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

aflatoxin contamination in groundnut- Control of plant diseases by biological agents is environmentally safe and compatible with sustainable agriculture. *Trichoderma spp* are well-known biocontrol agents against several plant pathogens (Elad et al. 1982).

Rhizosphere soil samples were collected from major groundnut-growing areas of four districts (Anantapur, Chittoor, Cuddapah, and Kurnool) in Andhra Pradesh, and two districts (Kolar and Tumkur) in Karnataka, India under the National Agricultural Technology Project (NATP) on "Aflatoxin contamination in groundnut: mapping and management in Gujarat, Andhra Pradesh, and adjoining areas". *Trichoderma* spp were isolated from the soil samples. The isolates were characterized for morphological traits for speciation, and evaluated for their antagonism against *Aspergillus flavus* to identify highly antagonistic isolates that could be used as potential biocontrol agents for pre-harvest aflatoxin contamination of groundnut.

Isolation of *Trichoderma* isolates

Five hundred ml of each soil sample at 10^{-3} and 10^{-4} aqueous dilutions was spread on petri dishes containing *Trichoderma* specific medium (TSM: glucose 3 g, ammonium nitrate 1 g, sodium dihydrogen phosphate 0.9 g, magnesium sulfate 0.2 g, potassium chloride 0.15 g, ferrous sulfate 20 mg, zinc sulfate 20 mg, manganese sulfate 20 mg, rose bengal 30 mg, agar 10 g, and distilled water 1000 ml). After autoclaving at 121°C for 20 min the medium was cooled to 50°C. Then 50 mg streptomycin sulfate, 50 mg chloramphenicol, 10 mg metalaxyl, and 10 mg PCNB (penta-chloro nitro-benzene) were added. Tw< plates were maintained for each dilution. The plate were incubated *for* 4 days in dark at 28°C *and typical Trichoderma* colonies were isolated. The colonies wer white or whitish-green to green, conidiophores long am thick, with or without sterile branches, side branche mostly thick bearing short and plump phialides phialospores globose or ellipsoidal rough or smootl walled.

Of 386 soil samples analyzed, 156 (40.4%) yielded *Trichoderma,* with the maximum (64.1%) *Trichodermi* isolates obtained from soil samples of Anantapur am the minimum (11.1%) from the samples of Kolar (Table 1) A total of 212 isolates of *Trichoderma* spp were obtained (Table 1).

Evaluation for in vitro antagonism

The dual culture method (Denis and Webster 1971c was used to study the antagonism against a highly aggressive and toxigenic strain of A. *flavus* (Af 11-4). Of the 212 isolates tested, 145 were antagonistic to Af 11-4. Among these, only 39 isolates showed clear inhibition zone against Af 11-4 (Fig. 1). These isolate were examined for species identification and furthe evaluated for antagonism, involving production of vola-tile and non-volatile antibiotics, and hyphal interaction with Af 11-4.

Species identification. Thirty-nine antagonistic *Trichoderma* isolates were identified according to the identification key (Rifai 1969) based on branching of

Table 1. Isolation of *Trichoderma* isolates from soil samples collected from major groundnut-growing districts of Andhra Pradesh and Karnataka, India, rainy season 2000.

District	No. of soil samples analyzed	No. of soil samples with *Trichoderma* isolates	No. of *Trichoderma* isolates obtained	Soil samples with *Trichoderma* (%)
Andhra Pradesh				
Anantapur	53	34	40	64.1
Chittoor	97	48	54	49.5
Cuddapah	23	11	19	47.8
Kurnool	26	15	20	57.6
Karnataka				
Kolar	72	8	35	11.1
Tumkur	115	40	44	34.8
Total	386	156	212	40.4

conidiophores, shape of the phialides, emergence of phialospores, and shape of phialospores. These isolates were identified into six species, *T. harzianum* (11), *T. hamatunt* (1), *T. viride* (9), *T. longibrachiatum* (5), *T. koningii* (9), *T. pseudokoningii* (3), and unknown species (1) (Table 2).

