Journal of Biological Control, 24 (2): 164–167, 2010

Research Article

Evaluation of *Pseudomonas fluorescens* and *Trichoderma viride* as seed and soil treatment against root knot nematode, *Meloidogyne arenaria* infecting groundnut

S. RAMAKRISHNAN[1*] and G. RAJENDRAN

Department of Plant Nematology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India.
[1]Central Tobacco Research Institute, Research Station, Hunsur 571105, Karnataka, India.
**Corresponding author: E-mail: ramkictri@yahoo.com*

ABSTRACT: Studies on evaluation of biocontrol agents against *Meloidogyne arenaria* on groundnut under glasshouse conditions revealed that application of *Pseudomonas fluorescens* as seed treatment+soil application recorded significant increase in plant growth characters and groundnut pod yield compared to untreated control. Groundnut plants treated with *P. fluorescens* as both seed treatment+soil application recorded least number of galls / plant, egg mass/plant, eggs/egg-mass and soil population/200 g soil with corresponding reduction of 70.9, 34.4, 20.3 and 68.1 per cent, respectively, over untreated control. It was followed by *P. fluorescens* as soil application alone and carbofuran in reducing the root knot nematode infection in groundnut.

KEY WORDS: *Meloidogyne arenaria*, *Pseudomonas fluorescens*, *Trichoderma viride*, groundnut, seed and soil treatment

(Article chronicle: Received: 09.02.2010; Sent for revision: 22.03.2010; Accepted: 06.04.2010)

INTRODUCTION

Groundnut, *Arachis hypogea,* is an important commercial crop grown worldwide for its edible oil. More than 55 pathogens have been reported to affect groundnut crop, of which several plant parasitic nematodes, *viz., Aphelenchoides arachidis, Aphelenchus avenae, Belonolaimus* spp., *Criconemella oronata, Criconemoides spp., Helicotylenchus* spp., *Hoplolaimus* spp., *Longidorus* spp., *Meloidogyne arenaria, M. incognita, M. javanica, Pratylenchus coffeae, Tylenchorhynchus* spp., and *Rotylenchulus reniformis* were found to be associated. Root-knot disease caused by *M. arenaria* (Neal, 1989) race 1 is the most widespread and destructive (Patel *et al*., 1996). Root knot nematodes enter and establish in the developing pegs and pods, often preventing seed development. Infested pods are weaker, and detached from the plant before or during digging. Chemicals like aldicarb, carbofuran or DBCP are widely used for nematode management. But indiscriminate use of chemical nematicides may be of limited practical value due to their high costs and environmental hazards they pose. Fluorescent pseudomonads associated with plant rhizosphere have emerged as potential biocontrol agents against plant parasitic nematodes (Oostendrop and Sikora, 1989). Hence, an attempt was made to study the bio-efficacy of *Pseudomonas fluorescens* and *Trichoderma viride* against *M. arenaria* in groundnut under glass house conditions.

MATERIALS AND METHODS

An experiment was conducted under glasshouse conditions for the management of *M. arenaria* on groundnut cv. Co3 using talc based commercial formulation of *P. fluorescens* (Pf1 strain) and *T. viride* along with carbofuran 3G and phorate 10G as standard chemical check. The experiment consisted of the following nine treatments, *viz*., T_1: seed treatment with *P. fluorescens* (Pf1) @ 10 g kg^1 seed, T_2: seed treatment with *T. viride* @ 4 g kg^1 seed, T_3: soil application with *P. fluorescens* (Pf1) @ 2.5 kg ha^1 at the time of sowing, T_4: soil application with *T. viride* @ 2.5 kg ha^1 at the time of sowing, T_5: seed treatment @ 10g kg^1 seed + soil application @ 2.5 kg ha^1 of *P. fluorescens,* T_6: seed treatment @ 4 g kg^1 seed + soil application @ 2.5 kg ha^1 of *T. viride,* T_7: carbofuran 3G @ 1 kg a.i ha^1, T_8: phorate 10 G @ 1 kg a.i ha^1 and T_9: untreated control.

