J. Biol. Control, 22(2): 425-432, 2008

# Microbial consortium and its effect on controlling coffee root-lesion nematode (*Pratylenchus coffeae*) under nursery conditions

**P. PANNEERSELVAM, M. THANGARAJU[1], M. SENTHILKUMAR and JAYARAMA[2]**
Indian Institute of Horticultural Research
Hessaraghtta Lake PO, Bangalore 560 089, Karnataka, India.
E-mail: panneerccri@yahoo.co.in

**ABSTRACT:** **In India, the coffee root-lesion nematode (*Pratylenchus coffeae*) is one of the most destructive pests in many coffee growing areas. The present study was attempted to understand the effects of native microbial consortium (*i.e.* combination of *Azospirillum*, *Azotobacter*, Phosphobacteria, *Gluconacetobacter* and *Pseudomonas*) along with arbuscular mycorrhizal (AM) fungi on the control of coffee root-lesion nematode under nursery conditions. The results indicated that application of microbial consortium (5.0 g seedling$^{-1}$) plus AM fungi (5.0 g seedling$^{-1}$) on arabica coffee seedlings found to be superior in increasing all the growth parameters *viz.*, leaf area, shoot length, shoot girth, root length, dry weight, leaf chlorophyll, total phenol of leaf and roots with significant reduction in the nematode populations after three months (18.0 number 100 ml$^{-1}$ of sterile soil and 18.6 number 100 ml$^{-1}$ of non-sterile soil) and after six months (14.8 number 100 ml$^{-1}$ of sterile soil and 17.1 number 100 ml$^{-1}$ non sterile soil) compared to control. Similar to soil nematode population, the microbial consortium plus AM applied treatments recorded significantly lower nematode population in roots after six months of inoculation compared to other treatments.**

**KEY WORDS:** Coffee, root-lesion nematode, microbial consortium, AM fungi

## INTRODUCTION

In coffee, nematode is one of the major pests, occurring in patches on coffee plantations in most of the coffee producing countries including India. In coffee, yield loss ranging from 15-25 per cent in Brazil (Lordello, 1986) and to 50 per cent in Asia (Campos *et al.*, 1990) has been attributed to nematode damage. The migratory endoparasitic nematode, *Pratylenchus* sp. are the most commonly observed and destructive nematodes on coffee (Campos *et al.*, 1990). In areas infested with nematodes, the establishment of young arabica coffee plants may be impossible due to high mortality and poor development of root system of the transplanted seedlings (Campos *et al.*, 1990). Studies in other crops have demonstrated that AM fungi and fluorescent pseudomonads played key

1. Department of Agricultural Microbiology, TNAU, Coimbatore - 641003, India
2. Central Coffee Research Institute, CRS --Po, Chikmagalore - 577 117, India

role by increasing plant growth even in the presence of sedentary endoparasitic nematodes. However, in India, no studies have been undertaken so far on the interaction of AM fungi and fluorescent pseudomonads against nematodes in coffee. Soil has enormous untapped potential antagonistic microbes, which are helpful in reducing pathogen through different modes of action such as competition for nutrients and space, antibiosis, mycoparasitism, production of siderophores and lytic enzymes (Rangaswami, 1975). At present, a similar type of approach is very much essential to control nematode in coffee. Hence, the present study was undertaken to understand the effect of native biological agents on nematode control in the host system.

