CP 2522

INTERACTIVE EFFECT OF VESICULAR ARBUSCULAR MYCORRHIZAE (VAM) WITH *RADOPHOLUS SIMILIS* ON COCONUT SEEDLINGS.

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ABSTRACT : Significant increase in lamina width and leaf area was recorded in plants that received mycorrhizae alone. Plants inoculated with nematodes alone showed maximum reduction in growth, fresh shoot and root weights which proves the pathogenic effect of *R. similis* on coconut seedlings. Increase in shoot weight, root weight and decrease in lesion indices was noticed in plants inoculated with mycorrhizae prior to nematode. Prior inoculation of VAM was found to be more effective against *R. similis* than simultaneous inoculation of VAM and nematode. Eventhough root infection and spore production of VAM was slightly reduced in nematode inoculated plants, the lesion index and root nematode population could be successfully lowered with mycorrhiza infection. A mixture of VAM consisting of multiple endophytes *viz. Acaulospora bireticulata, Glomus fasciculatum, G. macrocarpum, G. mosseae, G. versiforme, Sclerocystis rubiformis and Scutellospora nigra* was found effective in improving the plant growth and reducing the *R.similis* population infesting coconut seedlings.

INTRODUCTION

Vesicular arbuscular mycorrhizal fungi, obligate symbionts on most plant roots generally have a stimulatory effect on plant growth, increasing general growth characters, shoot, root and fruit weight of a wide varierty of crops. In addition to this, the antagonistic effect of VA mycorrhizae to plant parasitic nematodes within the host roots is also well established (Fox & Spasoff, 1972; Hussey & Roncadori, 1978; Jain & Sethi, 1988). Though many nematodes have been reported from the coconut basins, the only economically important endoparasitic nematode reported from the coconut root in India is the burrowing nematode, *Radopholus similis* (Cobb, 1893 Thorne, 1949). It causes severe root rotting leading to general decline in the number of healthy feeder roots there by leading to reduction in plant growth, loss of plant vigour delay in flowering and reduction in yield. (Koshy *et al.*, 1975; Koshy & Sosamma, 1994). Lily (1975) was the first to report VA mycorrhizal association on coconut. Later nineteen species of vesicular arbuscular mycorrhizae have been isolated and identified from the coconut rhizosphere (Sosamma *et al.*, 1990). After a preliminary screening trial on account seedlings, a mixture of VA mycorrhozae *viz., Acaulospora bireticulata, Glomus fasciculatum, G. macrocarpum, G. mosseae, G. versiforme, Sclerocystis rubiformis and Scutellospora nigra* were selected to study their combined effect on coconut seedlings inoculated with *R. similis*. No earlier reports are available on the effect of VAM on burrowing nematode infesting coconut.

MATERIALS AND METHODS

Raising seedlings

Seednuts from healthy West Coast Tall (WCT) coconut mother palms were selected and the nuts were dehusked leaving a tuft of fibres (mesocarp) intact above the fertile eye.

these nuts were sown in steam sterilized soil in 35 cm earthern pots in the green house (Koshy & Sosamma, 1978) and were watered with boiled, cooled water. After germination, at three leaf stage, coconut seedlings of uniform growth were selected for the experiment.

Mycorrhizal Inoculum

Mycorrhizal inoculum consisted of a mixture *viz. Acaulospora bireticulata, Scutellospora nigra, Glomus fasciculatum, G. macro carpum, G. mossene, G. versiforme and Sclerocystis rubiformis*. Fifty gram soil containing 100 resting spores of each sp and 5 g each of roots of infected sorghum nurse plants formed the inoculum. Healthy high yielding coconut palms in the root (wilt) tract in Kerala formed the source for the mycorrhizal species which were maintained on sorghum nurse plants as pure cultures.

Radopholus similis inoculum

Coconut isolate of *R. similis* (C17) axenically cultured on carrot discs was used and the nematode inoculum consisted of 100 numbers of active juveniles and females.

