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# Effect of *Paecilomyces lilacinus*, *Pasteuria penetrans* and VAM on the growth of coconut seedlings infested with *Radopholus similis*

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## Abstract

The burrowing nematode, *Radopholus similis* causes serious damage to coconut. Lesions and rotting of roots, defoliation and poor yield are the symptoms due to this nematode attack. Though the application of nematicides is effective in reducing the nematode population, these chemical pesticides destroy the beneficial soil fauna. Vesicular Arbuscular Mycorrhizal (VAM) fungi can protect the coconut seedlings from nematode invasion. Investigation was carried out for three years (1994-1998) to assess the effect of biocontrol agents (BCA) namely *Pasteuria penetrans*, *Paecilomyces lilacinus* and Vesicular Arbuscular Mycorrhizal fungi on the growth of coconut seedlings infested with *R. similis*. The results showed that application of these biocontrol agents reduced the nematode multiplication significantly, while ameliorating the ill effects caused by the nematode. These biocontrol agents were more effective when they were applied together compared to their individual applications. Prior protection of the plants with BCAs either individually or in combination was better compared to simultaneous inoculation with the nematode. *P. penetrans* and *P. lilacinus* suppressed the nematode population effectively also under field conditions. Among VAM fungi, *Acaulospora bireticulata*, *Glomus mosseae* and *G. fasciculatum* multiplied well under field conditions. Since these organisms were found to multiply and survive under field conditions for more than three years, they need to be introduced to the root zone of perennial crops after every three to five years.

*Key words: Radopholus similis, Paecilomyces lilacinus, Pasteuria penetrans*, VAM, burrowing nematode, biocontrol agents

## Introduction

*Radopholus similis* is the endoparasitic nematode that has been reported as a major problem in coconut in India (Weischer 1967 ; Koshy *et al.*, 1975) and Sri Lanka ( Ekanayake, 1964 ; Gnanapragasam *et al.*, 1991; Koshy 2000). *R. similis* infestation causes root rotting, growth suppression and reduction in yield of coconut palms (Koshy and Sosamma, 1987; 1996). Effective control of burrowing nematode could be achieved by nematicide application (Koshy and Sosamma, 1979; Koshy *et al.*, 1985). Antagonistic fungus like *Paecilomyces lilacinus* is a promising egg parasite of cyst, root-knot, reniform, and citrus nematodes. *Pasteuria penetrans* has also been accepted as a potential bio-control agent against the cyst and the root-knot nematodes. Application of VAM (*A. bireticulata*) reduced *R. similis* multiplication and promoted the vegetative growth of coconut seedlings (Sosamma *et al.*, 1998; Koshy *et al.*, 1998). Apart from these, no other fungal or bacterial bio-control agents were tested on *R. similis* on coconut under field conditions. Hence, an attempt was made to study the effect of bio-control agents *viz. P. lilacinus, P. penetrans* and VAM ( *Acaulospora bireticulata*, *Glomus mosseae* and *G. fasciculatum*) on the burrowing nematode.

## Materials and Methods

One hundred seedlings of coconut cv. West Coast Tall were raised in methylbromide fumigated soil in polyethylene basins and the seedlings were treated with bio-control agents *viz Paecilomyces lilacinus*, *Pasteuria penetrans* and VAM, either individually or in combinations as per the treatments *viz.* Control ($T_1$); VAM ($T_2$); *R. similis* ($T_3$); VAM followed by *R. similis* ($T_4$); VAM +*P. lilacinus* followed by *R. similis* ($T_5$); VAM +*P. penetrans* followed by *R. similis* ($T_6$); VAM +*P. lilacinus*+ *P. penetrans* followed by *R. similis* ($T_7$);

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*P. lilacinus* followed by *R. similis* ($T_8$); *P. penetrans* followed by *R. similis*($T_9$); *P. lilacinus* +*P. penetrans* followed by *R. similis* ($T_{10}$) and *R.similis* followed by nematicides($T_{11}$).