## MATERIALS AND METHODS

A nursery experiment was conducted during 2005 - 2006 at Regional Coffee Research Station, Thandikudi, Tamil Nadu, India. In this experiment, the following efficient isolates isolated from different coffee growing regions in India *viz.*, *Azospirillum* – ASW 13, *Azotobacter* – AZK 9, *Gluconacetobacter* – GDT 13, Phosphobacteria – PBY 23, *Pseudomonas fluorescens* – PKK 9 and arbuscular mycorrhizal fungi like *Glomus mosseae*, *G. fasciculatum* and *Glomus* spp (species unidentified) were used for the study. The authentic strain *P. fluorescens* – Pf 1 obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India was used as standard check for experiment. In arbuscular mycorrhizal fungi, the mixed cultures were multiplied using sterile vermiculite as substrate and maize as host plant. These root parts and soil containing chlamydospores were used as inocula (40 – 50 spores $g^{-1}$ of inoculum) for this experiment. The selected bacterial isolates were multiplied in respective broth and with cell load of $10^8$ cfu $ml^{-1}$ were used for inoculam preparation. Lignite was used as the carrier material. The microbial consortium was prepared by mixing equal volumes of broth (above mentioned five bacterial isolates from coffee plantations) with carrier material. Similar manner, *P. fluorescens* – PFK 9 and *P. fluorescens* – Pf 1 were prepared separately and used in this experiment.

For preparation of nursery, top fertile soil from virgin forest lands were collected and dried thoroughly. Latter the dried soil was sieved. A nursery mixture (6 :2 :1) comprising of six parts of sieved jungle soil, two parts of well decomposed and sieved compost and one part of fine river sand was prepared for filling up the nursery bags. Poly bags of 22.5 x 15 cm size and 150 gauge thickness with adequate number of 3 mm holes (10-12 per bag) at the bottom were used. The prepared nursery mixture was moistened with water and firmly filled in nursery bags. In this experiment both sterilized and non-sterilized soils were used. The sterilized and non-sterilized nursery mixture was filled in one kg capacity nursery bags separately. The forty five day old coffee seedlings *i.e.* at topee stage (S.795) were transplanted at the rate of one seedling per bag. The details of treatment are as follows $T_1$ – Sterile soil + *Pseudomonas* – PFK 9, $T_2$- Sterile soil + *P. fluorescens*- Pf1, $T_3$- Sterile soil + microbial consortium, $T_4$ -Sterile soil + AM fungi, $T_5$- Sterile soil, $T_6$ – Non-sterile soil + *Pseudomonas* – PFK 9, $T_7$- Non -sterile soil + *P. fluorescens* - Pf1, $T_8$- Non-sterile soil + Microbial consortium, $T_9$ –Non sterile soil + AM fungi, $T_{10}$- Non-sterile soil. Five replications of each treatment were arranged in completely randomized block design. In each treatment fifty seedlings were taken. At the time of planting, soil inoculation of *Pseudomonas* and microbial consortium was given @ 5 g/ bag and AM fungi @ 10 g / bag. In microbial consortium treatment, AM fungi were also inoculated @ 5 g per poly bag. Followed by addition of microbial inoculants, the second stage juvenile of *P. coffeae* were inoculated @ 500 nematode / nursery bag (Kumar, 1982) by making holes in the soil around the plant stem except control and the same were covered with sterilized soil.

The plants were pulled out 6 months after transplanting and the plant growth parameters such as shoot length, root length and dry weight; physiological parameters like leaf area, total chlorophyll content of leaves (Hiscox and Isrealstam, 1979), biochemical changes like phenol

content in leaves as well as in roots (Zieslin and Ben - Zaken, 1993) and plant uptake of N (Humphries, 1956), P, K, Zn, Cu, Fe and Mn (Jackson, 1973) were recorded by adopting standard methods. Population of nematodes (Kumar, 1982) and *Pseudomonas* and AM colonization (Gerdemann and Nicolson, 1963) were recorded after three and six months of nematode inoculation by using standard methods.

## RESULTS AND DISCUSSION

The growth performances of nematode infested coffee seedlings were assessed after six months and the results are presented in Table 1 and 2. In both sterile and non-sterile soil, the microbial consortium treatments were found to be superior in increasing all the growth parameters *viz.*, leaf area (286.4 and 281.6 $cm^2$ $plant^{-1}$), shoot length (25.3 and 24.9 cm), at collar region girth (3.9 and 3.6 mm), root length (20.1 and 19.8 cm), root dry weight of plant (1.28 and 1.21 g $plant^{-1}$) and dry weight of plant (4.8 and 4.7 g $plant^{-1}$), chlorophyll content (1.93 and 1.89 mg $g^{-1}$ of fresh leaf), total phenol (179.32 mg $g^{-1}$ and 174.63 mg $g^{-1}$ of leaf; 190.12 mg $g^{-1}$ and 187.21 mg $g^{-1}$ of root) in sterile and non sterile soil respectively compared to individual inoculant treatment.

