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Biointensive management of collar rot of groundnut caused by *Aspergillus niger*

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ABSTRACT: The collor rot incidence in groundnut was reduced by the application of *Trichoderma viride* as seed treatment along with fungicides. *In vitro* studies of fungal and bacterial antagonists, *viz., Trichoderma* spp. and *Pseudomonas fluorescens* indicated that *T. viride* was more effective in inhibiting the pathogen *A. niger*. In pot culture experiment, the combined effect of seed treatment with *T. viride* and captan resulted in significant reduction of collor rot. Combination of antagonist and fungicide also improved the growth parameters like length of the plant, biomass and yield besides decreasing the disease incidence. Biointensive disease management of collar rot is not only effective but also economical.

KEY WORDS: *Aspergillus niger*, biocontrol agents, collar rot, fungicides, groundnut.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the important oilseed crops grown in India which is affected by several diseases. Among the diseases that occur during seed and seedling stages, pre-emergence seed rot and post-emergence collar rot caused by *A. niger* is economically very important. Collar rot caused by *A. niger* is a widespread disease, which causes rotting of seed, pre-emergence soft rot of hypocotyls and post-emergence collar rot of seedlings (Mehan *et al.,* 1995). Though fungicides offer certain degree of protection against soil borne pathogens, they are not so effective because of the multiplication and continual persistence of pathogens in the soil. The application of fungicides may give effective protection for up to 20-25 days, but it adversely affects beneficial rhizosphere organisms besides causing soil and air pollution. Integrated disease management through cultural operations, disease resistant cultivars and biological control agents is gaining importance in recent times (Ghewande, 1990). The species of *Trichoderma* (Harman *et al.,* 1981), *Bacillus* spp. (Capper and Campbell, 1980) and *Pseudomonas* (Vidyasekharan and Muthamilan, 1995) have been used as biocontrol agents. In the present study, the influence of *Trichoderma* spp. in combination with chemical fungicides for sustainable management of collar rot of groundnut is reported.

MATERIALS AND METHODS

Isolation of the pathogen

Collar rot affected seedlings of groundnut were collected from the field and were used for the isolation of pathogen. The pathogen was isolated by tissue segment method (Rangaswami, 1958) on potato dextrose agar medium. The culture was purified by hyphal tip culture method on plain agar medium.

Isolation of biocontrol agents

Trichoderma spp. from rhizosphere soil samples was isolated using *Trichoderma* specific medium. *Trichoderma* isolates were identified to species using the keys proposed by Rifai (1969).

Fluorescent Pseudomonas was isolated from the rhizoplane of healthy groundnut roots on King's medium B (King *et al.,* 1954). Characterization of beneficial cultures was done using microbiological and biochemical tests as listed in Bergey's Manual (Unnamalai and Gnanamanickam, 1984).

In vitro screening and selection of antagonists

The antagonistic potential of selected biocontrol agents was studied by dual culture method (Morton and Straube, 1955). Culture discs (0.5cm) of *A. niger* and antagonists were placed 3.5cm apart from the center of potato dextrose agar (PDA) plate and incubated at 25 ± 1ºC. After 5-7 days of incubation, radial growth of the pathogen was measured at three places in each Petri plate and the average was obtained. Three replications along with control (without bio-agent) were maintained and per cent inhibition over control was calculated. Based on *in vitro* screening, the potential isolate was selected and maintained on PDA slants for further study.

Production and formulation of *Trichoderma viride*

Trichoderma viride was grown in Molasses-yeast medium (molasses 30g, yeast extract 5g, distilled water 1000ml) for 10 days at 27 ± 1°C. Subsequently, broth cultures were homogenized using a mixer grinder. The homogenized liquid cultures were formulated using talc as a carrier material (Talc: liquid broth culture of *Trichoderma* spp. @ 2: 1 w/v) with 10g of carboxyl methyl cellulose (CMC) per kilogram of carrier material as adhesive. The moisture content was 8% and population of *T. viride* was 2 x 10^7 cfu g^{-1} immediately after preparation. *A. niger* was grown in sand-sorghum medium (sand: sorghum: water @ 8: 1: 1 w / w / v). The medium was inoculated with mycelial discs of *A. niger* taken from the margin of actively growing culture and incubated at 25 ± 1°C for 8 days. The bags were carefully shaken periodically in order to permit uniform growth.

Greenhouse studies

Aspergillus niger grown on sand-sorghum medium was applied @ 10g / pot and thoroughly mixed with the soil. The seeds of groundnut cultivar TMV2, susceptible to collar rot, were first surface sterilized and then treated with talc-based formulation of *T. viride* @ 4g kg^{-1} seed. For combination treatments, seeds were first coated with captan @ 2g kg^{-1} followed by *T. viride*. Ten seeds were sown in each pot and watered whenever required. Seeds treated with captan served as fungicidal control. The design adopted for the experiment was a Completely Randomized Design (CRD). Observations on germination, root and shoot lengths, dry weight of the plant and the disease incidence were recorded 30 days after sowing.

