

ORIGINAL RESEARCH ARTICLE

Plant Growth Promotion and Management of Root-Knot Nematode (*Meloidogyne incognita*) through *Glomus aggregatum* and *Bacillus coagulans* and Vermicomposting in Tomato

P. Serfoji*¹, P. Smithra², K. Saravanan¹ and K. Durai Raj¹

¹PG & Research Department of Zoology, Government Arts College (A), Kumbakonam – 612 001, Tamil Nadu, India

²PG & Research Department of Microbiology, Srimath Andavar College, Trichy, Tamil Nadu, India

Received 23 Mar 2013; Revised 06 Jun 2013; Accepted 14 Jun 2013

ABSTRACT

A glass house experiment was conducted for the effectiveness of Vermicomposting and rhizotrophic microorganisms (*Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans*) for the management of *Meloidogyne incognita* on tomato CV. Pusa Ruby, (*Lycopersicon esculentum* Mill.). Among the different treatments evaluated, vermicompost and *Glomus aggregatum* alone and in combination with *B. coagulans* recorded the maximum growth, biomass and nutrients of tomato with decreased root-knot nematode population and root-knot index. But, amending the soil with application of vermicomposting + *B. coagulans* + *G. aggregatum* in tomato plant showed significantly increased the plant growth, biomass and nutrients. Similarly, reduction in root-knot nematode population, root-knot index (RKI), number of galls and egg masses per plant were recorded in the same treatment. Highest mycorrhizal colonization of 92.5 per cent and minimum nematode population of 265.4 / 250 Cc soil was observed in the same treatment. It can be concluded that application of vermicompost + *G. aggregatum* + *B. coagulans* increased plant growth characters and reduced RKI, nematode reproduction rate (NRR), number of galls and egg masses on tomato plants.

Key words: *Meloidogyne incognita*, Vermicomposting, *Glomus aggregatum*, *Bacillus coagulans*, Tomato and Biological control.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the important commercial and widely grown vegetable crops in both tropics and sub-tropics, which is often severely attacked by root-knot nematode, *Meloidogyne incognita*, a predominant and widely prevalent species inflicting serious loss in tomato [1]. An yield loss of 35-39.7 percent has been reported due to root-knot nematode infestation [2, 3]. Chemicals that are being used for controlling plant parasitic nematodes are costly and hazardous in nature. Researchers all over the world are engaged in standardizing the nematode management strategies by non-chemical and eco-friendly approaches viz., cropping systems, soil amendments (botanicals) [4, 5], organic soil amendment [6, 7, 8], biological control agents [9, 10, 11, 12] and judicious use of nematicides [13] to stabilize crop production. Among the various biocontrol agents, arbuscular mycorrhizal fungi (AMF) are being widely used in nursery seedlings

as it enhances nutrient availability [14] and in reducing harmful effect of root infection by many parasitic nematodes in crop plants is well recognized [15, 16, 17, 18]. The use of vermicompost, as a source of organic manure in supplementing chemical fertilizer is becoming popular among the farmers of the country. Increase in crop yield, soil nutrient status, and nutrient uptake was reported due to application of vermicompost [19]. Investigations carried out, so far, had been mostly on the management of root-knot nematode by utilizing AMF which have introduced from other centers and made to utilize indigenous isolates [20, 21]. Limited efforts have been to utilized plant growth promoting rhizobacteria and organic manures against virulent population of root-know nematode [22]. Hence, the present investigation was conducted to evaluate the effectiveness of vermicompost AM fungus, *Glomys aggregatum* and mycorrhiza helper bacterium (MHB), *Bacillus*

coagulans against *M. incognita* on tomato CV. Pusa Ruby under glass house condition.

