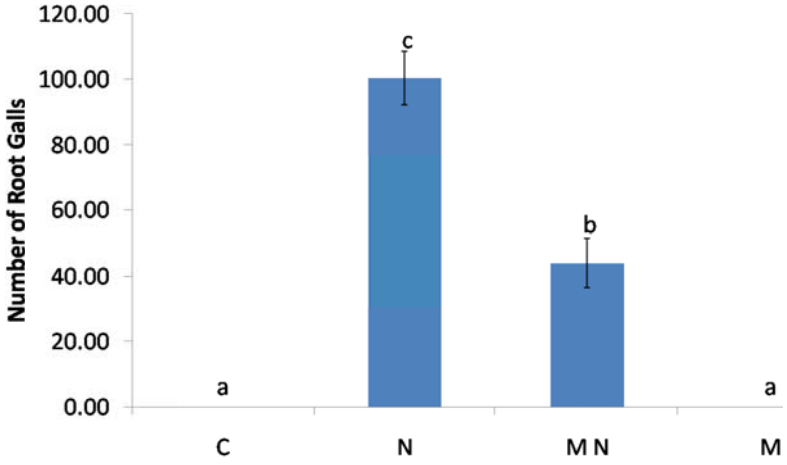

Figure 4. Effect of *Glomus intraradices* on the nematodes originated root galls at 20 days after inoculation (DAI). (C = control, N = nematodes, MN = mycorrhiza nematodes, M = mycorrhiza). Bars represent standard error, based on three replications. Different letters indicate significant differences (P<0.05) between the treatments.

Effect of *G. intraradices* on nematode egg masses, eggs and on adult females

Infection of *M. incognita* second-stage juveniles in tomato roots increased significantly from 10 to 30 DAI which was achieved by increasing root galls in nematodes infected roots. At 10 DAI, no root galls were seen while at 20 DAI, clear root swelling was observed, 56.15% reduction in root galls was observed in nematode (N) infected roots when compared with mycorrhiza nematode (MN) infected roots (Figure 4). Egg masses, eggs and adult females were significantly lower in nematodes infected roots as compared to mycorrhizal roots at 30 DAI (Figure 5 and 6). Mycorrhizal inoculated plants recorded lower root gall index (RGI) and egg mass index (EMI) (Table 1). About 64.13% reductions of egg numbers and 46.03% reduction of adult females with mycorrhiza nematodes (MN) inoculated plants was recorded (Figure 6).

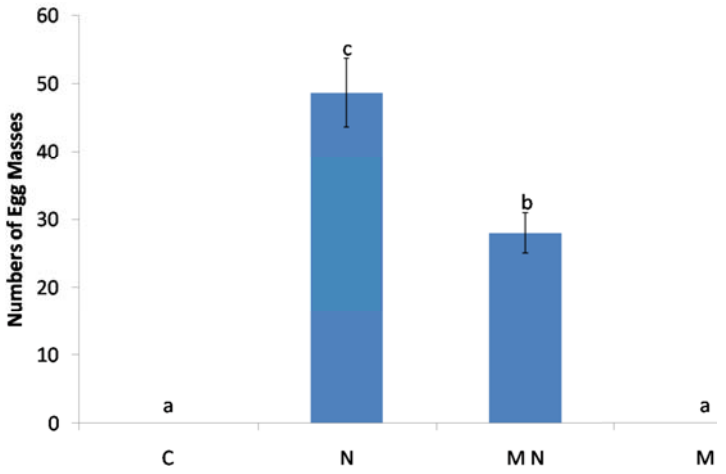

Figure 5. Effect of *Glomus intraradices* on the nematodes originated egg masses at 30 days after inoculation (DAI) (C = control, N = nematodes, MN = mycorrhiza nematodes, M = mycorrhiza). Bars represent standard error, based on three replications. Different letters indicate significant differences (P<0.05) between the treatments.