Experimantal lay out

Thirty coconut seedlings selected were grouped under six different treaments, each replicated five times. The tratments were T1- control, T2- *R.similis*, T3-VAM, T4 - VAM + *R. similis* (simultaneously), T5 - VAM -> *R. similis*, (sequential) and T6 - *R.similis* -> VAM (sequential). Soil around the roots were removed to expose the root system and the nematode and mycorrhizal inoculations were carried out as per the respective treatment. In T4 - mycorrhizae and nematodes were inoculated simultaneously. In treatments T5 and T6 there was 35 days interval between the mycorrhizal and nematode inoculations. After inoculation, the roots were again covered with soil. All the pots were kept in the green house with even sunlight and watered daily. The temperature in the green house varied from 27 to 34°C.

Eight months after inoculation, the plants were depotted without damaging the root system and washed thoroughly to remove the adhering soil particles. Plant growth parameters such an height, girth, total member of leaves, leaf length and breadth, lesion index, fresh root and shoot weight were recorded. The soil is each pot was mixed well and three aliquots of 250 g soil were processed and the average of three was used for estimating the soil nematode population. Another three, 50 g soil samples were processed for the recovery of resting spores of VAM. The average of three samples was used for estimating the total spores.

Estimation of soil and root nemotode population

Two hundred and fifty gram of soil was processed according to Cobb's sieving and sifting method. The nematode population was assessed under a stereoscopic microscope.

A sample of 50 g tender, fresh main roots were cut into one inch long bits and then split longitudinally into 4-8 pieces. The split root bits were left in water in petri plates at a temperature of 14-20°C in a refrigerator or BOD for 72 hours for the extraction of nematodes (Koshy *et al.*, 1975). The nematodes were separated after passing the suspension through 20 mesh (840µm) to remove the root pieces and then through 60 mesh (250 µm) and finally through 400 mesh (38 µm) sieves. The sieving was washed into a 150 ml beaker for observation and counting.

Estimation of va mycorrhizal infection in soil and root

Fifty gram soil was mixed and evenly suspended in water and then passed through 20 mesh sieve to remove root bits and bigger soil particles and then through 150 mesh (105 µm) and 200 (75 µm) mesh sieves. The sievings in 150 and 200 mesh sieves were

collected in beakers and the number of resting spores of each species present were recorded by observing under a stereomicroscope using a nematode counting dish.

Five gram coconut root was cut into one centimeter bits. Thick primary roots were then cut longitudinally into four pieces and processed along with the tertiary roots. The root bits were processed by a slight modification of the methods of Philips and Hayman (1970) and Koske and Jemma (1989) as given below. The root bits were put overnight in 2.5 per cent Potassium hydroxide solution. The roots were then washed in water, bleached in alkaline Hydrogen peroxide, rinsed again in water and acidified by soaking in one per cent HCI (20-50 vol) for 1-24 hours depending upon the hardness of the roots. Then the roots were stained using Trypan blue in acidic glycerol. The stained root bits were pressed between microscopic slides and the percentage root infection was assessed.