Production of volatile antibiotics. This study was done following the method of Dennis and Webster (1971b). The plates were incubated at 28°C for 72 h. The assembly was opened to measure colony diameter of Af 11-4 in each plate. Twenty-one of the 39 *Trichoderma* isolates showed inhibition of Af 11-4 colony by producing volatile antibiotics compared with the control. In the control plate, the colony diameter of Af 11-4 was 60 mm whereas in other plates it was 10-45 mm. Isolate T 102

Table 2. Species identification of *Trichoderma* isolates antagonistic to *Aspergillus flavus* (Af 11-4).

Trichoderma species	*Trichoderma* isolate number
T. harzianum	T 2, T 10, T 11, T20, T 42, T 53. T 58, T 72, T 109, T 129, T 170
T. hamatum	T 47
T. viride	T 16, T 24, T 50, T 51, T 60, T 62, T 179, T 188, T 205
T. longibrachiatum	T 6, T 34, T 56, T 102, T 110
T. koningii	T 12, T 13, T21, T 33, T 49, T 70, T 83, T 143, T 161
T. pseudokoningii	T 29, T 37, T 206
Trichoderma sp (unknown)	T 142

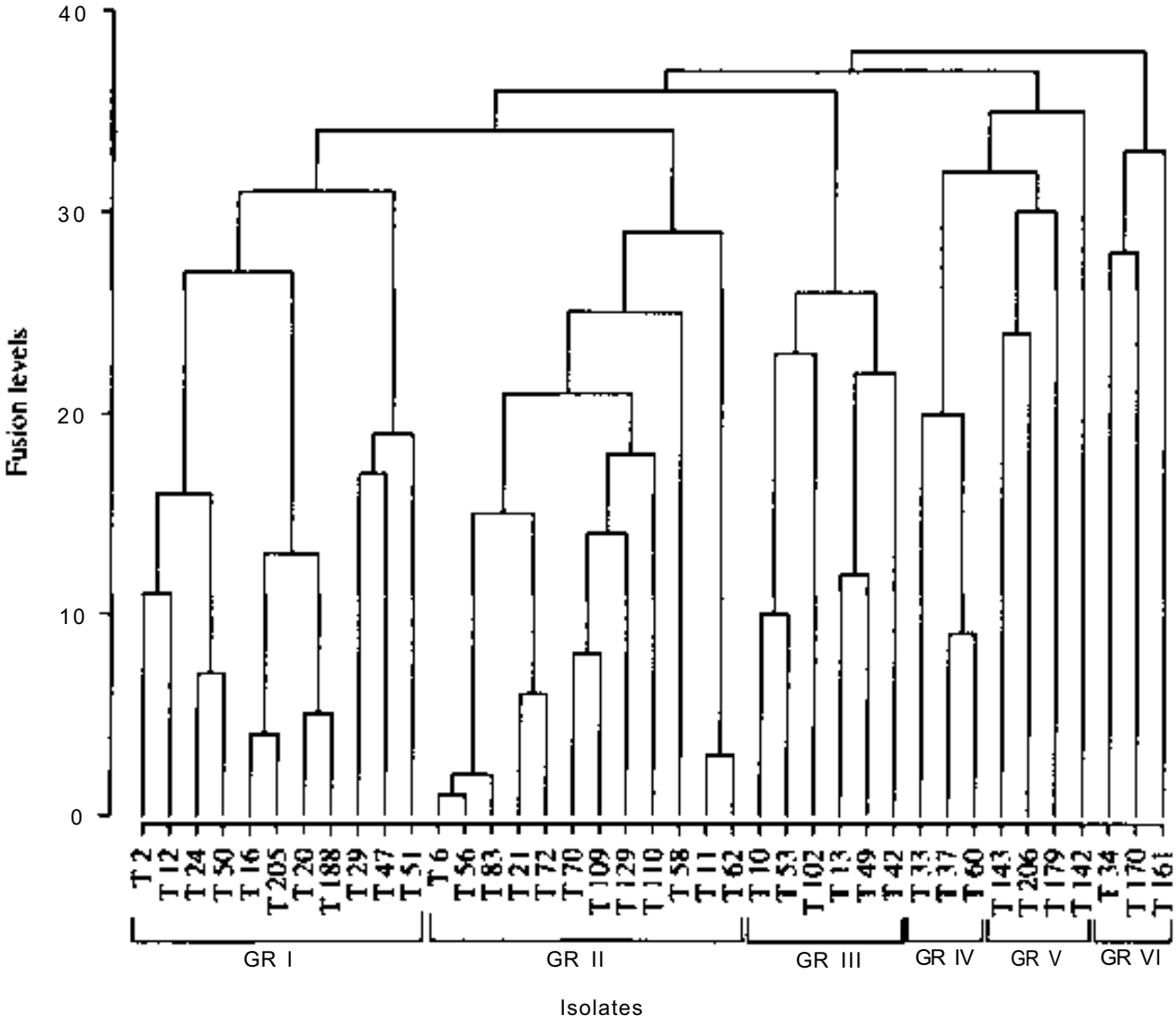

Figure 1. Dendrogram showing 39 *Trichoderma* isolates classified into six groups based on morphological traits and inhibition zone against *Aspergillus flavus* (Af 11-4).

(T.longibrachiatum) showed the maximum inhibition compared with other *Trichoderma* species.

Production of diffusible antibiotics. This study was done following the method of Dennis and Webster (1971a). The plates were incubated for two days and the colony diameter and sporulation of Af 11-4 were compared with the control. Fifteen of the 39 *Trichoderma* isolates showed inhibition of Af 11-4 colony by producing diffusible antibiotics compared with the control. Colony diameter of Af 11-4 in the control plate was 55 mm compared with 10-50 mm in plates with *Trichoderma* isolates. Isolate T 29 *(T.pseudokoningii),* T 42 *(T.harzianum),* and T 83 *(T.koningii)* showed significant inhibition of Af 11-4 growth.

