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Management of seed and collar rots caused by *Aspergillus niger* Van Tieghem in groundnut (*Arachis hypogaea* L.) by biocontrol method

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Abstract: Groundnut is affected by pre and post emergence rots caused by *Aspergillus niger* Van Teigham. Management of seed and collar rots caused by A. *niger* with *Trichoderma* species, *Pseudomonas fluorescens* and *Bacillus subtilis* and organic amendments like neem cake, groundnut cake, gingelly cake, mahua cake and sawdust + NPK was evaluated. The incidence of the disease was significantly reduced in seed treatment with *T. viride* along with soil application with *T. viride* and neem cake. The root and shoot dry weight and pod yield (Kg/ha) was also maximum in this treatment followed by seed treatment with *T. viride* and carbendazim.

Key words: Biological control, Aspergillus niger, organic amendments

Introduction

Groundnut *(Arachis hypogaea)* is an important commercial crop in India. It serves as a good oil yielder with rich protein content. Many fungal diseases affect the crop during its growth. Seed borne and soil borne pathogen *Aspergillus niger* causing seed rot and collar rot diseases reduces the yield attributes. Biological control with bio-agents and organic amendments finds a promising approach to manage the disease. The present attempt was made to further analyse the effect of bio-agents in combination with organic amendments on disease incidence and yield of the crop.

Materials and Methods

Lab studies

The experiment was carried in Department of Plant Pathology, S.V. Agricultural College, Tirupati during the year 1998-2000.

The *in vitro* screening of the species of *Trichoderma, Pseudomonas fluorescens and Bacillus subtilis* against *Aspergillus niger* by dual culture techniques (Dennis and Webster, 1971). The pathogen, *Aspergillus niger* was subcultured on PDA medium. The effective bio-agent, *Trichoderma viride* was mass multiplied in molasses yeast medium (consisting of molasses 30 g, yeast extract 5 g in 1000 ml of water and sterilized at 1.1 kg/m^2 for 20 minutes). The flask was incubated at room temperature for 10 days. After 10 days, the suspension was pooled and to one litre of *Trichoderma* suspension was mixed with 2 kg of talc powder. To one kg of this mixture, 10 g of carboxy methyl cellulose (CMC) was added and shade dried for 3-4 days and packed in poly bags. These talc-based formulations were further used for the seed treatment and soil application.

The organic amendments like neem cake, groundnut cake, gingelly cake, mahua cake and sawdust + NPK were taken, powdered and sieved through 1 mm^2 mesh. They were filled in polypropylene bags at the rate of 100 g/bag and the moisture adjusted to 50 per cent. They were autoclaved at 1.4 kg/cm^2 for one hour on two successive days (Elad *et al.* 1980). They were inoculated with 9-mm mycelial disc of *T. viride* and incubated at room temperature to evaluate growth and multiplication of the antagonist.

One gram of the substrate was drawn aseptically, after thorough mixing, after seven and fifteen days of incubation, diluted with water serially and spores were counted using haemocytometer. The number of colony forming units (cfu) were also recorded by dilution plate technique on PDA medium.

Field studies

The field experiment was carried out in wet land of S.V.Agricultural College, Tirupati.

ble 1. Effect of fungal and bacterial antagonists on growth of *Aspergillus niger* in dual culture method

ıatments	Growth of *A.niger* (mm)	Percentage inhibition over control
choderma viride	37.92	52.06
choderma harzianum	40.42	49.48
choderma hamatum	47.75	40.31
choderma koningii	44.58	44.28
choderma reesei	67.58	15.52
udomonas fluorescens	72.75	6.98
cillus subtilis	78.25	2.19
ntrol	80.00	0.0
M ±		1.06
at 5%		2.25