Treatment application in glass house and experimental design

For seed treatment, groundnut seeds were soaked in water containing the talc based product of *P. fluorescens* (Pf1) @ 10 g kg^{1} seed for 12 h. Excess water was drained off and the treated seeds were incubated in the dark for 24 h before sowing (Karthikeyan *et al.,* 2000). For treatment with *T. viride*, seeds of groundnut were surface sterilized with mercuric chloride @ 0.1 per cent for two minutes, washed with sterile distilled water and treated with *T. viride* @ 4 g kg^{1} seed as dry treatment just before sowing. For soil application, talc based product of *P. fluorescens* (Pf1) and *T. viride* were incorporated into the soil @ 2.5 kg ha^{1} at the time of sowing. Egg masses of *M. arenaria* were collected from pure cultures maintained on groundnut plant and kept for hatching. At seven days after sowing, freshly hatched second stage juveniles of *M. arenaria* were inoculated to the plant at the rate of one per gram of soil. All the treatments were replicated thrice in randomized block design. The experiment was terminated at 105 DAS and observations were made on plant growth characters, besides nematode population and microbial colonization at the time of termination of the experiment.

The colonization of *P. fluorescens* (Pf1) in roots was estimated by taking a homogeneous sample of 1g root bits from *P. fluorescens* treated plants. The root bits were ground treatment-wise separately in a sterile pestle and mortar with 10 ml of sterile distilled water. The extract thus obtained was serially diluted up to 10^{-8}. One ml of aliquot was transferred to a sterile Petri dish to which molten Kings'B medium was added, gently rotated and incubated at 28 ± 2%C for 24h. The colonies were counted under UV lamp at 366 nm. Colonization of *T. viride* in roots was estimated by taking a homogeneous sample of 1g root bits from *T. viride* treated plants. The root bits were ground treatment-wise separately in sterile pestle and mortar with 10 ml of sterile distilled water. The extract thus obtained was serially diluted up to 10^{-3}. One ml aliquot was transferred to a sterile Petri dish to which molten *Trichoderma* special medium was added, gently rotated and incubated at room temperature for five to seven days and colonies were counted.

RESULTS AND DISCUSSION

Application of *P. fluorescens* and *T. viride* as seed treatment+soil application, seed treatment alone and soil application alone recorded significant increase in groundnut plant growth characters and pod yield compared to untreated check (Table 1). *P. fluorescens* as seed treatment+ soil application significantly increased shoot length, shoot length, shoot weight, root length and root weight to the tune of 58.3, 145.2, 53.4 and 70.1 per cent, respectively, over untreated control. The use of carbofuran recorded 48.6 per cent increase in shoot length compared to untreated control and was on par with *P. fluorescens* as seed treatment + soil application. *P. fluorescens* as seed treatment + soil application recorded 115.7 and 107.3 per cent increase in fresh and dry pod yield respectively, over control. *T. viride* as seed treatment + soil application recorded 98.6 per cent increase in fresh pod yield and was on par with carbofuran. In accordance with the present results, increase in plant growth and yield by the plant growth promoting rhizobacterium *P. fluorescens* was reported in various crop plants. (Santhi and Sivakumar, 1995). Karthikeyan *et al*. (2000) earlier recorded an increase in dry shoot and root weight in chilli and brinjal as observed in the present study when *T. viride* was used as seed treatment. The plant growth regulators, including gibberellins, cytokinins and indole-3 acetic acid (IAA) produced by the plant growth promoting bacterium were reported to constitute a mechanism for plant growth promotion (Lifshits *et al*., 1987).