Data presented in Table 3 revealed that the coffee seedlings inoculated with microbial consortium had recorded significantly higher uptake of all the nutrients *i.e.* 51.5 to 52.6mg of N $plant^{-1}$, 21.0 to 21.6 mg of P $plant^{-1}$, 20.6 to 21.3 mg of K $plant^{-1}$, 15.2 to 15.6mg of Zn $plant^{-1}$, 3.1 to 3.4 mg of Cu $plant^{-1}$, 27.2 to 28.6 mg of Fe and 50.2 to 56.8 mg of Mn in both sterile and non sterile soil as compared to all other treatments.

In the present study, the microbial consortium treatment contains known nitrogen fixing and growth promoting organisms like *Azospirillum, Azotobacter; Gluconacetobacter, P. fluorescens*, phosphorus solubilizing bacteria and AM fungi and the combined effect of all might have increased growth parameters of coffee seedlings. In the present study, the total chlorophyll and phenol content had significantly increased in coffee seedlings inoculated with microbial consortium compared to that in seedlings inoculated with individual inoculant. This is in agreement with the earlier findings of Jothi (1999) and Kumutha (2001). The rhizosphere populations of *Pseudomonas*, AM fungi and nematode in the nursery soil infested with coffee root lesion nematode (*P. coffeae*) were assessed after 3 months and 6 months of inoculation and the results are presented in Table 4. Coffee seedlings inoculated with microbial consortium recorded significantly lower population of nematode after three months (18.0 number 100 $ml^{-1}$ of sterile soil and 18.6 number 100 $ml^{-1}$ of non-sterile soil) and six months (14.8 number 100 $ml^{-1}$ of sterile soil and 17.1 number 100 $ml^{-1}$ non sterile soil) followed by AM fungi inoculation and similar trend was also observed in root population of nematode after six months of inoculation (Table 4). In treatments ($T_5$ and $T_{10}$) inoculated with nematode alone recorded significantly higher nematode population of 32.4 to 34.2 number 100 $ml^{-1}$ and 34.6 to 35.2 number 100 $ml^{-1}$ of soil after three months and six months respectively compared to other treatments. With regard to rhizosphere population of *Pseudomonas* and AM fungi, the microbial consortium treatment had significantly higher population of *P. fluorescens* and AM fungal infection compared to other treatments (Table 4).

In the present investigation, the application of microbial consortium was found to be superior in reducing the root-lesion nematode population in coffee as seen from the results. It may be due to the synergistic effect of rhizobacteria with AM fungi which resulted in reduction of the nematode population. The results of the present study agree with the earlier findings by several investigators in other crops (Kloepper *et al.*, 1980; Gamilel and Katan, 1991; Shanthi and Sivakumar, 1995). In the present study, the increase in phenol content due to microbial consortium in coffee plants might have reduced the nematode population. This is in accordance with the earlier findings of Pitcher *et al.* (1960), Farkas and Kiraby, (1962) and Jothi (1999). Some phenols are known to form complex amino acids and chlorogenic acid complexes (Clarke *et al.*, 1959) which are highly toxic to the parasites.

