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Biological Control of Crown Rot of Groundnut by *Trichoderma harzianum* and *T. viride*

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Crown rot of groundnut (*Arachis hypogaea*) caused by *Aspergillus niger* is prevalent in warm and dry climatic zones and its incidence ranges from 2% to 14% (Pande and Narayana Rao 2000). The pathogen attacks groundnut plants at all the growth stages and causes pre-emergence rotting in seeds, soft rot in emerging seedlings, and crown rot in mature plants. Thus, management of crown rot by fungicides is difficult and expensive. Biological control of plant diseases is cost effective and environmentally safe compared to fungicides. Also, the biocontrol agent once established persists in the soil for longer periods and offers disease protection even in the consecutive crop seasons (Mew and Rosales 1986). *Trichoderma* spp are antagonistic to a wide range of phytopathogenic fungi and are able to control economically important diseases in several crop plants (Papavizas 1985). *Trichoderma harzianum* and *Bacillus subtilis* AF 1 were tested to control the incidence of crown rot in groundnut and varying levels of disease control were obtained with these biocontrol agents (Lashin et al. 1989, Podile 2000). *Bacillus subtilis* AF 1 induced production of lipoxygenase and altered the phytoalexin metabolism in groundnut seedlings (Podile 2000). We report the results of the in vitro antagonistic potential of 16 *Trichoderma* isolates against *A. niger* and the efficacy of the selected isolates to control *A. niger* infection under greenhouse conditions in comparison with a fungicide.

Sixteen *Trichoderma* isolates were obtained from the rhizosphere soil of groundnut plants collected from experimental fields at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India and these were identified into four species aggregates, *hamatum, harzianum, longibrachiatum,* and *viride. Aspergillus niger* was isolated from the groundnut plants that wilted due to crown rot infection in the experimental field at ICRISAT. The antagonistic activity of *Trichoderma* isolates against *A. niger* was determined by a dual-culture technique on potato dextrose agar (PDA) and the antagonistic potential of the strains was rated on a 1-5 scale (Bell et al. 1982). Of the 16 *Trichoderma* isolates tested, two *T. harzianum* isolates A 3 and A 11, and one *T. viride* isolate A 14 were highly antagonistic to *A. niger* and were rated 1. Among the remaining 13 isolates, 9 were rated 2, and 4 were rated 3. The production of diffusible antibiotics by the three potent antagonistic isolates was confirmed following the standard procedure of Dennis and Webster (1971).

Tolerance of biocontrol agents to commonly used fungicides is desirable for integration with the modern production practices. In addition, fungicide tolerance enhances the competitiveness of biocontrol agents in soils amended with fungicides. In this study, we tested the tolerance of *T. harzianum* isolates A 3 and A 11, and *T. viride* A 14 to thiram, the common groundnut seed dressing fungicide. This was done by amending the PDA with thiram at concentrations of 100, 200, 300, and 500 mg ml^{-1}. All the three *Trichoderma* isolates were sensitive to thiram at all the concentrations and hence cannot be used in combination with thiram.

Trichoderma harzianum A 3 and A 11, and *T. viride* A 14 were further evaluated for control of pre-emergence and post-emergence rotting under greenhouse conditions. Fifteen-day-old culture of *A. niger* grown on sorghum *(Sorghum bicolor)* grains was used as pathogen inoculum. Sorghum grain-culture was added to a mixture of *red* soil, farmyard manure, and sand (2:1:2) at 25 g kg^{-1} and mixed well. The *A. niger* infested soil was filled to top one-third portion of 20-cm diameter pots. The pots wvre watered, left for 48 h in the greenhouse and then were used for planting. The temperature in the greenhouse was maintained at 30 ± 2^0C throughout the experimentation. Seeds of the groundnut genotype TMV 2 were coated with *Trichoderma* (10^8 conidia ml^{-1}) using 0.5% carboxy methyl cellulose (CMC). Groundnut seeds treated with thiram at 2 g kg^{-1} were used as one of the treatments. Seeds treated with 0.5% CMC served as control. For soil amendment of *Trichoderma,* 15-day-old culture grown on sorghum grain was mixed in the top layer of soil at

5 g kg^{-1} before planting. Ten seeds were planted in each pot and five pots were considered as one replication. Three replications were maintained for each treatment and the experiment was repeated twice. The pots were observed for pre-emergence rotting at 7 days after sowing, and for post-emergence rotting at 25 days after sowing. The incidence of pre- and post-emergence rotting was found insignificant between the three experiments and hence the data for the three experiments was pooled and analyzed.

The *Trichoderma* isolates were effective in reducing the pre-emergence rotting both when applied as seed treatment and soil amendment compared with control. Soil amendment of *Trichoderma* was significantly more effective than seed treatment in controling post-emergence rotting (Table 1). This could be due to the poor survival of *Trichoderma* in the soil or poor rhizosphere competence when applied as seed treatment (Papavizas 1985). When compared with *Trichoderma* isolates, seed dressing with thiram offered maximum protection to groundnut seedlings both from pre- and post-emergence rotting. Among the *Trichoderma* isolates tested, *T. viride* A 14 was effective in controlling *A. niger* infection and the disease protection obtained was comparable with that of thiram. The effectiveness of *T. viride* A 14 to control crown rot under field conditions is currently being investigated.

Table 1. Effect of antagonistic *Trichoderma* isolates on the incidence of pre-emergence and post-emergence *Aspergillus niger* infection in groundnut seedlings.

	Crown rot infection[1] (%)	
Treatment	Pre-emergence	Post-emergence
T. harzianum A 3 (seed treatment)	14.2	22.2
T. harzianum A 3 (soil amendment)	13.8	18.4
T. harzianum A 11 (seed treatment)	15.1	25.3
T. harzianum A 11 (soil amendment)	15.8	19.8
T. viride A 14 (seed treatment)	10.7	20.9
T, viride A 14 (soil amendment)	11.3	15.8
Thiram 2 g kg^{-1} (seed treatment)	9.1	13.3
Control	45.6	34.4
LSD (*P* = 0.01)	3.1	3.6

1. Data are means of nine replications in three sets of experiments.

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Identification of *Trichoderma* Species and their Antagonistic Potential Against *Aspergillus flavus* in Groundnut

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Contamination of groundnut *{Arachis hypogaea)* seed by aflatoxins produced by *Aspergillus flavus* is a major problem affecting quality and trade of groundnut and its products. Among several management options, biological control can play a significant role in reducing pre-harvest