On the contrary, in susceptible cultivars, although the levels of ortho-dihydroxy phenols were initially high it reduced progressively from 5 DAI (Table 2). It is reported that ortho-dihydroxy phenols are highly fungitoxic and accumulate rapidly in resistant cultivars following infection by the pathogen (Bhatia et al. 1972) as observed in the present study.

Activity of polyphenol oxidase (PPO) was higher in the infected leaves of resistant cultivars throughout the growth period than in the susceptible cultivars, which recorded a decline in the enzyme activity within 2 DAI (Table 3). Kosuge (1969) reported that phenols are oxidized to quinones by PPO and there exists direct correlation between the accumulation of phenols and the activity of PPO. This supports the present study.

Based on the above studies it is clear that phenols are induced following rust infection and play a major role in groundnut rust resistance. Such an induction may be a general defense response or a phytoalexin per se. Interestingly, it was observed that susceptible cultivars had shorter incubation period, greater infection frequency, and lesion diameter than the resistant cultivars (data not provided). Hence, it is probable that induction of phenols may be more of a phytoalexin response than the elicitation of general defense. Further studies should focus on identification of specific phenolic compound(s) associated with phytoalexin activity and understanding mechanism(s) of induction.

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Characterization of Isolates of *Trichoderma* for Biocontrol Potential Against *Aspergillus flavus* Infection in Groundnut

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Groundnuts are infected in the field, during processing, and in storage by *Aspergillus flavus* resulting in accumulation of aflatoxins in the seeds, thus rendering them unfit for consumption and trade. Aflatoxins have been reported to be immunosuppressive, carcinogenic, and teretogenic in nature. In the absence of acceptable levels of host plant resistance, use of biocontrol agents could be a promising alternative for the management of aflatoxin contamination. *Trichoderma* spp are well known for their biocontrol ability, especially against soilborne plant pathogens, and these have several modes of action. A systematic characterization and cataloging of isolates for different modes of biocontrol ability will help in deployment of a biocontrol agent for effectively managing plant pathogens. Preliminary observations have indicated that some isolates of *Trichoderma* are effective against *A. flavus.* We report the in vitro antagonistic characteristics of some *Trichoderma* isolates against *A. flavus.*

A total of 26 *Trichoderma* isolates, belonging to five species aggregates, *viride, hamatum, harzianum, auroviride,* and *longibrachiatum,* obtained from different sources were used in the study (Table 1). The *A. flavus* isolate Af 11-4, which is a highly aggressive seed colonizer and is toxigenic was used as the test pathogen. The *Trichoderma* isolates were characterized for growth in broth culture, antagonism in dual culture, production of volatile and non-volatile substances that are inhibitory to *A. flavus,* and tolerance to commonly used seed dressing fungicides (carbendazim and thiram).

All *Trichoderma* isolates were grown on potato dextrose broth for seven days at 28 ± 1°C with a 12-h photoperiod. After the harvest, dry weight of mycelium of the various isolates was recorded. The isolates differed significantly in their growth. Maximum mycelial dry weight was produced by *T. viride* - NARDI (366 mg), followed by *T. harzianum* - APDRC 19 (353 mg), and the least growth was recorded for *T hamatum* - T049 (75 mg) (Table 1). This character would be useful for mass multiplication of the fungus for use in product formulation.

The ability of *Trichoderma* isolates to suppress the growth of *A. flavus* was tested in vitro by dual-culture method (Deacon 1976) and their effectiveness was scored on a numerical scale (Bell et al. 1982) with slight modifications as: 1 = *Trichoderma* overgrowing the colony of *A.flavus;* 2 = *Trichoderma* covering 2/3rd of the plate and progressing

Table 1. In vitro growth of 26 isolates of *Trichoderma* spp as mycelial dry weight and influence of non-volatiles produced by *Trichoderma* isolates on growth of *Aspergillus flavus.*

Trichoderma species	Identity	Source[1]	Mycelial dry weight[2] (mg)	Colony diameter[3] (mm)
T. viride	T071	NRCG, India	191	22
T. viride	T219	NRCG, India	229	20
T. hamatum	T049	NRCG, India	75	19
T. hamatum	T166	Dornach, Switzerland	230	21
T. hamatum	354	Giessen, Germany	253	22
T. harzianum	043	NRCG, India	261	17
T, harzianum	126	NRCG, India	155	17
T. harzianum	127	NRCG, India	167	22
T. harzianum	144	NRCG, India	226	18
T. harzianum	250	NRCG, India	182	24
T. harzianum	295	NRCG, India	220	19
T. harzianum	390	ATCC, USA	277	22
T. harzianum	391	ATCC, USA	167	17
T. longibrachiatum	TL-3	RAU, India	152	18
T. viride	TV4	RAU, India	177	21
T. auroviride	TA-2	RAU, India	197	21
T. harzianum	TH-1	RAU, India	284	27
T. viride	APDRC3	PKV, India	255	21
T. harzianum	APDRC4	PKV, India	246	22
T. viride	APDRC 12	PKV, India	279	22
T. harzianum	APDRC 19	PKV, India	353	18
T. harzianum	OPTNAB	Philippines	172	23
T. viride	Bca6	ICRISAT, India	238	23
Trichoderma sp	MPH	ICRISAT, India	314	19
T. viride	NARDI	NARDI, India	366	17
Trichoderma sp	Ananthapur	ICRISAT, India	312	20
A. flavus (control)			-	85
SEm			±21.08	±13.3
LSD (P = 0.05)			59.9	38.4