Mean of three replication

ble 2. Effect of certain organic amendments on sporulation of *Trichoderma viride*

ganic amendment	Spores (10^5/ml)		cfu (10^5/g)	
	7 DAI	15 DAI	7 DAI	15 DAI
eem cake (100 g/bag)	1.89	282.62	26.00	324.00
roundnut cake (100 g/bag)	1.40	229.37	19.00	275.67
ahua cake (100 g/bag)	0.89	14.70	9.00	199.67
awdust + NPK (100 g/bag)	0.43	3.05	6.00	71.67
ingelly cake (100 g/bag)	1.14	209.87	12.67	222.33
EM +	0.24	3.03	0.84	6.18
D at 5%	0.05	6.75	1.879	13.763

Mean of three replication
DAI = Days After Inoculation

licro plots of size 1 x 1 m^2 were laid. The llowing treatments were followed:

Seed treatment with *T. viride*
Seed treatment with Carbendazim
Seed treatment with *T. viride* and Carbendazim
Soil application of neem cake
Seed treatment with *T. viride* and soil application of neem cake
Seed treatment with Carbendazim and soil application of neem cake
Seed treatment and soil application of *T. viride*
Seed treatment with Carbendazim and soil application of *T. viride* and neem cake
Seed treatment with *T. viride* and soil application of *T. viride* and neem cake
T_{10} Control

Neem cake and *T. viride* were applied to the plots 15 days before sowing. Seed treatment with *T. viride* was done 24h before sowing at the rate of 4g/kg seed. Fungicide treatment was done at the rate of 2g/kg seed before sowing. The disease incidence percent was determined 15 days after sowing. The root and shoot dry weight were recorded 45 days after sowing. The pod yield was also recorded. Three replications were imposed with Randomised Block Design.

Results and Discussion

Lab studies

In order to select suitable isolates of antagonist against *Aspergillus niger*, five species

Table 3. Effect of seed and soil treatment of *Trichoderma viride* and neem cake on growth parameters of groundnut in *Aspergillus niger* infested soil

Treatments	Shoot length (cm)	Root length (cm)	No. of branches per plant	Nodule (no. per plant)
Seed treatment with *T.viride* (4 g/kg seed)	12.06	8.90	4.67	25.0(
Seed treatment with carbendazim (2 g/kg seed)	13.17	9.20	4.00	15.3
Seed treatment with *T. viride* (4 g/kg seed) + carbendazim (2 g/kg seed)	16.63	8.67	4.00	23.0(
Soil application of neem cake (100 g/m²)	15.43	9.30	3.30	24.33
Seed treatment with *T.viride* (4 g/kg seed) + soil application of neemcake (100 g/m²)	15.37	8.13	4.67	12.33
Seed treatment with carbendazim (2 g/kg seed) + soil application of neem cake (100 g/m²)	15.93	7.70	4.00	15.66
Seed treatment (4 g/kg seed) + soil application of *T.viride* (100 g/m²)	15.83	7.87	4.67	12.33
Seed treatment with carbendazim (2 g/kg seed) + soil application of *T.viride* (100 g/m²) + neem cake (100 g/m²)	13.13	8.30	4.33	28.00
Seed treatment with T.viride (4 g/kg seed) + soil application of T.viride (100 g/m²) + neem cake (100 g/m²)	21.23	9.90	4.00	44.00
Control (pathogen alone)	12.57	7.40	4.00	15.66
SEM ±	1.51	0.69	0.44	11.77
CD @ 5%	3.17	1.45	NS	24.73

* Mean of three replication

of *Trichoderma viz., T. viride T. harzianum, T hamatum, T koningii, T reesii, Pseudomonas fluorescens and Bacillus subtilis* were tested by dual culture method. The per cent inhibition of growth of the pathogen was 52.06 in presence of *T. viride* followed by *T. harzianum* (49.48%) (Table 1).

