Integrated Management of Aflatoxin Contamination: Progress towards Enhancing Food and Feed Quality and Human and Animal Health

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1. The Problem

Aflatoxins are the secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus* in several crops and commodities. They are carcinogenic, teratogenic and immunosuppressants that are implicated in growth retardation in children, immune-suppression, interference in micronutrient metabolism, liver cirrhosis, liver and esophageal cancers and decreased human and animal productivity[1,2] (Figure 1). Acute severe intoxication of aflatoxin can result in death[3]. Research in Asia and Sub-Saharan Africa (SSA) has shown that aflatoxin contamination is widespread in staple crops like groundnut, maize, millet, chillies, sorghum, etc., and even in milk from animals fed with contaminated feed[2]. In the developed countries, aflatoxins are regulated through good production and post-harvest practices and stringent food safety monitoring. However, application of such strategies in the developing countries are difficult due to differences in production practices, food insufficiency, lack of resources and technology for monitoring and poor awareness about the problem[2]. Consequently, people in these countries are chronically exposed to aflatoxins[4]. The ICRISAT and CGIAR recognizes the economic and health implications of aflatoxins as an important constraint to the goal of improving human health and well-being through agriculture, and pursue various strategies to eliminate aflatoxin contamination in food and feed (CGIAR Priority 2C: Enhancing Nutritional Quality and Safety)[5,6].

ICRISAT's efforts are primarily focused on preventing aflatoxin contamination in groundnut, a key commodity in the livelihood of the rural poor in the semi-arid tropics (SAT). It is one of the most susceptible crops to the invasion by *Aspergillus sp.* Due to high incidence of aflatoxin contamination, groundnut export shares in Asia and SSA region have declined during the last four decades[7]. Moreover farmers in poor countries trade best quality produce, while poor quality nuts are consumed locally thereby harming their health[3]. Therefore, a major emphasis has been placed on reducing the risk of aflatoxin contamination in groundnut grown in the SAT[5].

2. ICRISAT Strategy for Reducing the Risk of Aflatoxin Contamination in Groundnut, and Human Exposure to Aflatoxins

Aflatoxin contamination in groundnut is influenced by several interactions between host plant, fungal population, environment and farming practices. Pre-harvest aflatoxin contamination is most frequent in groundnut cultivated under rain-fed conditions. Drought and heat stress at maturity stage exacerbates the fugal invasion, and high temperature and high humidity during harvesting and storage lead to further invasion of the fungi and higher production of the toxin in the kernels. This situation necessitates a need for series of strategies to prevent the fungal infection at pre- and post-harvest stages and during the storage. Due to inadequate level of host-plant resistance available, our research emphasis has been on integrated genetic and natural resource management (IGNRM) approach that combines genetic resistance, cultural practices and biocontrol to suppress the fungal infection and aflatoxin contamination in a convenient and affordable packages, classified as no-cost, low-cost, and high-cost options, appropriate for farmers in diverse agro-eco regions[8] (Figure 2). Simple and low-cost diagnostic test was developed for monitoring aflatoxin contamination. In addition, emphasis was placed on promotion of technology through participatory knowledge sharing activities. Recently, we have developed a diagnostic test to assess the human exposure to aflatoxin that will aid in conducting field studies to identify aflatoxin-exposed populations, determine the source of contaminated food and stimulate appropriate interventions to minimize human and animal exposure to aflatoxins and consequent health hazards (Figure 2).