Field studies

Field trials were conducted using susceptible cultivar TMV2 in 3 x 5 m^2 plots in a randomized block design. The talc-based products of antagonist *T. viride* @ 4 g kg^{-1} of seed used for seed treatment. Pre-emergence rotting at 10 days (DAS) after sowing and disease incidence was recorded at 25 and 45 DAS. Plant biomass was taken at 45 days and pod yield was recorded at harvest.

RESULTS AND DISCUSSION

The antagonistic nature of *Trichoderma* spp. against *A. niger* was studied. The per cent inhibition of the pathogen was 68.0% by *T. viride* followed by *T. harzianum* (54.0%), *T. koningii* (42.0%), *T. hamatum* (42.0%), *T. virens* and *P. fluorescens* (Table 1). The maximum inhibition of growth of *A. niger* was observed in presence of *T. viride* followed by *T. harzianum*. Similar results were reported by earlier workers (Hussain and Lane, 1992; Sukanta *et al.,* 1998). The inhibition of growth of *A. niger* could be attributed mainly due to antibiosis or hyperparasitism. Most fungi have chitin and ß- 1,3 glucanase an essential constituent in their cell wall. *Trichoderma* spp. produce chitinase and ß- 1,3 glucanase, which degrades the cell wall leading to lysis of pathogens as reported by Wu *et al.* (1986).

Table 1. Per cent inhibition of growth of *A. niger* by dual culture technique

Treatment	Inhibition of growth (%)
T. hamatum	42.0
T. viridae	68.0
T. harzianum	54.0
T. virens	38.0
T. koningi	42.0
P. fluorescenss	34.0
CD (P = 0.05)	3.45
CV%	4.10

Pot culture studies

Seed treatment with biocontrol agent and fungicide showed significantly higher percentage of germination, compared to control, followed by seed treatment with *T. viride* alone and captan. Seeds treated with *T. viride* (4g kg^{-1}) + captan (2g kg^{-1}) showed maximum shoot length, root length and dry matter production of 36.3cm, 22.6 and 1.9g, respectively, as compared to 19.0cm, 16.3cm and 1.5cm in control.

Table 2. Efficacy of the biocontrol agent against *A. niger* in greenhouse

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Root Dry Wt. (g)	Shoot Dry Wt. (g)	Disease incidence (%)
T. viride	88.5 (74.1)	35.3	26.3	0.9	1.8	72.3 (58.8)
Captan	80.7 (63.7)	31.7	21.2	0.8	1.5	64.7 (52.8)
T. viride + Captan	100.0 (90.0)	36.3	22.7	0.9	1.9	50.3 (45.1)
Control	75.3 (60.2)	19.0	16.33	0.6	0.9	97.7 (85.7)
CD (P = 0.05)	4.5	6.2	5.0	-	-	4.7

Figures in parentheses indicate transformed values.

Table 3. Effect of *T. viride* and captan on collar rot incidence and yield under field conditions

Treatment	Germination (%)	Disease incidence (%)	Pod yield (g)
T. viride	84.5 (66.6)	76.6 (61.2)	1940
Captan	88.7 (74.3)	74.3 (59.5)	1820
T. viride + Captan	98.6 (84.3)	66.6 (55.8)	2120
Control	70.0 (57.7)	98.3 (84.8)	1683
CD (P = 0.05)	4.6	5.8	130.0

Figures in parentheses indicate transformed values

The effect of *T. viride* was similar to that of captan in increasing the shoot length, root length and dry matter production. Seed treatment with antagonists both individually and in combination significantly increased the shoot length, root length and dry matter production of groundnut seedling. Seed treatment with both the antagonist and captan significantly reduced the collar rot compared with control (Table 2).

Field studies

Seed treatment with *T. viride* increased germination percentage, dry matter production and pod yield. Collar rot incidence was significantly reduced (66.7%) with seed treatments of *T. viride* and captan, followed by seed treatment with *T. viride* (72.3%) and captan (74.3%) when compared to control where the incidence was 98.3% (Table-3).

Similar report by Alagarswamy *et al.* (1987) stated that seed pelleting with *T. viride* recorded more shoot length, root length and dry matter production. Samiyappan *et al.* (1987) reported that *Trichoderma* sp. significantly increased the shoot and root length, dry weight and nodule number in green gram. The enhanced growth of plants induced by antagonists might be due to biological control of plant pathogens in soil or due to the growth regulatory metabolites produced by biocontrol agents.

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