2. MATERIALS AND METHODS

The experiment was conducted in PVC pots (20 cm × 16 cm) containing 5 kg autoclaved sandy loam soil (pH 6.8, P (Na HCO₃ extractable) 9.2 mg/kg, total N 0.4 g/kg, silt 110g/kg) and mixed with rock phosphate @ 21.40 mg P/kg. The soil was made nematode sick by thoroughly mixing freshly hatched second stage Juveniles (J2) of *Meloidogyne incognita* @ 4000 J2/ plant. The AM fungus, *Glomus aggregatum* was mass multiplied on onion (*Allium cepa* L.) and an inoculum rate of 1000 chlamydo spores / plant was used. The vermicompost used has 11.5 per cent organic carbon, 1.3 per cent total N, 1.2 per cent P and 2.6 per cent K, Vermicompost @ of 650 g/pot was thoroughly mixed with the soil before filling the pots [23]. *Eudrilus eugeniae* was the earthworm species used for making compost. *B. coagulans* was grown in Pikovskaya medium [24] for 3 days at 30 ± 2°C to a cell density of 2.3 × 10⁷ cells/ml. Inoculation by various combinations of *B. coagulans* under study was done by soaking the surface sterilized seeds of tomato CV. Pusa Ruby in the liquid culture of an organism was mixed in equal proportion and then the seeds were soaked in it. Tomato seedlings were raised in PVC pots and later one seedling was transplanted per pot. Inoculation of *G. aggregatum* and application of vermicompost were done at the root -zone of each 21-day-old tomato seedlings. The treatments included nematode infested soil check, vermicompost (Vc), *Glomus aggregatum* (Ga), *B. coagulans* (Bc), Vc+Ga, Vc+Bc, Ga+Bc and Vc+Ga+Bc. Autoclaved soil without infestation served as non-infested soil check. The glass house temperature ranged between 27 and 32°C. They were watered daily with 100 ml tap water per pot. The plants were completely randomized block design with five replicates on glass house bench. After 60 days of transplantation, five plants from mycorrhiza treated pots were deputed; roots cleared of soil and washed with water. Rest of the plants harvested after 90 days of transplantation for plant height, dry weight of root and shoot, N, P, K content of plants, number and size of galls, number of egg masses per plant root-knot index and nematode reproduction rate. The AM fungal colonization of roots was estimated by trypan blue staining technique [25] along with root-knot index was scored by using 0-5 scale [26].

For estimating the N, P, K content, tomato plants inoculated with different treatments were harvested after 90 days and dried in oven at 60°C for three days. Nitrogen, phosphorus and potassium content of tomato plants were determined by Mikrokjeldahl, Vanadomolybdate – phosphoric yellow colour and flame photometry methods [27], respectively. The soil was also analyzed for mycorrhizal Chlamydo spores through wet-sieving and decantation technique [28]. The data were statistically analysed for variance [29].

3. RESULTS AND DISCUSSION

Application of vermicompost and *Glomus aggregatum* alone significantly increased plant growth, biomass and nutrients of tomato plants compared to that of nematode infested soil (Table 1). However, the magnitude of increase in each character under report varied with the combinations of *G. aggregatum* and Vermicomposting with *B. coagulans*. Plant height under *B. coagulans* alone treatment was same as that of Vermicompost alone indicating that these treatments individually give increased or stimulation to the plant in terms of plant height, whereas, *G. aggregatum* individually as well as in combination with vermicompost and *B. coagulans* increased plant height, biomass and nutrients significantly compared to infested and non-infested soil. Similar results also have been reported by Singh *et al.* [30] in tomato and Vedhara *et al.* [31] in ginger. Further, *B. coagulans* did not individually enhanced shoot and root dry weights significantly over the non-infested soil, although it increased biomass over that of infested soil (Table 1). These results are in conformity with the findings of previous reports of Sumana *et al.* [32], who also reported that inoculation of *B. coagulans* individually did not influence the shoot and root biomass significantly in neem plants. Maximum plant height, biomass and nutrient uptake were recorded in plants treated with *B. aggregatum* + Vermicompost + *B. coagulans* followed by *G. aggregatum* + Vermicompost. Results further indicated that integration of vermicompost + *G. aggregatum* + *B. coagulans* promoted better growth than their individual applications. Similar observations on enhanced plant response to AM fungi in combination with organic manures and *B. coagulans* were reported by Singh *et al.* [33] in tomato, Reddy *et al.* [34] in acid lime and Nagesh and Reddy [35] in *Crissandra undulaefolia*.

At harvest, the root-knot nematode populations in soils were minimum in plants treated with vermicompost + *G. aggregatum* + *B. coagulans* followed by plants inoculated with *G. aggregatum* + Vermicompost, combination of Vermicompost + *G. aggregatum* + *B. coagulans* had minimum number of egg masses, root galls and root-knot index (Table - 2). Application of vermicompost and *G. aggregatum* individually also resulted in lower RKI and number of egg masses under *B. coagulans*. However, RKI and number of egg masses under individual application of vermicompost and *G. aggregatum* were significantly lower as compared to RKI and number of egg masses of infested soil (Table - 2). Further, nematode reproduction rate was also significantly lower in vermicompost + *G. aggregatum* + *B. coagulans* treated plants followed by *G. aggregatum* + Vermicompost treated plants (Table - 2). These results are in conformity with the findings of previous reports of Vedhera *et al.* [36], who reported that application of different organic manures inhibited reproduction in females and penetration of second stage juveniles into ginger roots besides increasing the yield as per findings of Goswani

and Vijayalakshmi [37], Rajendran and Saritha [38] and Kantharaju *et al.* [39] in tomatoes.