Similar results were reported by Sukanta Dasgupta *et al.* (1998) where reduction in *A. niger* growth was seen in the presence of *T. harzianum*. The inhibition of growth of *A. niger* would be attributed mainly due to antibiosis or hyperparasitism. *Trichoderma spp* produced chitinase and β 1,3 glucanase enzymes which

le 4. Effect of seed and soil treatment of *Trichoderma viride* and neem cake on yield parameters of groundnut in *Aspergillus niger* infested soil

ıtments	No. of plants	100-seed weight (g)	Shelling (%)	Harvest index	Pod yield (kg/ha)	Dryshoot weight (g)	Dry root weight (g)
d treatment with *T.viride* g/kg seed)	23.00	38.10	66.83	34.93	1911	6.99	0.78
d treatment with carbendazim g/kg seed)	20.67	38.18	65.70	33.87	1829	6.98	0.75
d treatment with *T. viride* g/kg seed) + carbendazim g/kg seed)	25.00	38.33	68.27	35.80	2043	7.23	0.83
l application of neem cake 0 g/m²)	18.00	38.31	63.40	32.67	1794	6.27	0.61
d treatment with *T.viride* g/kg seed) + soil application neemcake (100 g/m²)	21.00	38.51	64.17	33.67	1785	6.78	0.69
d treatment with carbendazim g/kg seed) + soil application neem cake (100 g/m²)	20.33	38.10	63.27	33.27	1803	6.52	0.68
ed treatment (4 g/kg seed) + il application of *T.viride* 00 g/m²)	19.67	38.04	62.87	33.50	1815	6.43	0.65
ed treatment with carbendazim g/kg seed) + soil application of *viride* (100 g/m²)	23.67	38.33	67.97	34.70	1965	7.07	0.79
ed treatment with T.viride g/kg seed) + soil application T.viride (100 g/m²) + neem ke (100 g/m²)	27.00	39.03	69.63	37.00	2163	7.55	0.88
ontrol (pathogen alone)	16.00	37.63	62.30	31.20	1773	4.55	0.58
EM ±	0.93	0.63	0.31	0.67	0.30	0.09	0.03
D @ 5%	1.96	NS	0.64	1.40	0.64	0.19	0.05

Mean of three replication

egrade the cell wall leading to lysis *of hizoctonia solani* as reported by Wu *et al.* (1986).

In the present study, neem cake proved o be an effective and best substrate for nultiplication of *T. viride* followed by groundnut ake. *T. viride* sporulated well in neem cake used as substrate, which recorded 324 x 105 cfu/g 15 days after inoculation, followed by groundnut cake (275.67 x 105 cfu/g) (Table 2). Padmakumari and Balakrishnan (1987), Krishnamoorthy and Bhaskaran (1987) reported that addition of neem cake to soil enhances the population of *Trichoderma* spp.

Field studies

The biological control of the fungus, *A. niger* has been reported by Bansal and Sobti (1990), Karthikeyan (1996) and Sukanta Dasgupta *et al.* (1998). Papavizas and Davey (1960), Gautam and Kolte (1979), Chakrabarti and Sen (1991) reported the efficacy of organic amendment as a source of substrate for growth and multiplication of the bio-agent.

The per cent disease incidence of pre-emergence seedling rot was reduced to 88.05% in seed treatment with *T. viride* along with soil application of *T. viride* and neem cake followed by seed treatment with *T. viride* and carbendazim (70.66%). Similar results was reported by Karthikeyan (1996) where neem cake + *T. viride* + carbendazim seed treatment gave good disease control against collar rot.

In the present investigation, seed treatment with *T. viride* along with soil application of *T. viride* and neem cake recorded shoot length (21.3 cm), root length (9.9 cm) (Table 3) with pod yield of 2163 kg/ha, dry shoot weight 7.55g, dry root weight 0.88g/plant significantly higher than control (Table 4). Backman and Kabana (1975) reported sclerotial blight of peanut was decreased and pod yield increased due to application of *Trichoderma* spp.

Two mechanisms have been stated to explain the increased growth response induced by certain soil microflora. The first hypothesis was that enhanced growth of plants induced by antagonists might be due to biological control of plant pathogens in the soil. The other is not clearly demonstrated that a microbial agent produces regulatory metabolites, thus increases germination rate, dry shoot and root weights (Windham *et al.* 1986).

Alagarsamy *et al.* (1987) stated that seed pelleting *T. viride* recorded more shoot length, root length and dry matter production. Similar reports in increase in vegetative growth by *Trichoderma* species were made by Manomohandas and Sivaprakasam (1994) during their studies on damping off disease in chilli nursery.

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