RESULTS AND DISCUSSION

VAM colonized plants (T3) recorded the highest value of lamina width and total leaf area closely followed by plants, that received VAM inoculum prior to nematode inoculation(T5). There was significant differences in lamina width and total leaf area. Minimum lamina width and leaf area was recorded in plants inoculated with nematode alone (T2). Maximum growth in terms of total plant height, girth, total number of leaves, leaf area, fresh root and shoot weight *etc* was recorded in the plants inoculated with mycorrhizae alone (T3) (Table 1 and 2). This supports the fact that mycorrhiza improves the general growth characters of coconut seedlings. There was no significant difference in the number of main (primary) roots (Table 2). The increase in shoot weight in the mycorrhizae inoculated plants (T3-T6) was found associated with increased root infection O'Bannon and Nemec (1979) reported significantly greater growth in mycorrhizal lemon seedlings than that of non-mycorrhizal, or seedlings inoculated with *R.similis*. Nematode population in the root was highest in the seedlings inoculated with *R.similis* alone (T2). Reduction in growth, shoot and root weights was also maximum in these plants which again proves the pathogenic effect of *R. similis* on coconut plants. Nematode population in the soil and root was less in seedlings where the plants were protected with VAM prior to nematode inoculation (T5). This supports the fact that prior colonisation of VAM in plant roots can reduce nematode multiplication. This is in agreement with the reports of Umesh *et al.*, (1988) and Smith and Kaplan (1988). Umesh *et al.*, (1988) reported that prior inoculation of *G. fasciculatum* was effective in reducing the ill-effects of *R. similis* infestation on banana. Smith and Kaplan (1988) found out reduction in the burrowing nematode population (*R. citrophilus*) in the roots of rough lemon seedlings inoculated with VAM, *Glomus intraradices*. Maximum VA mycorrhizal root infection and resting spores were recorded in the mycorrhizal seedlings inoculated with VAM alone (T3). This again indicates that burrowing nematode infestation reduces the infection and multiplication of VAM. Disease symptoms like lesion index and reduction in growth was less severe in plants when mycorrhizae was inoculated prior or simultaneously with nematodes. The resting spores recovered from 50 g soil from the experimental pots were as follows - *A. bireticulata* - 46, *G. nigra* - 17, *G. fasciculatum* - 17, *G. macrocarpum* - 16, *G. mosseae* -26, *G. versiforme* - 25 and *S. rubiformis* - 3. Root colonisation of mycorrhiza was reduced in plants inoculated with nematodes but in turn *R. similis* population in roots was also significantly reduced as a result of mycorrhization which shows both burrowing nematode and VAM are mutally inhibitory to each other in coconut roots.

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Table 1. Effect of interaction of VAM and *R. similis* on shoot growth characters of coconut.

Treatments	Height (cms)	Girth (cms)	No. of leaves	Lamina length (cms)	Lamina breadth (cms)	Leaf Area (m^2)	Shoot wt(g)
Control	215.4	12.8	8.2	103.5	25.0	1.33	454.0
Nematode	206.0	12.6	8.4	95.8	22.2	1.28	298.0
VAM	251.8	14.6	9.6	108.4	26.8	1.77	494.0
VAM + N	228.2	13.4	9.0	100.2	24.0	1.43	406.0
VAM -> N	247.6	13.6	9.6	107.6	26.4	1.76	444.0
N -> VAM	207.2	13.8	9.0	100.6	24.0	1.42	322.0
Gen. mean	226.0	13.5	9.0	102.7	24.7	1.48	403.0
CV(%)	14.5	10.3	12.1	8.1	7.8	16.12	11.4
F. ratio	1.85	1.35	1.45	1.67	3.85*	4.91**	14.35**
CD at P=0.05	NS	NS	NS	Ns	2.52	0.31	59.73

** significant at P= 0.01 * significant at P = 0.05 NS = Not significant.

Table 2. Effect of interaction of VAM and *R. similis* on root growth characters, nematode and VAM population.

Treatments	No. of roots	Maximum root length (cm)	Root weight (g)	Lesion index	R. similis population soil(250g)	R. similis population Root(50g)	VAM Resting spores (50g) soil	VAM Percentage root infection
Control	6.6	102.0	102.0	0	0	0	0	0
Nematode	7.4	77.0	83.8	4.0	15.0	2289.0	0	0
VAM	7.2	97.0	133.2	0	0	0	376.0	80.0
VAM + N	7.8	66.0	96.0	2.4	7.6	1108.0	137.0	33.0
VAM -> N	7.4	79.0	107.2	1.8	6.2	600.0	130.0	48.0
N -> VAM	6.2	90.0	79.2	3.2	43.8	1007.0	150.0	28.0
Gen. mean	7.1	85.2	100.2	1.9	12.1	828.8	132.3	31.5
CV %	15.7	16.0	8.4	41.3	277.1	89.5	24.9	22.8
F ratio	1.39	4.96*	26.48**	21.02**	1.21	6.51**	87.38**	89.49**
CD at P = 0.05	NS	17.78	10.94	1.02	NS	966.11	42.94	9.37

**significant at P = 0.01 * significant at P = 0.05 NS - Not significant

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