Root colonization by *G. aggregatum* and number of chlamyospores / 50 cc soil was significantly increased when integrated with vermicompost and *B. coagulans* than its individual applications (Table 2). Among different treatments, *B. coagulans* enhanced root colonization by *G. aggregatum* to the maximum and the number of chlamyospores. The observed difference in the root colonization and spore number of *G. aggregatum* among its different treatment combinations could be possibly due to the presence of nitrogen, potassium and phosphorus content in vermicompost and *B. coagulans*. This suggest a synergistic activity were in *B. coagulans* enhances the activity of *G. aggregatum* by producing organic acids which serve as a carbon source to the fungus or by hydrolytic enzymes thus enabling the AM fungus to penetrate and ramify in the root system of the host [40]. It can be concluded that application of vermicompost + *G. aggregatum* + *B. coagulans* increased plant growth characters and reduced RKI, NRR, number of galls and egg masses on tomato CV. Pusa Ruby in sandy loam acidic soils.

Table 1: Influence of vermicompost, *Glomus aggregatum* and *Bacillus coagulans* on growth of tomato cv Pusa Ruby infested with *Meloidogyne incognita*

Treatment*	Plant height, cm per plant	Plant dry weight, g per plant			Nitrogen uptake, %/plant	Phosphorus uptake, %/plant	Potassium uptake, %/plant
		Root	Shoot	Total			
Infested soil check	22.45	7.20	34.50	41.70	0.9	0.4	2.8
Non-infested soil check	30.26	9.85	41.50	51.35	1.0	0.5	3.2
Vermicompost (Vc)	32.06	17.75	45.00	62.75	1.2	0.6	4.4
<i>Glomus aggregatum</i> (Ga)	34.65	18.04	46.50	64.54	1.3	0.6	4.2
<i>Bacillus coagulans</i> (Bc)	31.65	15.16	43.25	58.41	1.0	0.6	4.0
Vc+Ga	36.25	28.50	52.00	80.50	1.2	0.6	4.6
Vc+Bc	33.45	26.65	51.55	78.20	1.2	0.6	4.3
Ga+Bc	34.75	26.05	50.50	76.55	1.2	0.6	4.6
Vc+Ga+Bc	35.15	28.68	58.50	87.18	1.4	0.6	4.8
SEM±	0.49	0.34	2.4	1.42	0.02	0.02	0.02
CD (P=0.05)	1.40	1.02	4.8	3.72	0.08	0.08	0.18

Table 2: Effect of vermicompost, *Glomus aggregatum* and *Bacillus coagulans* on multiplication of *Meloidogyne incognita*, % mycorrhizal root colonization and spore number in Rhizosphere soil

Treatments*	Number/plant		Root-knot index (RKI)	Final nem. Popula./ 250 cc soil	Nematode reproduction rate	AM fungal root colonization, %	AM fungal spores/100 cc soil
	Galls	Egg masses					
Infested soil check	132.5	68.22	4.00	676.4	3.46	-	-
Non-infested soil check	-	-	-	-	-	-	-
Vermicompost (Vc)	59.25	42.65	3.48	486.4	2.51	-	-
<i>Glomus aggregatum</i> (Ga)	48.50	22.50	3.12	424.2	2.36	69.35	704
<i>Bacillus coagulans</i> (Bc)	66.24	46.24	3.82	462.3	2.42	-	-
Vc+Ga	38.50	20.25	2.72	382.2	2.12	74.65	865
Vc+Bc	39.65	32.08	2.68	383.4	1.98	78.25	922
Ga+Bc	39.25	28.65	2.68	383.4	1.98	78.25	922
Vc+Ga+Bc	18.25	19.62	2.65	265.4	1.42	92.05	992
SEM±	1.42	0.72	-	4.08	0.06	1.20	5.00
CD (P=0.05)	4.11	2.08	-	16.06	0.84	3.50	17.00

ACKNOWLEDGEMENT

Authors are thankful to the Secretary and Correspondent and the Principal of A.V.V.M. Sri Purshpam College (Autonomous) for

encouragement, providing necessary facilities and thank the chief editor and an anonymous referee for their valuable comments on the manuscript.