After three months, fifty five seedlings of almost uniform growth and vigour, were selected, uprooted carefully with out causing any damage to the root system and transplanted in 35 cm diameter earthen pots filled with 10 kg steam sterilized soil, one plant per pot. The plants were grouped under 11 treatments as mentioned above with five replications each. The second application of bio-control agents and the nematode inoculation was done at transplanting in earthen pots. The nematode inoculum consisted of 100 active juveniles and females of *R. similis* ($C_{17}$) extracted from the axenic culture on carrots. *P. penetrans* inoculum consisted of 1 g of powdered brinjal root having 2.6 x $10^7$ spores 500 mg root powder (applied in two split doses). The fungal inoculum consisted of 1g of *P. lilacinus* infected wheat grain containing 3.6 x $10^8$ conidia /g (applied in two split dose one gram each at germination and transplantation). The VAM inoculum consisted of a mixture containing equal quantity of eight different VAM *viz. Acaulospora bireticulata, A. laevis, Glomus fasciculatum, G. mosseae, Scutellospora coralloidea, S. margarita, Sclerocystis coremioides* and *S. rubiformis*, 100 g of soil and root from the VAM culture pot containing 4500-5000 spores and heavily infested root bits of sorghum host plants was used as inoculum . The nematode and biocontrol agents were introduced directly on to the root system, after exposing the roots by removing the surface soil. After inoculation, the roots were covered with wet soil. These were kept in a greenhouse where the temperature varied from 24-34°C. Fifteen plants were left without inoculation for treatments $T_1$, $T_3$, and $T_{11}$. Pits of 1m x 1m x 1m (length x breadth x depth) were taken in the field for planting the seedlings. Soil samples were taken from each pit at 1m depth and initial nematode population and population density/presence of biocontrol agents in each pit was estimated prior to planting. The seedlings grown in pots were planted in October 1994 in a completely randomized design. Girth was marked around the base of the plumule with red paint, as a 1 cm wide band. For treatment $T_{11}$ the nematicides Phorate @ 3 g a.i./ seedling was appled in the soil one month after nematode inoculation at transplanting. The nematicide application was repeated again in October-November and June-July every year. Growth parameters *viz.* the height of the plant, girth, number of leaves, length and breadth of individual leaf were recorded. The number of leaves up to the spindle were counted, and a red paint mark was put on the petiole of the youngest opened leaf. For the next observation, the number of leaves produced were counted from the marked leaf up to the spindle. Growth parameters were recorded at the termination of the experiment. The experiment was terminated in July 1998. The basins of all the fifty five coconut seedlings were cleared free off weeds in one metre radius. One-fourth sector in the basin of the seedling was exposed by removing the soil without disturbing the roots. This soil was mixed well and three aliquots of 250 g soil were collected for nematode extraction and 50 g soil was used for estimating the population of Vesicular arbuscular mycorrhizal spores. Cobb's sieving and sifting method was used for nematode extraction from soil.

The roots in the 1/4$^{th}$ sector of the palm basin were completely cut out from the bole of the palm. All the roots were collected, washed thoroughly with water and were separated into primary, secondary and tertiary roots and the weights of these roots were recorded separately. The number of primary roots from the 1/4$^{th}$ sector of the bole of the palm was also recorded . Lesion index was recorded by taking into account the lesions and rotting present on ten young, fleshy, creamy white primary roots selected randomly from the whole lot. The nematodes were extracted from the roots following the method of Koshy *et al.*,1975. Root bits from each plant were plated on PDA for the isolation of *P. lilacinus*. The tertiary roots were cut into 1 cm long bits and processed for the estimation of VAM infection. Another 50 g soil was processed for the extraction of VAM resting spores (Sosamma, *et al.*, 1998). Soil was also plated on PDA to ascertain the presence of *P. lilacinus* in soil.

## Results and Discussion

Effect of biocontrol agents and *R. similis* alone and in combination on shoot and root growth characters of coconut seedling in the field for three years are presented in Table 1 and 2. Treatment differences were found to be significant for production of number of primary and secondary roots. Maximum nematode population and root lesion index was noticed in $T_3$ where burrowing nematode was inoculated and the plants were not protected with biocontrol agents (Table 3). With reference to nematode multiplication, there was significant reduction in plant growth characters viz. height, girth, leaf area, fresh root and shoot weights. Thus, the results support the fact that *R. similis* infestation on coconut reduces plant growth significantly (Koshy and Sosamma 1987) and delays flowering and reduction in

yield under field conditions (Koshy and Sosamma, 1996). Fresh root and shoot weight was maximum in $T_2$ which received mycorrhizal inoculum only, supporting that VAM enhances growth of plants. Among the treatments that received nematode inoculum the total nematode population was lowest in $T_7$ where all the bio-control agents viz. *P. lilacinus, P. penetrans* and VAM were introduced. When applied individually, *P. penetrans* was most effective in reducing nematode population followed by VAM. This supports the fact that prior colonization of VAM in roots can reduce nematode multiplication (Koshy *et al.*,1998; Sosamma *et al.*,1998). In combination, maximum reduction in population was recorded in the application of *P. lilacinus* and *P. penetrans*. VAM and *P. penetrans* when applied together proved to be the next best combination. Combination of *P. lilacinus, P. penetrans* and VAM proved to be the most effective treatment. Among the three bio-control agents, *P. penetrans* proved to be most potential against *R. similis* either alone or in combination with VAM and *P. lilacinus*.