3. ACHIEVEMENTS AND CURRENT STATUS

3.1. Genetic Enhancement

3.1.1. Genetic enhancement through conventional breeding: Research on groundnut breeding for aflatoxin resistance is focused on the identification and utilization of genetic resistance to pre-harvest seed infection, seed colonization and aflatoxin production by *A. flavus* under field conditions. These efforts have resulted in the identification of over 30 germplasm accessions as resistant/tolerant sources. Several resistant germplasm lines identified include, ICG 51 (RS 149), 1323 (HG 1), 4440 (E 15), 6101 (NCAC 898), 6222 (RCM 466), 9610 (VRR 538), 10020 (PI 476149), 10094 (S4), 10609 (PI 337342), 10933 (PI 476166), 11682 (PWA 3). Some of these genotypes, including 55-437, Tamnut 74, PI 365553 (for resistance to seed infection), and PI 337394 F, PI 337409, UF 71513, Ah 7223, J 11, U 4-47-7, Var 27, Faizpur and Monir 240-30 (for resistance to *in vitro* seed colonization), and U 4-7-5 and VRR 245 (for resistance to aflatoxin production) were used for developing elite aflatoxin resistant varieties, like ICGV # 88145, 89104, 91278, 91283 and 91284 released as improved germplasm[9,10,11]. Three lines, ICGV 87084, 87094 and 87110 were found resistant to seed infection in Niger, Senegal and Burkina Faso in West Africa. Studies indicated that the three components (seed infection, seed colonization and aflatoxin production) of resistance are inherited independently[10]. Despite considerable efforts for genetic enhancement, stable resistance to *A. flavus* infection /aflatoxin contamination was limited due to the high G × E interaction[9]. Current efforts are focused to combine the various components of resistance into a single genotype to augment host resistance; utilizing 32 newly identified resistant germplasm sources in the breeding programs; and evaluating the groundnut mini-core collection for pre-harvest seed infection[12].

3.1.2. Interspecific hybrids: Interspecific derivatives obtained from highly resistant wild *Arachis* accessions [*Arachis cardenasii* (ICG 8216) and *A. chiquitana*] are being evaluated for *A. flavus* seed colonization under in vitro and field screening assays at Patancheru[13]. Results from 2006 trials indicated availability of good resistance (0-10% seed colonization) in a few interspecific hybrids. These lines will be evaluated during 2007, and stable and high levels of resistance sources will be used in backcross breeding to transfer resistance.

3.1.3. Transgenic resistance: Transgenic approach was undertaken to deploy the resistance against fugal invasion and toxin production. A rice chitinase (*RChi*) gene that has anti-fungal properties was introduced into a popular groundnut variety JL 24 via transgenic approach[14]. Thirty transgenic events were selected and assessed for resistance against *A. flavus* seed colonization by in vitro seed inoculation assay. Seeds of events that showed 0-10% incidence were advanced up to T_5 generation. Nine events that consistently showed <10% seed infection during T_3-T_5 seed generations were selected for evaluation under contained field conditions in *A. flavus* sick plots at ICRISAT, Patancheru. The best events will be used for further field evaluations and subjected to environmental and food safety testing. Efforts are also being made to introduce *13S LOX* (*lipoxygenase*) gene, which has been shown to down-regulate aflatoxin production, and/or RNAi technology to down-regulate/knockout 9S Lox gene that enhances the sporulation and aflatoxin production in groundnut[15].

3.2. Biological control

Biological control is emerging as one of the promising approaches for aflatoxin management in groundnut. Our research has used selected isolates of *Trichoderma* [*T. viride* (Tv 47), *T. harzianum* (Th 23), *T. harzianum* (Th 20), *T. koningii* (Tk 83)], geocarposphere bacterial strains of *Pseudomonas* [*P. aeruginosa* CDB35, *P. cepacia* and *P. fluorescens*] and Actinomycetes [strain CDA19)] for reducing groundnut seed colonization by competitive exclusion/inhabitation of *Aspergilli*[16]. Biocontrol agents were used as seed dressing and soil application to determine their effects on population dynamics of *A. flavus* in the geocarposphere and subsequently on preharvest kernel infection of groundnut. Significant reduction (20-90%) in *A. flavus* seed infection was recorded with several treatments over control[17,18]. Present studies are focused on use of these biocontrol agents singly or in combination with compost, crop residues or gypsum under field conditions to determine the most efficient field application strategy. These agents are also being evaluated on-station and on-farm field trials. Emphasis is also placed on commercial production of most potent biocontrol agents under public-private partnerships.