4. REFERENCE

1. Sasser JN. 1990. Economic importance of *Meloidogyne* in tropical countries In: Systematics Biology and Control [Lamberti F and Taylor CE. (Eds)] Academic Press, New York, p.477.
2. Reddy DDR. 1985. Analysis of crop losses in tomato due to *Meloidogyne incognita* *J Nematol* 15: 55-59.
3. Jonathan EI, Kumar S, Devrajan K and Rajendran G. 2001. Fundamentals of plant Nematology. Devi Publications, Tiruchirappalli, India, p.229.
4. Sukul NC, Sinhababu SP, Datta SC, and Sukul A. 2001. Nematode effect of *Acacia auriculiformis* and *Artemisia nilagrica* against root-knot nematode Allelopathy *J* 8: 65-72.
5. Rajendran G and Saritha V. 2005. Effect of plant extracts and their potential doses against root-knot nematode, *Meloidogyne incognita* on tomato *Indian J Nematol* 35: 38-31.
6. Singh YP, Singh RS, and Sitaramaiah K. 1990. Mechanism of resistance of mycorrhizal tomato against root-knot nematode In: Current Trends in Mycorrhizal Research [Jalali BL and Chand H. (Eds)] Haryana Agricultural University, Haryana, India, p.210.
7. Vedhera I, Tiwari SP, and Dave GS. 1998. Integrated management of root-knot nematode *Meloidogyne incognita* in ginger *Indian Phytopathol* 51: 161-163.
8. Nagesh M and Reddy PP. 1997. Management of *Meloidogyne incognita* on *Crossandra undulaefolia* using vesicular arbuscular mycorrhiza (*Glomus mosseae*) and oil cakes Mycorrhiza News 9: 12-14.
9. Babu RS, Nageswari Poornima K and Suguna N. 2000. Biological control potential of *Glomus fasciculatum* against *Meloidogyne incognita* on tomato and Okra *Proc First Nat Sym Pest Manage Horti Crops* Environmental implication and thrusts. Bangalore, India. p.348.
10. Krishnappa K. 2002. Integrated management of root-knot on tomato and burrowing nematode on banana by utilizing indigenous types of mycorrhizal bioagent, *Glomus fasciculatum*, University of Agricultural Sciences, Bangalore, India, p.71.
11. Kantharaju V, Krishnappa K, Ravichandra NG and Karuna K. 2005. Management of root-knot nematode *Meloidogyne incognita* on tomato by using indigenous isolates of AM fungus *Glomus fasciculatum* *Indian J Nematol* 35: 32-36.
12. Sumathi M, Parthiban S, and Sameul SD. 2006. Effect of graded doses of nitrogen and VAM on herbage oil yield and nematode population on patchouli (*Pogostemon patchouli* Pellet.) *Indian J Nematol* 36: 209-213.
13. Taylor AL. and Sasser JN. 1978. Biology Identification and Control of root-knot nematode (*Meloidogyne* spp). North Carolina State Univ. and United States Agency for International Development Raleigh, USA, p.111.
14. Jeffries P. 1987. Use of Mycorrhiza in agriculture *Critical Review Bitech* 5: 319-357.
15. Mahaveer P, Sharma S, Bhargava Verma MK, and Alokadnoleya A. 1994. Interaction between the endomycorrhizal fungus *Glomus fasciculatum* and root-knot nematode, *Meloidogyne incognita* on tomato *Indian J Nematol* 24: 133-139.
16. Jothi G and Sunderababu R. 2002. Nursery management of *Meloidogyne incognita* by *Glomus mosseae* in egg plant *Nematologia Mediterranea* 30: 154-157.
17. Kantharaju V, Krishnappa K, Ravichandra NG and Karuna K. 2005. Management of root-knot nematode *Meloidogyne incognita* on tomato by using indigenous isolates of AM fungus *Glomus fasciculatum* *Indian J Nematol* 35: 32-36.
18. Shreenivasa KR, Krishnappa K, Ravichandra NG, Ravikumar B, Kirankumar KC, and Karuna K. 2007. Optimization of arbuscular mycorrhizal fungus *Glomus fasciculatum* culture against root-knot nematode *Meloidogyne incognita* on tomato *Asian J Micro Biotech Environ Sci* 9: 117-121.
19. Ansari AA and Ismail SA. 2001. Vermitechnology in organic solid waste management *J Soil Biol Ecol* 21: 21-24.
20. Mishra A and Shukla BN. 1997. Interactions between *Glomus fasciculatum* and *Meloidogyne incognita* on tomato *J Mycol Plant Pathol* 27: 199-202.
21. Kantharaju V, Krishnappa K, Ravichandra NG and Karuna K. 2005. Management of