**Table 1. Effect of microbial consortium on plant growth parameters of coffee seedlings infested with root lesion nematode after six months of planting**

| Treatments | Leaf area ($cm^2$ $plant^{-1}$) | Shoot length (cm) | Shoot girth (mm) | Root length (cm) | Root dry weight (g $plant^{-1}$) | Dry weight of plant (g $plant^{-1}$) |
|---|---|---|---|---|---|---|
| $T_1$- *Pseudomonas* PFK 9* | 254.8 | 21.4 | 3.2 | 16.4 | 0.98 | 3.9 |
| $T_2$- *P.fluorescens* Pf1* | 246.6 | 20.8 | 3.3 | 16.0 | 0.92 | 3.8 |
| $T_3$- Microbial consortium* | 286.4 | 25.3 | 3.9 | 20.1 | 1.28 | 4.8 |
| $T_4$- AM fungi* | 260.2 | 22.4 | 3.5 | 18.3 | 1.16 | 4.3 |
| $T_5$-Control* | 108.6 | 16.2 | 2.9 | 6.8 | 0.42 | 2.2 |
| $T_6$- *Pseudomonas* PFK 9 ** | 248.5 | 20.6 | 3.0 | 15.8 | 0.92 | 3.6 |
| $T_7$- *P.fluorescens* Pf1** | 242.8 | 20.1 | 3.0 | 15.0 | 0.90 | 3.5 |
| $T_8$- Microbial consortium** | 281.6 | 24.9 | 3.6 | 19.8 | 1.21 | 4.7 |
| $T_9$- AM fungi** | 256.5 | 22.0 | 3.2 | 18.0 | 1.08 | 4.2 |
| $T_{10}$-Control** | 101.2 | 15.2 | 2.4 | 6.0 | 0.38 | 1.9 |
| SEd | 11.48 | 0.99 | 0.15 | 0.77 | 0.05 | 0.18 |
| CD (P=0.05) | 23.27 | 2.01 | 0.30 | 1.57 | 0.10 | 0.37 |

* Sterilized soil ** Non-sterilized

**Table 2. Effect of microbial consortium on total chlorophyll and total phenol content of coffee seedling infested with root lesion nematode after six months of planting**

| Treatments | Total chlorophyll (mg $g^{-1}$ of fresh leaf) | Total phenol (ìg $g^{-1}$) | |
|---|---|---|---|
| | | Leaves | Roots |
| $T_1$- *Pseudomonas* PFK 9* | 1.59 | 153.21 | 167.21 |
| $T_2$- *P. fluorescens* Pf1* | 1.53 | 150.42 | 163.23 |
| $T_3$- Microbial consortium* | 1.93 | 179.32 | 190.12 |
| $T_4$- AM fungi* | 1.68 | 162.00 | 172.84 |
| $T_5$-Control* | 1.26 | 118.53 | 130.26 |
| $T_6$- *Pseudomonas* PFK 9 ** | 1.57 | 151.22 | 170.18 |
| $T_7$- *P. fluorescens* Pf1** | 1.53 | 148.41 | 161.46 |
| $T_8$- Microbial consortium** | 1.89 | 174.63 | 187.21 |
| $T_9$- AM fungi** | 1.61 | 157.07 | 170.62 |
| $T_{10}$-Control** | 1.24 | 114.60 | 129.64 |
| SEd | 0.10 | 7.12 | 7.75 |
| CD (P=0.05) | 0.21 | 14.44 | 15.71 |

* Sterilized soil ** Non-sterilized

**Table 3. Effect of microbial consortium on total nutrient uptake of coffee seedling infested with coffee root lesion nematode after six months of planting**