Hyphal interaction. This study was done following the dual culture method (Dennis and Webster 1971c). A block of cellophane (10 mm x 20 mm) was cut from the juncture of the two colonies and mounted in trypan blue-lactophenol, and examined under microscope for hyphal interactions. Isolates T 16 (71 *viride),* T 109 *(T.harzianum),* and T 188 (T.*viride)* showed clear hyphal coiling with Af 11-4 mycelia.

The data of inhibition zone and morphological characters were subjected to average linkage cluster analysis using Euclidian distance as dissimilarity association of GENSTAT Statistical Package (Rothamsted Experiment Station, Herpenden, Herts, UK). The dendrogram prepared from the above classified the 39 *Trichoderma* isolates into six groups (Fig. 1). Further studies are in progress to determine the biological control potential of these isolates against *A. flavus.*

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Mechanisms of Resistance to Groundnut Rosette

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Groundnut *(Arachis hypogaea)* is an important crop in sub-Saharan Africa (SSA) and is mostly grown by smallholder farmers as a subsistence crop under rainfed conditions. Groundnut rosette is endemic to SSA and the impact of the disease can be devastating under the conditions that favor epidemics (Subrahmanyam et al. 1991, 1997, Naidu et al. 1999a). During the 1999/2000 cropping season in Malawi, the average rosette incidence on a national scale was 21.1%, estimated to cause crop losses of nearly US$ 10 million.

The causal agents of rosette are groundnut rosette assistor virus (GRAV), groundnut rosette virus (GRV), and satellite RNA. GRAV is asymptomatic but it acts as a helper virus in vector transmission of GRV and its satellite RNA as they must be packaged in the coat protein of GRAV to form particles that can be transmitted by the aphid, *Aphis craccivora.* The satellite RNA is largely responsible for symptom expression and depends on GRV for replication while GRV depends on satellite RNA for aphid transmission. However, GRV can replicate independently. All three agents must occur together for transmission by the aphid vector and subsequent disease development. Prolonged probes by the aphid vector are