Table 1. Effect of *G. intraradices* on the nematodes root galls and egg masses

Treatments	20 DAI		30 DAI			
	Number of root galls	RGI**	Number of root galls	RGI**	Number of egg masses	EMI**
C	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0
N	100.33 ± 4.63c	5	199.00 ± 7.64c	5	48.67 ± 2.90c	4
M N	44.00 ± 4.35b	4	99.67 ± 2.33b	4	28.00 ± 1.73b	3
M	0.00 ± 0.00a	0	0.00 ± 0.00a	0	0.00 ± 0.00a	0

Numbers of root galls and egg masses based on three replicates (C, control; N, nematodes; MN, mycorrhiza nematodes; M, mycorrhiza). Different letters indicate significant differences (P<0.05) between the treatments. **Root gall index (RGI) and egg mass index (EMI) were scored on each treatment on a 0-5 basis with 0 = no galls or egg masses, 1= 1 or 2 galls or egg masses, 2 = 3-10 galls or egg masses, 3 = 11-30 galls or egg masses, 4 = 31-100 galls or egg masses and 5 = >100 galls or egg masses.

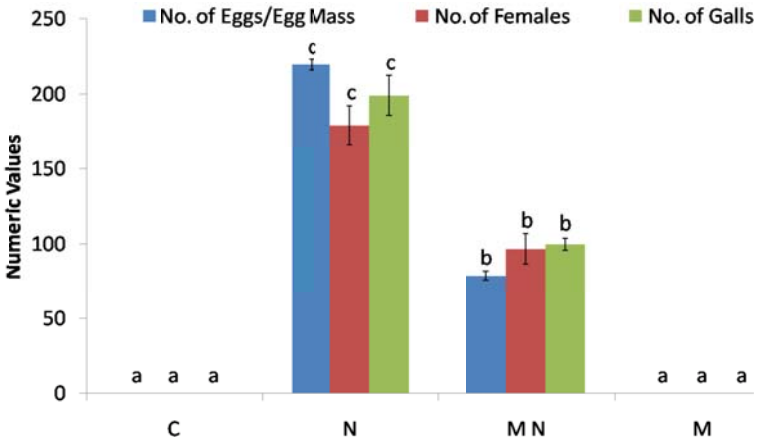

Figure 6. Effect of *Glomus intraradices* on the nematodes eggs, adult females and on nematodes originated root galls at 30 days after inoculation (DAI). (C = control, N = nematodes, MN = mycorrhiza nematodes, M = mycorrhiza). Bars represent standard error, based on three replications. Different letters indicate significant differences (P<0.05) between the treatments.

DISCUSSION

The shoot/root length in tomato var. Pant-T3 was significantly lower in nematodes (N) inoculated plants as compared to pre-colonized mycorrhizal (M) plants. Similar observations were demonstrated in previous experiments (Bhagawati et al., 2000; Talavera et al., 2001; Diehiou et al., 2003; Shreenivasa et al., 2007; Peregrin et al., 2012; Vos et al., 2012a, b; Peregrín et al., 2012).

Penetration by *M. incognita* second-stage juveniles on tomato var. Pant-T3 roots was significantly lower in mycorrhizal roots as compared to nematode infected roots. In the nematodes infected roots, the adult females reduced a maximum of 46.03% at 30 DAI, which is in accordance with previous experiments for this nematode on tomato (Vos et al., 2012a, b). The reduction in nematodes infection by mycorrhiza might have several causes, it may affect motility of the second-stage juveniles in the soil (Jones et al., 2004; Lioussanne et al., 2009). Wuyts et al. (2006) reported negative effects on nematode chemotaxis and on motility due to the presence of several phenolic compounds. Different soil micro-organisms which depend on the AMF might be involved in the nematodes reductions (Lioussanne et al., 2009).

In conclusion, this study demonstrated that reduction of RKN is due to AMF and *G. intraradices* is a potential biocontrol tool for the management of root-knot nematodes.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Azcon-Aguillar C, Barea JM (1996). Arbuscular mycorrhizas and biological control of soil-borne plant pathogens-an overview of the mechanisms involved. Mycorrhiza 6:457-464.

Bhagawati B, Goswami BK, Singh CS (2000). Management of disease complex of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *Lycopersici* through bioagent. Ind. J. Nematol. 30: 16-22.

Byrd DW, Kirkpatrick JRT, Barker KR (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. J. Nematol. 15:142-143.

