References

Csinos, A.S., and Bell, D.K. 1989. Pathology and nutrition in the peanut pod rot complex. Pages 124-136 *in* Management of diseases with macro- and microelements (Engelhard, A.W., ed.). St. Paul, Minnesota, USA: American Phytopathological Society.

Marinelli, A., March, G.J., Rago, A., and Giuggia, J. 1998. Assessment of crop loss in peanut caused by *Sclerotinia sclerotiorum, S. minor,* and *Sclerotium rolfsii* in Argentina. International Journal of Pest Management 44:251-254.

Prevalence of Aflatoxin Contamination in Groundnut in Tumkur District of Karnataka, India

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Aflatoxin contamination in groundnut *(Arachis hypogaea)* is a serious problem worldwide affecting trade and human health. Contamination of groundnut seed occurs in the field, during transportation, and in storage by *Aspergillus flavus* group of fungi. Abiotic stresses, such as end-season drought, improper drying, and moist storage conditions form the major predisposing factors for infection of groundnut seed by *A. flavus.* The National Agricultural Technology Project (NATP) "Aflatoxin contamination in groundnut: mapping and management in Gujarat, Andhra Pradesh, and adjoining areas" was launched in August 2000 in collaboration with the National Research Centre for Groundnut (NRCG) (Junagadh, Gujarat), ICRISAT (Patancheru), and the Gujarat Agricultural University to assess the extent of awareness among farmers, traders, and oil producers about aflatoxin and its associated problems, to assess the extent of pre- and postharvest aflatoxin contamination in the target districts, to determine the influence of on- and off-farm practices on the toxin contamination, and finally to evolve an integrated management strategy for combating the problem. The study reported here is a part of this project in Tumkur district of Karnataka, India.

Awareness among farmers

To assess the problem of aflatoxin awareness among farmers, traders, and oil millers, a survey was conducted at the end of the rainy season in 2000 across the four major groundnut-growing taluks of Tumkur district: Koratagere, Madhugiri, Pavagada, and Sira. Information was obtained by closely interacting with 137 farmers on the crop details, on- and off-farm practices, socioeconomic aspects of groundnut cultivation, problematic pests and diseases, and awareness on aflatoxin contamination. The survey results revealed that farmers were ignorant of the aflatoxin problem. Many farmers opined that the lack of visual indication on the seed was the major factor for their being unaware about aflatoxin contaminated seeds. *Helicoverpa armigera,* white grubs, and leaf spots were the major production constraints. Farmers thought that end-season drought was a major factor for reduced yields and bitterness in seeds. It is known that any delay in harvesting the crop under end-season drought could severely reduce yields and increase aflatoxin contamination.

Pod and soil sampling

A stratified sampling method was followed to collect one sample representing 800-1000 ha of groundnut-growing area. We collected 137 pod samples (1 kg pods per sample) and 133 soil samples from the estimated 80,000 ha groundnut area in Tumkur district during the 2000 rainy season crop.

Pod samples were collected randomly from 5 spots in each field from the mature/harvested plants in the field and bulked. The soil in the geocarposphere region from the same spots was collected and pooled to make a bulk sample (250 g per sample).

Analysis for seed infection and soil population of *A. flavus*

Pods were shelled and the seeds were surface sterilized before plating them on Czapek Dox agar (CDA) fortified with rose bengal, and incubated at 25°C for 4 days in dark for determining seed infection. For each sample, 100 apparently healthy seeds were used. Number of seeds colonized by typical *A. flavus* was counted and percentage seed infection determined. Soil samples were sieved to fine powder and serially diluted in sterilized distilled water to $10^{-3}$and 10^{-4} concentrations and plated on AFPA (*Aspergillus flavus* and *parasiticus* agar) medium (Pitt et at. 1983). The plates were incubated for two days

at 28°C in dark and typical *A.flavus* colonies were counted and population density determined as colony forming units (cfu) g^{-1} of soil.

Seed infection studies revealed that of the 137 samples, 39 had no infection, 64 had 1-5% infection, 10 had 6-10% infection, and 24 had more than 10% seed infection (Fig. 1). The results of soil analysis revealed that of the 133 samples, only 31 were free from *A.flavus* propagules, 75 samples had 1 x 10^3 to 5 x 10^3 cfu g^{-1} soil, 14 had 6 x 10^3 to 10 x 10^3 cfu g^{-1}, and 13 had more than 10,000 cfu g^{-1} soil (Fig. 1).