| Treatments | mg plant$^{-1}$ | | | $\mu$g plant$^{-1}$ | | | |
|---|---|---|---|---|---|---|---|
| | N | P | K | Zn | Cu | Fe | Mn |
| $T_1$- *Pseudomonas* PFK 9* | 38.6 | 16.4 | 15.8 | 9.8 | 2.2 | 20.6 | 41.0 |
| $T_2$- *P. fluorescens* Pf1* | 36.2 | 14.3 | 15.0 | 8.4 | 2.0 | 20.2 | 39.6 |
| $T_3$- Microbial consortium* | 52.6 | 21.6 | 21.3 | 15.6 | 3.4 | 28.6 | 56.8 |
| $T_4$- AM fungi* | 42.2 | 18.2 | 19.2 | 13.8 | 2.8 | 24.4 | 48.2 |
| $T_5$-Control* | 13.8 | 2.58 | 5.8 | 1.2 | 0.80 | 2.4 | 3.1 |
| $T_6$- *Pseudomonas* PFK 9 ** | 35.2 | 16.0 | 14.6 | 9.0 | 2.1 | 19.6 | 39.0 |
| $T_7$- *P. fluorescens* Pf1** | 34.4 | 15.2 | 14.0 | 7.8 | 1.9 | 18.2 | 38.2 |
| $T_8$- Microbial consortium** | 51.5 | 21.0 | 20.6 | 15.2 | 3.1 | 27.2 | 50.2 |
| $T_9$- AM fungi** | 38.8 | 17.8 | 18.8 | 13.6 | 2.4 | 24.0 | 41.6 |
| $T_{10}$-Control** | 11.2 | 2.18 | 5.1 | 1.0 | 0.76 | 1.8 | 2.6 |
| SEd | 1.95 | 0.79 | 0.81 | 0.53 | 0.11 | 1.03 | 1.98 |
| CD (P=0.05) | 3.94 | 1.60 | 1.64 | 1.08 | 0.23 | 2.08 | 4.01 |

* Sterilized soil ** Non-sterilized

**Table 4. Population of *P. fluorescens*, AM fungi and nematode in coffee nursery soil infested with root lesion nematode**

| Treatments | Nematode population in soil and root | | | After three months | | After six months | |
|---|---|---|---|---|---|---|---|
| | After three months( No. Per 100 ml soil ) | After six months( No. Per 100 ml soil ) | After six months (No. Per 10 g roots) | *P.fluorescens* (cfu x $10^5$) | AM fungi (%) | *P.fluorescens* (cfu x $10^5$) | AM fungi (%) |
| $T_1$- *Pseudomonas* PFK 9* | 24.2 | 20.0 | 34.0 | 23.2 | 4.3 | 13.2 | 2.8 |
| $T_2$- *P.fluorescens* Pf1* | 24.8 | 20.4 | 35.1 | 22.6 | 3.8 | 13.8 | 3.0 |
| $T_3$- Microbial consortium* | 18.0 | 14.8 | 20.2 | 25.8 | 53.2 | 16.2 | 54.2 |
| $T_4$- AM fungi* | 21.4 | 18.0 | 28.0 | 0.02 | 43.6 | 0.01 | 45.6 |
| $T_5$-Control* | 32.4 | 34.6 | 48.4 | 0.06 | 3.2 | 0.04 | 3.0 |
| $T_6$- *Pseudomonas* PFK 9 ** | 26.0 | 21.8 | 36.6 | 21.6 | 8.2 | 8.6 | 6.5 |
| $T_7$- *P.fluorescens* Pf1** | 26.2 | 22.4 | 35.2 | 21.2 | 8.6 | 8.3 | 6.1 |
| $T_8$- Microbial consortium** | 18.6 | 17.1 | 24.6 | 23.8 | 51.0 | 12.8 | 52.8 |
| $T_9$- AM fungi** | 22.0 | 19.8 | 29.2 | 0.06 | 38.0 | 0.02 | 36.0 |
| $T_{10}$-Control** | 34.2 | 35.2 | 50.2 | 0.08 | 6.0 | 0.03 | 4.0 |
| SEd | 1.12 | 0.99 | 1.43 | 0.84 | 1.56 | 0.43 | 1.25 |
| CD (P=0.05) | 2.27 | 2.01 | 2.91 | 1.69 | 3.27 | 0.87 | 2.62 |

* Sterilized soil ** Non-sterilized

The phenols in mycorrhizal roots are associated in the reduced reproduction of nematode (Singh *et al.*, 1990). Thus the present findings had clearly proved that applications of microbial consortium (5.0 g seedling$^{-1}$) along with AM fungi (5.0 g seedling$^{-1}$) on coffee seedlings found to be superior in reducing the root-lesion nematode population in coffee with significant increase in plant growth compared individual treatment or control plants.