All the treatments evaluated significantly reduced *M. arenaria* population in soil and root compared to untreated control (Table 2). *P. flourescens* application as seed treatment + soil application significantly reduced number of galls, egg mass and soil population to the tune of 70.9, 82.7 and 68.1 per cent, respectively, over untreated control. *P. flourescens* as soil application alone recorded reduction of 62.4 and 63.9 per cent in gall number and soil population respectively, and were on par with chemical nematicide carbofuran. Application of *T. viride* as seed treatment + soil application caused 52.9 per cent reduction in soil population compared to 62.4 per cent in carbofuran treated plants. Similar reduction in potato cyst nematodes in potato roots by 47.7 per cent due to application of *P. flourescens* @ 10 kg ha^{-1} was reported earlier by Mani *et al*. (1998). Maximum colonization of rhizobacteria, *P. fluorescens* on roots was observed in plants treated with *P. fluorescens* as both seed treatment and soil application with an average of 66.3 x 10^{8} CFUs g^{-1} root and it was followed by *P. fluorescens* as soil application alone (61.3 x 10^{8} CFUs g^{-1}) and *P. fluorescens* as seed treatment alone (53.6 x 10^{8} CFUs g^{-1}). Application of *T. viride* as both soil application and seed treatment recorded the highest root colonization in groundnut roots with an average of 22.67 x 10^{3} CFUs g^{-1} root and it was followed by the treatment, with *T. viride* as soil application alone and *T. viride* as seed treatment alone with an average of 20.67 and 18.0 x 10^{3} CFUs g^{-1} root respectively. The mechanism responsible for the reduction of nematode population may be related to the ability of the bacterium to envelop or bind in the root surface lectins (carbohydrate – lectin bindings) thereby interfering with normal host recognition (Oostendorp and Sikora, 1990). The production

Table 1. Effect of bio control agents on plant growth and yield of groundnut cv. Co 3 infested with root knot nematode, *Meloidogyne arenaria* under glasshouse conditions

Treatments	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Fresh pod yield (g)	Per cent increase over control	Dry pod (g) control	Per cent increase over
Pseudomonas fluorescens as ST @ 10 g / kg seed	33.33 (46.1)	12.06 (15.6)	6.90 (20.0)	1.00 (14.9)	8.00	90.5	2.65	76.6
Trichoderma viride as ST @ 4 g / kg seed	6.23 (15.0)	11.40 (9.3)	6.16 (7.1)	0.90 (3.4)	6.90	64.2	2.48	65.3
Pseudomonas fluorescens as SA @ 2.5 kg / ha	34.29 (50.4)	14.20 (36.1)	10.50 (82.6)	1.25 (43.7)	8.73	107.8	2.92	94.7
Trichoderma viride as SA @ 2.5 kg / ha	28.40 (24.5)	11.60 (11.2)	6.30 (9.6)	0.90 (3.4)	7.71	83.6	2.58	72.0
Pseudomonas fluorescens as ST + SA	36.10 (58.3)	16.00 (53.4)	14.10 (145.2)	1.48 (70.1)	9.06	115.7	3.41	107.3
Trichoderma viride as ST + SA	32.20 (41.2)	12.53 (20.1)	8.40 (46.0)	1.05 (20.7)	8.34	98.6	2.75	83.3
Phorate 10G @ 1 kg a.i / ha	30.10 (32.0)	12.03 (15.3)	6.80 (18.2)	1.00 (14.9)	8.03	99.6	2.75	83.3
Carbofuran 3G @ 1 kg a.i / ha	33.90 (48.6)	.13.80 (32.3)	10.20 (77.4)	1.05 (20.7)	8.70	107.1	2.85	90.0
Untreated control	22.8	10.43	5.75	0.87	4.20	–	1.50	–
CD (P = 0.05)	3.11	2.08	1.29	0.14	0.65		0.32	

* Figures in parentheses are per cent increase over control; ST- Seed treatment; SA – Soil application

Table 2. Effect of biocontrol agents on root knot nematode, *Meloidogyne arenaria* in groundnut cv. Co 3 under glasshouse conditions