In general, there was no clear correlation between *A. flavus* soil population density and seed infection among samples from each taluk. However, in six villages of three taluks, there was good correlation between *A. flavus* soil populations (6 x 10^3 to 44 x 10^3 cfu g^{-1}) and seed infection (10-22%) (Table 1).

Table 1. Groundnut areas with high soil density of *Aspergillus flavus* and seed infection in Tumkur district of Karnataka, India.

Taluk	Village	*A. flavus* population (x 10^3 cfu g^{-1} soil)	Seed infection (%)
Koratagere	Agrahara Thanda	12	10
Koratagere	Arasapura	6	19
Madhugiri	Nagenahalli	7	13
Madhugiri	Bhaktharahally	26	16
Pavagada	Shilapura	44	17
Pavagada	Kotagudda	10	22

Aflatoxin in market samples

Aflatoxin contamination under market conditions was determined in 42 pod samples collected from Madhugiri (11 samples) and Pavagada (31 samples) market yards, the major oil milling centers in Tumkur district. The aflatoxin content in the collected pod samples was estimated using enzyme-linked immunosorbent assay (ELISA), a simple and quick immunoassay protocol for monitoring allatoxins (Devi et al. 1999). Seeds from all 42 samples showed aflatoxin contamination, though the levels were below 20 µg kg^{-1} seed. Anatoxin content was 8.51 (range 3-11) µg kg^{-1} seed in Madhugiri samples and 6.78 (range 5-18) µg kg^{-1} seed in Pavagada samples. The fact that all the samples contained aflatoxin is of concern because the levels could go up under humid storage conditions.

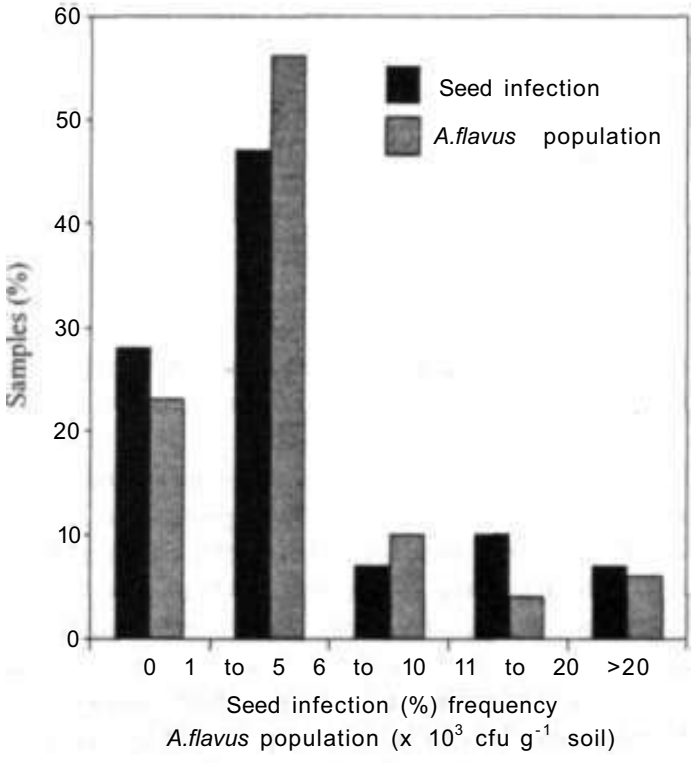

Figure 1. Extent of seed infection and soil population of *Aspergillus flavus* in groundnut samples from Tumkur district, Karnataka, India.

Conclusion

From these preliminary results six villages in three taluks of Tumkur district were identified with relatively higher population density of *A. flavus* and higher seed infection, and thus likely to be aflatoxin risk prone areas. However, these results need confirmation from pod and soil samples and aflatoxin estimation in the seed samples in 2001. An integrated management practice will be developed and on-farm evaluations will be conducted in the high-risk areas to reduce aflatoxin contamination in groundnut during the rainy season 2001.

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References

Devi, K.T., Mayo, M.A., Reddy, K.L.N., Delfosse, P., Reddy, G., Reddy, S.V., and Reddy, D.V.R. 1999. Production and characterization of monoclonal antibodies for aflatoxin B_1 Letters in Applied Microbiology 29:284-288.

Pitt, J.I., Hocking, A.D., and Glenn, D.R. 1983. An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus.* Journal of Applied Bacteriology 54:109-114.

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