**Table 1. Effect of biocontrol agents and *R. similis* on shoot growth characters of coconut seedlings in the field after three years (av.)**

| Treatment | Height (cm) | Girth (cm) | Total leaves produced | Existing no. of leaves | Leaf area (sq cm) |
|---|---|---|---|---|---|
| Control | 418.4 | 71.4 | 22.2 | 7.6 | 175905.7 |
| VAM | 441.2 | 62.6 | 23.4 | 7.8 | 189275.0 |
| N | 321.6 | 63.6 | 21.0 | 6.4 | 125313.1 |
| VAM→N | 427.4 | 71.2 | 22.0 | 7.2 | 156326.3 |
| Pl→N | 477.6 | 68.6 | 22.8 | 7.6 | 189826.1 |
| Pp→N | 421.2 | 64.2 | 23.6 | 7.8 | 173349.7 |
| VAM+Pl→N | 401.2 | 69.4 | 22.8 | 7.2 | 176497.6 |
| VAM+Pp→N | 381.4 | 54.8 | 21.4 | 7.6 | 141527.3 |
| Pl+Pp→N | 373.0 | 65.6 | 23.2 | 7.2 | 127324.7 |
| VAM+pl+pp→N | 424.2 | 75.4 | 24.2 | 8.2 | 214142.1 |
| N→Nematicide | 438.0 | 79.0 | 22.8 | 8.8 | 215774.9 |
| CD(P-0.05) | NS | NS | NS | NS | NS |

*VAM* - *Vesicular Arbuscular Mycorrhizae* *Pp* - *Pasteuria penetrans*
*N* - *Nematode* *Pl* - *Paecilomyces lilacinus*
→ - *followed by*

From the data presented in Table 1 it is seen that the treatment differences were not significant. However, the height was minimum in plants inoculated with nematodes alone and maximum in $T_5$ that received *P. lilacinus* followed by nematodes. This was followed by seedlings that received VAM alone of $T_2$ and $T_{11}$ nematode followed by nematicide. The per cent increase over initial height was maximum in plants that received *P. lilacinus* followed by nematode, which was closely followed by $T_7$ that received VAM and *P. lilacinus* together followed by nematode. Production of leaves and retention of leaves were minimum in $T_3$ that received nematode alone that clearly indicates the pathogenicity of the nematode on coconut. Combined application of all bioagents i.e. VAM, *P. lilacinus* and *P. penetrans* followed by nematode had produced the maximum number of leaves and had also retained higher number of leaves next to plants that received nematicide.

**Table 2. Effect of biocontrol agents and *R. similis* on root growth of coconut seedlings in the field after three years**

| Treatment | No. of primary roots in 1/4th sector | Fresh roots in 1/4th sector(kg) | | | Total fresh root wt.(kg) in 1/4th sector |
|---|---|---|---|---|---|
| | | Primary | Secondary | Tertiary | |
| Control | 205 | 1.9 | 0.1 | 0.4 | 2.4 |
| VAM | 241 | 1.9 | 0.1 | 0.5 | 2.5 |
| N | 148 | 1.0 | 0.04 | 0.3 | 1.3 |
| VAM→N | 221 | 1.6 | 0.2 | 0.4 | 2.0 |
| Pl→N | 208 | 2.5 | 0.1 | 0.6 | 3.1 |
| Pp→N | 256 | 2.1 | 0.1 | 0.5 | 2.6 |
| VAM+Pl→N | 212 | 2.1 | 0.1 | 0.4 | 2.6 |
| VAM+Pp→N | 149 | 1.6 | 0.1 | 0.3 | 2.0 |
| Pl+Pp→N | 221 | 1.9 | 0.1 | 0.8 | 2.8 |
| VAM+pl+pp→N | 220 | 1.9 | 0.1 | 0.4 | 2.4 |
| N→Nematicide | 304 | 2.2 | 0.1 | 0.7 | 3.1 |
| CD(P-0.05) | 83.52 | NS | 0.13 | NS | NS |

*VAM* - *Vesicular Arbuscular Mycorrhizae* *Pp* - *Pasteuria penetrans*
*N* - *Nematode* *Pl* - *Paecilomyces lilacinus*
→ - *followed by*

The data presented in Table 2 clearly indicates the effect of burrowing nematode population on the production of primary roots and the total root mass. Minimum number of primary and tertiary roots as well as root weight were recorded in plants that received nematode alone. Maximum root weight was recorded in plants that received nematicides and plants that received *P. lilacinus* followed by nematodes which indicate that *P. lilacinus* is also effective in controlling the nematode population. Data presented in Table 3 shows that root lesion index and nematode population was maximum in $T_3$ that received nematode alone. Root lesion index and total nematode population was at par in the case of application of nematicide or introduction of one or combination of two or three bioagents. These bioagents reduced the nematode population significantly though the resultant effect in enhancing the growth of seedlings was not significant under field conditions in three years. It is also interesting to note that VAM colonisation was maximum in plants that received VAM alone and minimum in plants that

