

Evaluation of an Integrated Management Package to Reduce Preharvest Seed Infection by *Aspergillus flavus* in Groundnut

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Aflatoxin contamination in groundnut (*Arachis hypogaea*) is one of the major problems that can occur at preharvest and postharvest stages affecting the quality of the produce and thus trade. Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus*, which can invade the pods during crop growth, when the conditions are congenial for the pathogen (Hill et al. 1983). An integrated approach through combining chemical, cultural, and biological management options could be a viable option for reducing preharvest contamination of seed in groundnut production systems. The efforts to subdue preharvest aflatoxin problem should be based on the principles of greater ecological sustainability in the long run keeping in view minimal use of pesticides. Through a collaborative project, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Indian Council of Agricultural Research (ICAR) evaluated an integrated package at ICRISAT, Patancheru, Andhra Pradesh, India during the rainy season in 2001 to demonstrate the effectiveness of improved package vis-a-vis farmers' practice.

Methodology

Two treatments, integrated aflatoxin management package (IAMP) and farmers' practice were compared in Alfisol fields at ICRISAT, Patancheru. Each treatment was conducted on a 0.1-ha plot. The IAMP comprised: summer plowing of the field; seed treatment with carbendazim (Bavistin 50 WP) at 4 g kg⁻¹ seed; furrow application of

Trichoderma harzianum at 50 g culture mixed in 50 kg farmyard manure before sowing [to make a final population of 1 x 10⁶ colony forming units (cfu) g⁻¹ soil]; spray of Nimbuclidin (250 ml in 50 L water) for controlling foliar diseases and insects; and a second spray of carbendazim (50 g) + Dithane M-45 (250 g) in 50 L of water, if required; harvesting plants at 75% pod maturity; drying the harvested plants by inverted windrows method for 3 days to avoid contact between the pods and wet soil; and removing insect-damaged and diseased pods. In both practices, hand weeding was done twice, at 20 and 45 days after sowing.

The farmers' practice (as a control) included summer plowing, harvesting pods at full maturity, drying pods by leaving them in the field, and removing damaged pods, but did not include any chemical and biological treatments.

Application of *A. flavus* inoculum. To ensure infection, a highly toxigenic strain of *A. flavus* (Af 11-4) was multiplied on pearl millet (*Pennisetum glaucum*) seeds, mixed with farmyard manure and applied in both the practices when the crop was at 50% flowering stage (the most susceptible stage of the crop).

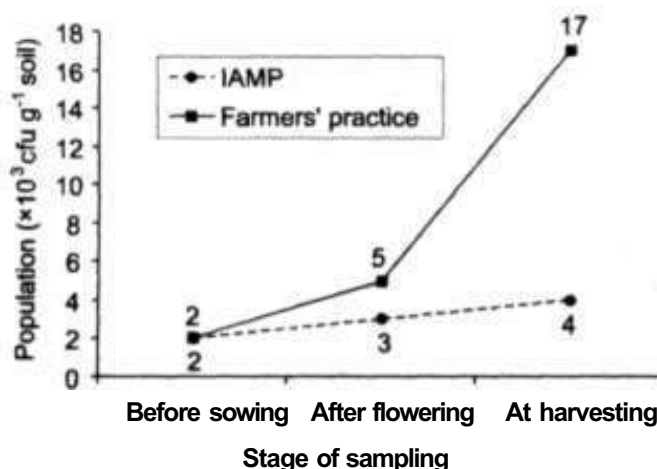


Figure 1. Soil population of *Aspergillus flavus* (Af 11-4) at different crop growth stages in plots with IAMP (integrated aflatoxin management practice) and farmers' practice during the rainy season, 2001 at ICRISAT, Patancheru, India.

Soil and pod sampling. Soil was sampled at three stages to monitor the levels of *A. flavus* population. Initial sampling was done just before sowing; the second sampling was done prior to application of the pathogen inoculum (at 50% flowering stage), and the final sampling at harvest in both experimental plots. Pods from the improved package plot were harvested at 75% maturity level and dried by inverted windrows method for three days. On the other hand, pods in the control plot (farmers' practice) were harvested at full maturity and dried by leaving them in the field.

Seed infection, aflatoxin content, and *A. flavus* population in soil. Pods were shelled and seeds were surface sterilized before plating them on Czapek Dox agar (CDA) fortified with rose bengal (25 mg L⁻¹) and incubated at 25°C for four days in dark. From each plot, 100 apparently healthy seeds were selected. Number of seeds colonized by typical *A. flavus* was counted and expressed as percent seed infection. Seed samples (50 g) were soaked in sterile distilled water for 4 h, later dried and incubated at 25°C overnight prior to aflatoxin estimation by using enzyme-linked immunosorbent assay (ELISA), a simple and quick immunoassay protocol for estimation of aflatoxins (Devi et al. 1999).