Treatments	No. of galls / plant	No. of egg mass/ plant	No. of eggs / egg mass	Soil population / 200 g soil	No. of colony forming units / g root
Pseudomonas fluorescens as ST @ 10 g / kg seed	31.66 (32.6)	10.67 (44.8)	259.33 (10.1)	115.67 (48.5)	53.67 x 10
Trichoderma viride as ST @ 4 g / kg seed	40.33 (16.5)	15.33 (20.7)	274.33 (4.9)	130.33 (41.9)	18.0 x 10^6
Pseudomonas fluorescens as SA @ 2.5 kg / ha	17.67 (62.4)	5.67 (70.7)	240.67 (16.6)	81.00 (63.9)	61.33 x 10^8
Trichoderma viride as SA @ 2.5 kg / ha	36.67 (21.9)	12.67 (34.4)	265.33 (8.1)	128.67 (42.7)	20.67 x 10^6
Pseudomonas fluorescens as ST + SA	13.67 (70.9)	3.33 (82.7)	230.00 (20.3)	71.67 (68.1)	66.33 x 10^8
Trichoderma viride as ST + SA	26.33 (43.9)	8.33 (56.9)	250.67 (13.2)	105.67 (52.9)	22.67 x 10^6
Phorate 10G @ 1 kg a.i / ha	34.33 (26.9)	11.67 (39.6)	262.33 (9.1)	122.33 (45.5)	
Carbofuran 3G @ 1 kg a.i / ha	20.33 (56.67)	5.67 (70.67)	242.67 (15.9)	84.33 (62.4)	
Untreated control	47.00	19.33	288.67	224.67	
CD (P = 0.05)	3.88	1.76	13.76	9.3	

* Figures in parentheses are per cent decrease over control; ST – Seed treatment; SA – Soil application

of antibiotics and iron chelating agents (siderophores) were reported to be responsible for the suppression of plant pathogens (Kloepper *et al*., 1980). Secondary metabolites such as 2, 4 – diacetylphloroglucinol and lytic enzymes produced by *P. fluorescens* were chitinolytic in nature and were responsible for increase in premature hatching of eggs and subsequent reduction in viability and mobility of nematode juveniles (Elsherif and Grossmann, 1996). Thus, it can be inferred that *P. fluorescens* is an effective bioagent against *M. arenaria* on groundnut and can be used as both seed treatment and soil application.

REFERENCES

Elsherif, M. and Grossmann, F. 1996. Role of biotic factors in the control of soil-borne fungi by fluorescent pseudomonads. *Microbiology Research*, **151**: 355–357.

Karthikeyan, G., Sabitha Doraiswamy and Sivakumar, C. V. 2000. Biological and chemical control of *Pythium aphanidermatum – Meloidogyne incognita* disease complex in chilli and brinjal. *Madras Agricultural Journal*, **87**: 105–108.

Kloepper, J. W., Leong,J., Jeintze, M. and Schroth, M. N. 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature*, **286**: 885–886.

Lifshits, R., Kloepper, J. W., Kozlwski, M., Simonson, C., Cavison, J., Tipping, E. M. and Zaleska, I. 1987. Growth promotion of canola (rape seed) seedling by a strain of *Pseudomonas putida* under gnotobiotic conditions. *Canadian Journal of Microbiology*, **33**: 390–395.

Mani, M. P., Rajeswari, S. and Sivakumar, C. V. 1998. Management of the potato cyst nematodes, *Globodera* spp. through plant rhizosphere bacterium *Pseudomonas fluorescens* Migula. *Journal of Biological Control*, **12**: 131–134.

Oostendorp, M. and Sikora, R. A. 1989. Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. *Revue de Nematologie,* **12**: 77–83.

Oostendorp, M. and Sikora, R. A. 1990. *Invitro* interrelationship between rhizosphere bacteria and *Heterodera schachtii*. *Revuede Nematologie,* **13**: 269–274.

Patel, B. A., Patel , D. J., Sharma, S. B., Patel, H. V. and Patel, S. K. 1996. Nematode problems of groundnut and their management in Gujarat, India. *International Arachis Newsletter,* **16**: 38–39.

Santhi, A. and Sivakumar, C. V. 1995. Biocontrol potential of *Pseudomonas fluorescens* (Migula) against root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 on tomato. *Journal of Biological Control*, **9**: 113–115.