1. NRCG - National Research Centre for Groundnut, Junagadh, Gujarat, India; ATCC - American Type Culture Collection, Maryland, USA; RAU = Rajasthan Agricultural University, College of Agriculture, Udaipur, Rajasthan, India; PKV = Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharshtra, India; ICRISAT = International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India; NARDI - Nagarjuna Agricultural Research and Development Institute, Hyderabad, Andhra Pradesh, India.
2. Mycelial growth from 7-day-old culture in potato dextrose broth at 28 ± 1°C; mean of three replications.
3. Colony diameter of *A. flavus* (Af 11-4) recorded 10 days after incubation at 28±1°C; mean of three replications.

towards *A. flavus;* and 3 = *Trichoderma* and *A. flavus* meeting at halfway ofthe petri dish and producing inhibition zone. Seven isolates were fast growing and were rated 1, 16 isolates were rated 2, and three isolates produced inhibition zone with *A. flavus* and were rated 3.

To test the ability of these isolates for the production of volatile and non-volatile chemicals that are inhibitory to *A. flavus* the method of Dennis and Webster (1971a, 1971b) was followed. While assessing the production of volatiles, colony diameters of *Trichoderma* and *A. flavus* were recorded daily, for seven days. None of the isolates of *Trichoderma* inhibited the growth of *A. flavus* by production of volatiles. While assessing the production of non-volatile chemicals, initially, there was very slow growth of *A. flavus.* Even after 10 days of incubation, a maximum of only 27 mm colony diameter of *A. flavus* was recorded with *T. harzianum* - TH-1 as compared with 85 mm in the control (Table 1) indicating the production of non-volatile chemicals inhibitory to *A. flavus* growth by all *Trichoderma* isolates.

All 26 *Trichoderma* isolates were tested for their tolerance to common seed dressing fungicides, thiram and carbendazim (Bavistin®) following poisoned food technique. Potato dextrose agar was amended with either carbendazim at 0.005,0.05,1,2, and 10 µg mL^{-1} or thiram at 100, 200, 500, 1000, and 1500 µg mL^{-1}. All isolates were sensitive to the fungicides at all concentrations indicating that these isolates were not compatible with the fungicides, and thus cannot be used in combination with these seed dressing fungicides. Sensitivity of *Trichoderma* isolates to carbendazim has been reported by Desai and Schlosser (1993). Identification of *Trichoderma* isolates with proven biocontrol ability and tolerance to seed dressing fungicides would be desirable to utilize them to control *A. flavus* infestation. Selected *Trichoderma* isolates from this study are being used in greenhouse and field experiments to evaluate their biocontrol potential against aflatoxin contamination in groundnut.

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Mycotoxins from Groundnuts Marketed in Yemen

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One of the most serious aspects of the invasion of grain by some fungi is the production of toxic secondary metabolites known as mycotoxins. Many agricultural commodities and their products including feed have been shown to be contaminated by them. Mycotoxins are highly toxic to humans and livestock. Different fungi produce different types of mycotoxins, e.g., *Aspergillus flavus* and *A. parasiticus* produce aflatoxin, and *A. ochraceous* and *Penicillium viridicatum* produce ochratoxin. Groundnuts are most susceptible to the fungi that produce aflatoxin.

Surveys from several countries have reported considerable contamination of groundnut seeds, groundnut cake, and its feed with aflatoxin, ochratoxin, citrinine, zearalenone, trichothecens, T-2 toxins, deoxynivalenol (DON), nivalenol, diacetoxyscirphenol, and penicillic acid. Most of these studies were conducted in the areas of outbreaks of mycotoxicoses in farm animals or humans, while other representative samples studied had obvious mold damage (Bhat 1989). Mycotoxin contamination in groundnut can occur in the field during pre-harvest, harvest, and during postharvest handling (Nahdi 1997). In many countries, they are able to control the entry of contaminated groundnut in food chain by following strict regulatory programs. The maximum permissible limit of these mycotoxins varies from 0 to 100 µg kg^{-1} depending on the country and foodstuff and also whether the commodity is for human or animal consumption. Most of the groundnut for local consumption in Yemen is imported. Unfortunately, there are no regulatory mechanisms established in Yemen to prevent the entry of contaminated groundnut in food chain. The quarantine laboratories in the country are neither equipped nor have trained staff to undertake mycotoxin analysis.