Soil samples (200 g from a composite bulk of 1000 g soil from 5 random spots in a field) were sieved. The fine powder was serially diluted with sterile distilled water to 10⁻³ and 10⁻⁴ concentrations and plated on AFPA (*Aspergillus flavus* and *parasiticus* agar) medium (Pitt et al. 1983). The plates were then incubated for 2-3 days at 28°C in dark and typical *A. flavus* colonies were counted and population density was expressed as cfu g⁻¹ soil.

Other diseases. The crop was given supplemental irrigation to avoid moisture stress throughout the growing season. In both the plots, other important diseases were recorded by selecting ten blocks, each comprising 100 plants row⁻¹. Disease incidence was scored as percentage of infected plants for stem rot (*Sclerotium rolfsii*) and

on a 1-9 scale for late leaf spot. Bud necrosis was scored for the presence (+) or absence (-) of infected plants.

Results and Discussion

The indicators for the effectiveness of integrated management of aflatoxin contamination were fungal infection and aflatoxin content in the seed and *A. flavus* population in the soil. Despite the similar initial population levels in both the plots, cumulative gain in the cfu was observed in the plot where farmers' practice was followed indicating unremitting growth in *A. flavus* due to absence of any control measures. On the other hand, the beneficial effects of soil treatment with the antagonistic fungus *Trichoderma* sp was apparent in the plot with improved package, despite the addition of *A. flavus* inoculum during flowering stage, which produced spores at 1.82 x 10⁷ m⁻¹ row (Fig. 1). *Trichoderma* sp being a potential antagonist might have prevented the proliferation of *A. flavus* in the soil. *Trichoderma* sp has the ability to inhibit the growth of *A. flavus* in vitro by production of non-volatile antibiotics (Desai et al. 2000).

Seed infection studies revealed predominance of *A. flavus* infection in plot with farmers' practice (10%) over improved package (2%) (Table 1).

Table 1. Evaluation of an integrated aflatoxin management package and farmers' practice in groundnut (cv ICGS 11) during the rainy season, 2001 at ICRISAT, Patancheru, India.

Parameter	Integrated package	Farmers' practice
Seed infection by <i>Aspergillus flavus</i> (%)	2	10
Pod yield (kg ha ⁻¹)	555	544
Late leaf spot damage ¹	6.9 (±0.23)	7.7 (±0.15)
Stem rot incidence (%)	2.5 (±0.62)	31.0 (±4.70)
Bud necrosis incidence ²	+	+

1. Mean of 10 replications; 1-9 disease rating scale where 9 = susceptible.

2. + = Disease noticed.

This could be because of inhibition of initial rhizosphere soil population build up of *A. flavus* by seed treatment with systemic fungicide and application of biocontrol agent in the improved package. Although no aflatoxin contamination was recorded in seed samples in both practices, with 10% seed infection levels in farmers' practice it is likely that aflatoxin levels would be higher under farmers' storage conditions than under dry conditions.

The improved package recorded only a marginal increase in pod yield than farmers' practice, reflecting the fact that aflatoxin contamination is more of a qualitative problem in groundnut than quantitative. The concomitant effects of the improved package were evident in scanty incidence of late leaf spot and stem rot diseases over farmers' practice. Further, application of such a package in the long run would improve soil health and might result in improved yields as well.

These results need further confirmation, and relative economics of the two cultivation practices could be compared from on-farm evaluation trials at village level in aflatoxin risk sensitive areas in the target districts of Andhra Pradesh and Karnataka during the rainy season in 2002.

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Bio-efficacy of Fungicides for Control of Leaf Spots of Groundnut in Northeastern Dry Zone of Karnataka, India

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Groundnut (*Arachis hypogaea*) is an important oilseed crop of Karnataka in India. It is cultivated in an area of 1.3 million ha, the production being 1.0 million t and productivity 0.8 t ha⁻¹ (Directorate of Agriculture 1997). In recent years, irregularity in supply of water from Tungabhadra and Upper Krishna Project canals and unpredictable rainfall as well as disease problems have made groundnut cultivation risky, particularly in northeastern dry zone of Karnataka. Early leaf spot caused by *Cercospora arachidicola* and late leaf spot caused by *Phaeoisariopsis personata* are endemic diseases in the rainy season causing 90% defoliation. Besides causing quantitative losses, these diseases are responsible for reduction in protein content and oil recovery (Gupta et al. 1987). So far no variety has been identified as resistant or tolerant to these diseases and adapted to the agroclimatic conditions of the region. Therefore, use of fungicides is the only alternative for effective