- root-knot nematode *Meloidogyne incognita* on tomato by using indigenous isolates of AM fungus *Glomus fasciculatum* *Indian J Nematol* 35: 32-36.
22. Rao MS Mohandas S, and Reddy PP. 1993. Integrated management of root-knot nematode on egg plant in nursery beds with combination of *Glomus mosseae* and neem leaf *Indian J Nematol* 23: 14-18.
23. Shivaputra SS, Patil CP, Swamy GSK, and Patil PB. 2004. Effect of vesicular arbuscular mycorrhizal fungi and vermicompost on drought tolerance in papaya *Mycorrhiza News* 16: 12-13.
24. Pickovskya RI. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species *Microbiology* 17: 362-370.
25. Phillips JM and Hayman DS. 1970. Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection *Trans Brit Mycol Soc* 55: 156-161.
26. Taylor AL. and Sasser JN. 1978. Biology Identification and Control of root-knot nematode (*Meloidogyne* spp). North Carolina State Univ. and United States Agency for International Development Raleigh, USA, p.111.
27. Jackson ML. 1973. Soil Chemical Analysis, Prentice Hall of India, New Delhi, India.
28. Gerdemann JW and Nicolson TH. 1963. Spores of mycorrhizal *Endogone* species extracts from soil by wet-sieving and decanting *Trans Brit Mycol Soc* 46: 235-244.
29. Little TH and Hills JF. 1978. Agricultural Experimentation. John Willey and Sons, New York, USA.
30. Singh YP, Singh RS, and Sitaramaiah K. 1990. Mechanism of resistance of mycorrhizal tomato against root-knot nematode In: Current Trends in Mycorrhizal Research [Jalali BL and Chand H. (Eds)] Haryana Agricultural University, Haryana, India, p.210.
31. Vedhera I, Tiwari SP, and Dave GS. 1998. Integrated management of root-knot nematode *Meloidogyne incognita* in ginger *Indian Phytopathol* 51: 161-163.
32. Sumana DA, Bagynaraj DJ, and Arpana J. 2003. Interaction between *Glomus mosseae* *Azotobacter chroococcum* and *Bacillus coagulans* and their influence on growth and nutrition of neem *J Soil Biol Ecol* 23: 80-86.
33. Singh YP, Singh RS, and Sitaramaiah K. 1990. Mechanism of resistance of mycorrhizal tomato against root-knot nematode In: Current Trends in Mycorrhizal Research [Jalali BL and Chand H. (Eds)] Haryana Agricultural University, Haryana, India, p.210.
34. Reddy PP, Rao MS, Mohandoss S, and Magesh M. 1995. Integrated management of the *Citrus* nematode *Tylenchulus semipenetrans* Cobb. using VA-mycorrhiza *Glomus fasciculatum* and oil cakes *Pest Manage Horti Ecosy* 1: 37-41.
35. Nagesh M and Reddy PP. 1997. Management of *Meloidogyne incognita* on *Crossandra undulaefolia* using vesicular arbuscular mycorrhiza (*Glomus mosseae*) and oil cakes *Mycorrhiza News* 9: 12-14.
36. Vedhera I, Tiwari SP, and Dave GS. 1998. Integrated management of root-knot nematode *Meloidogyne incognita* in ginger *Indian Phytopathol* 51: 161-163.
37. Goswami BK and Vijayalakshmi K. 1981. Effect of some indigenous plant material and oil cakes amended soil on growth of tomato and root-knot nematode. *Indian J Nematol* 11: 12-16.
38. Rajendran G and Saritha V. 2005. Effect of plant extracts and their potential doses against root-knot nematode, *Meloidogyne incognita* on tomato *Indian J Nematol* 35: 38-31.
39. Reddy BMR, Krishnappa K, and Karuna K. 1996. Management of root-knot nematode on tomato by seedlings base-root dips in chemicals *J Soil Biol Ecol* 16: 145-147.
40. Dupannois R and Garbaye J. 1991. Effect of dual inoculation of *Doughlus fir* with the ectomycorrhizal fungus *Laccaria laccata* and mycorrhiza helper bacteria (MHB) in two bare root forest nurseries *Plant soil* 138: 169-176.