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Table 3. *R. similis* and mycorrhizal population in the roots of coconut seedlings in the field

| Treatment | Root lesion index | VAM (% infection) | *R. similis* population In 50 g root | In 1/4th sector of root |
|---|---|---|---|---|
| Control | 3.2 | 66 | 211.0 | 10240.0 |
| VAM | 2.0 | 96 | 125.0 | 6091.8 |
| N | 3.4 | 32 | 401.6 | 11659.6 |
| VAM→N | 2.4 | 78 | 131.6 | 4328.2 |
| Pl→N | 2.4 | 52 | 37.0 | 2095.0 |
| Pp→N | 2.0 | 52 | 27.4 | 1259.6 |
| VAM+Pl→N | 2.2 | 62 | 46.0 | 3340.4 |
| VAM+Pp→N | 2.2 | 60 | 41.0 | 1604.8 |
| Pl+Pp→N | 2.2 | 72 | 17.2 | 1634.8 |
| VAM+pl+pp→N | 2.2 | 72 | 12.2 | 711.6 |
| N→Nematicide | 1.4 | 60 | 25.8 | 1378.4 |
| CD(P-0.05) | 0.63 | 15.03 | 63.89 | 3972.51 |

*VAM - Vesicular Arbuscular Mycorrhizae* *Pp - Pasteuria penetrans*
*N - Nematode* *Pl - Paecilomyces lilacinus*
→ - *followed by*

## Conclusion

The results of this study clearly show the deleterious effect of burrowing nematode on the growth of coconut seedlings and the beneficial effect of VAM, *P. lilacinus* and *P. penetrans* in reducing the nematode population equally efficiently as the nematicide. Therefore, it is recommended to raise coconut seedlings with the addition of these bioagents to the potting mixture and to add all the contents of the poly bag to the planting

**it sample(pre inoculation) and from the root zone(post-inoculation) of coconut seedlings**

| Post inoculation Pit samples VAM Spores ( 50g soil) | Pre inoculation Nematode population (250g soil) | Post inoculation Nematode population ( from root zone)(250g soil) |
|---|---|---|
| Ab-50,Gf-17,Gm-26 | Hoplo-19,Meloi-7 | Hoplo-6,Meloi-4,Rs-2 |
| Ab-486,Gf-167,Gm-200,Scu mar-183 | Tylencho-8,Rr-112 | Hoplo-4,Meloi-7 |
| Ab-70,Gm-183,Gf-167 | Hoplo-10,Rr-95 | Hoplo-17,Rs-4,Rs-26,Longi-1 |
| Ab-600,Gm-134,Gf-217 | Meloi-11,Rr-22,Ximphi-4 | Hoplo-5,Tylencho-4,Rs-4 |
| Ab-83,Gm-50 | Tylencho-10,Rr-15,Hoplo-14 | Hoplo-6,Rs-1 |
| Ab-250,Gm-100,Gf-67,Scu cor-50 | Hoplo-22,Meloi-8,Rr-9 | Hoplo-4,Rs-2,Tylencho-9 |
| Ab-530,Gm-117,Gf-50 | Hoplo-18,Rr-14 | Hoplo-4,Meloi-7 |
| Ab-300,Gm-67,Gf-100 | Hoplo-41,Meloi-15,Crico-3 | Tylecho-14,Rs-4,Longi-1 |
| Ab-513,Gm-43 | Hoplo-41,Meloi-15 | Hoplo-6,Rr-2,Rs-1 |
| Ab-300,Gm-4117,Gf-33 | Hoplo-10,Rr-5,Xiphi-7 | Meloi-8,Xiphi-1, Hoplo-11 |
| Ab-333,Gm-133 | Meloi-14,Xiphi-2,Hoplo-21 | |

- *Meloidogyne incognita* Gm - *Glomus mosseae* *Scu cor* - *Scutellospora coralloidea*
- *Glomus fasciculatum* Rr - *Rotylenchulus reniformis* Crico - *Criconemoides Sp*
- Radopholus similis Surbi - Sclerocystis rubiformis Hoplo - *Hoplolaimus seinhorsti*
r - *Scutellopora margarita* *Longi* - *Longidorus sp.*

pits at the time of transplantation. Coconut being a perennial crop, it will be only advantageous to reintroduce these bioagents after an interval of 3 to 5 years in the root zone for sustaining higher population levels of these in the root zone and thus to decimate the population of the harmful nematodes in holistic and environmentally friendly way.

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