Diehiou PM, Hallmann J, Oerke EC, Dehne HW (2003). Effects of arbuscular mycorrhizal fungi and a non-pathogenic *Fusarium oxysporum* on *Meloidogyne incognita* infestation of tomato. Mycorrhiza 13:199-204.

Dong LQ, Zhang KQ (2006). Microbial control of plant-parasitic nematodes: a five-party interaction. Plant Soil 288:31-45.

Elsen A, Gervacio D, Swennen R, De Waele D (2008). AMF-induced biocontrol against plant parasitic nematodes in *Musa* sp.: a systemic effect. Mycorrhiza 18:251-256.

Harrier LA, Watson CA (2004). The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soilborne pathogens in organic and/or other sustainable farming systems. Pest Manage. Sci. 60:149-157.

Hoagland DR, Arnon DI (1950). The water-culture method for growing plants without soil. California Agriculture Experiment Station Circular 347, revised.

Hooper DJ, Hallman J, Subbotin S (2005). Methods for extraction, processing and detection of plant and soil nematodes. In: Luc M, Sikora R, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edn. CABI Publishing, Wallingford, pp. 53-86.

Jones DL, Hodge A, Kuzyakov Y (2004). Plant and mycorrhizal regulation of rhizodeposition. New Phytol.163:459-480.

Kaur DN, Sharma SK, Sultan MS (2011). Effect of different chemicals on root knot nematode in seed beds of tomato. Plant Dis. Res. 26:170-170.

Lioussanne L, Beauregard MS, Hamel C, Jolicoeur M, St-Arnaud M (2009). Interactions between arbuscular mycorrhizal fungi and soil microorganism.0s. In: Khasa D, Piché Y, Coughlan AP (eds) Advances in mycorrhizal science and technology. NRC Research Press, Ottawa. pp. 51-69.

National Horticultural Board (NHB) (2011). Indian Horticulture Database. National Horticultural Board. Ministry of Agriculture, Government of India.

Peregrín EF, Azcón R, Salmerón T, Talavera M (2012). Biological protection conferred by *Glomus* spp. and *Bacillus megaterium* against *Meloidogyne incognita* in tomato and pepper. IOBC-WPRS Bulletin 83:215-219.

Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-160.

Shreenivasa KR, Krishnappa K, Ravichandra NG (2007). Interaction effects of arbuscular mycorrhizal fungus *Glomus fasciculatum* and root-knot nematode, *Meloidogyne incognita* on growth and phosphorous uptake of tomato. Karnataka J. Agric. Sci. 20:57-61.

Smith GS, Read DJ (1997). Mycorrhizal Symbiosis. Academic Press, London.

Talavera M, Itou K, Mizukubo T (2001). Reduction of nematode damage by root colonization with arbuscular mycorrhiza (*Glomus* spp.) in tomato- *Meloidogyne incognita* (Tylenchida: Meloidogynidae) and carrot-*Pratylenchus penetrans* (Tylenchida: Pratylenchidae) pathosystems. Appl. Entomol. Zool. 36:387-392.

Taylor AL, Sasser JN (1978). Biology, identification and control of root knot nematodes (*Meloidogyne* spp.). North Carolina State Univ. and United State Agency for International Development, Religh, USA, p. 111.

Vos C, Geerinckx K, Mkandawire R, Panis B, De Waele D, Elsen A (2012a). Arbuscular mycorrhizal fungi affect both penetration and further life stage development of root-knot nematodes in tomato. Mycorrhiza 22:157-163.

Vos CM, Tesfahuna AM, Panis B, De Waele D, Elsen A (2012b). Arbuscular mycorrhizal fungi induce systemic resistance in tomato against the sedentary nematode *Meloidogyne incognita* and the migratory nematode *Pratylenchus penetrans*. Appl. Soil Ecol. 61:1-6.

WPTC (2012). World Production Estimate of Tomatoes for Processing.

Wuyts N, Swennen R, De Waele D (2006). Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant-parasitic nematodes *Radopholus similis*, *Pratylenchus penetrans* and *Meloidogyne incognita*. Nematology 8:89-101.