## ACKNOWLEDGEMENTS

First author (PP) acknowledges with gratitude, the financial assistance in the form of 'study leave' rendered by Coffee Board, Bangalore, India during his study period.

## REFERENCES

Campos, V. P., Sivapalan, P. and Gnanapragasam, N.C. 1990. Nematode parasites of coffee, cocoa, and tea. In: Luc, M., Sikora, R.A. and Bridge, J. (Eds.). *Plant parasitic nematodes of subtropical and tropical agriculture*. CAB International, Wallingford, pp. 387-430.

Clarke, R. A., Kuchenze, R.T. and. Quackenbush, F.W. 1959. The nature and fungitoxicity of an aminoacid addition product of chlorogenic acid. *Phytopathology*, **49**: 594-598.

Farkas, G. L. and Kiraby, Z. 1962. Role of phenolics compared in the physiology of plant disease and diseases resistance. *Phytopathology*, **44**: 105-115.

Gamilel, A. and Katan, J. 1991. Involvement of fluorescent pseudomonads and other microorganisms in increased growth response of plants in solarized soils. *Phytopathology*, **81**: 494-502.

Gerdemann, J. W. and Nicolson, T.H. 1963. Spore of mycorrhizal endogone species extracted from soil by wet sieving and decanding. *Transaction of British Mycological Society*, **46**: 234-244.

Hiscox, J. D. and Isrealstam, G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, **57**: 1332-1334.

Humphries, E.C. 1956. Mineral composition and ash analysis. In: Peach, K. and Tracey, M.V. (Eds.), *Modern Methods of Plant Analysis*. Vol. I Springer-Verlag, Berlin, pp. 468-502.

Jackson, M.C. 1973. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi. p.103.

Jothi, G. 1999. *Interaction of vesicular – arbuscular mycorrhizae and biochemical variation with Meloidogyne incognita (Kofoid and Whilte, 1999), Chitwood, 1949 on Brinjal (Solanum melongena L.)*. Ph.D thesis, Tamil Nadu Agricultural University, Coimbatore, p. 135.

Kloepper, J. W., Leong, J. and Schroth, M. N. 1980. *Pseudomonas siderophores*; A mechanism explaining disease suppressive soils. *Current Microbiology*, **4**: 317-320.

Kumar, A. C. 1982. Evaluation of Nemacur 5G against the coffee root-lesion nematode, *Pratylenchus coffeae*. *Journal of Coffee Research*, **12**: 1-7.

Kumutha, K. 2001. *Symbiotic influence of AM fungi and rhizosphere on biochemical and nutritional changes in mulberry (Morus alba L.)*. Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore. p. 276.

Lordello, L.G.E. 1986. Plant parasitic nematodes that attack coffee. In: *Plant –parasitic nematodes of banana, citrus, coffee, grapes, and tobacco*. Union Carbide Corp. North Carolina, pp. 33-41.

Pitcher, R. S., Patrick, Z .A. and Moutain, W. B. 1960. Studies on the hot parasite relations of *Pratylenchus penetrans* (Cobb) to apple seedlings. *Nematologica*, **5**: 309-314.

Rangaswami, G. 1975. *Disease of crop plants in India*. Prentice Hall (P) Ltd., New Delhi, p. 520.

Shanthi, A. and Sivakumar, C. V. 1995. Biocontrol potential of *Pseudomonas fluorescens* (Migula) against root-knot nematode, *Meloidogyne incognita* infecting tomato. *Journal of Biological Control*, **9**: 113-115.

Singh, Y. P., Singh, R.S. and Sitaramaiah, K. 1990. Mechanism of resistance of mycorrhizal tomato against root knot nematode, pp. 96-97. In: Jalali, B.L. and Chand, H. (Eds.), *Trands in Mycorrhizal Research*. Haryana Agricultural University, Hissar.

Zieslin, N. and Ben- Zaken, R. 1993. Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. *Plant Physiology Biochemistry*, **31**: 333-339.

**(Received: 16.02.2008; Revised: 03.04.2008; Accepted: 26.04.2008)**