3.3. Cultural practices

3.3.1. Habitat management**:** Certain cultural practices such as: summer ploughing, selection of appropriate planting date to take advantage of periods of rainfall to avoid end-season drought effects, seed dressing with systemic fungicides or biocontrol agents, maintaining good plant density in the fields, removal of premature dead plants, managing pest and diseases, timely harvesting, exclusion of damaged and immature pods, quick pod drying, controlling storage pests and storing the pod/seed with <10% moisture prevent fungal infection and proliferation[19,20,21]. The use of safe and efficient mechanical threshers, and seed storage bins are other cultural practices for reducing aflatoxin in groundnuts. Although most of the options are cost-effective and practical under subsistence farming conditions, they remain largely un-adopted by farmers. Our efforts resulted in devising appropriate combination of practices (timely harvesting, windrow drying and threshing) that are more compatible with socio-economic profiles and farming practices of the farmers of a particular region which contributing to good adoption in Mail and Niger in West Africa and in Andhra Pradesh state, India.

3.3.2. Control through soil amendments: Soil amendments with gypsum (as source of calcium), farmyard manure and cereal crop residues applied either singly or in various combinations at different cropping stages contributed to reduction in the preharvest *A. flavus* infection and aflatoxin contamination in groundnut[22]. Application of gypsum and farmyard manure at the time of sowing was most effective in reducing seed infection and aflatoxin contamination (mean reduction of 80% compared to controls). Lime and farmyard manure are cheap, easily available in most developing countries, including SSA.

3.3.3. Post-harvest practices: Our studies in West Africa have shown that aflatoxin contamination increased with the delay in pod removal after lifting the plants and during storage[23]. In addition, traditional heap drying enhance rapid fungal proliferation and toxin production. Small and immature seeds (gleans) contain the highest toxin and segregating such seeds reduced the contamination in the final product. Replacement of farmers' traditional practice of 'heap' drying with windrow drying of lifted plants has dramatically reduced the contamination. In Andhra Pradesh state, India, mechanical threshers were introduced for rapid threshing of pods to avoid the heaping. However, on-farm and household storage conditions in Asia and SSA are not adequate to store groundnut safely (lack of clean storage bins, frequent pest infestation) and creates conducive conditions for aflatoxin contamination even in a healthy produce from the field. This requires further study by profiling storage structures and practices in various regions to develop appropriate methods where proper temperature and humidity can be maintained to reduce the contamination risk.

3.2. Diagnostic Tools for Monitoring Aflatoxins and its Metabolites

3.2.1. Monitoring food and feed samples and capacity building: We have developed simple and cost-effective (US$ 1 per sample) ELISA for detection and estimation of aflatoxins in various commodities, including groundnut[24]. This method has lower detection limit of 2.5 g/kg or 50 pg/ml of aflatoxins in various samples. More than one hundred samples can be analyzed in a day and the results obtained are comparable with that of High-Performance Liquid Chromatography (HPLC) system. We are extensively using this ELISA system for evaluating germplasm/breeding lines/inter-specific derivatives for aflatoxin resistance; assess the effectiveness of intervention strategies; and routine monitoring of various commodities (crop/food/feed/fodder/milk) [25].

As of 2006, eight training programs were conducted to train >125 NARES in application of ELISA test for aflatoxin monitoring (See Annexure). The diagnostic reagents and the aflatoxin ELISA kit is being widely distributed to partners from public and private sector in Asia and SSA. We helped in setting up of 16 aflatoxin testing laboratories in India, Mozambique, Kenya, Malawi and Mali, that uses our ELISA method for monitoring groundnut and other commodities for aflatoxin contamination[26]. These laboratories are contributing to the quality certification of the farmers produce and thereby enhancing the competitiveness of the produce in domestic and international markets. For instance, the aflatoxin laboratory at Malawi contributed to the revival of groundnut exports to Europe and South Africa. Current efforts are focused on developing simple and inexpensive on-site screening test for aflatoxin estimation.

3.2.2. Diagnostic test for monitoring human exposure to aflatoxins: To identify the aflatoxin-exposed populations, a simple competitive ELISA has been developed[27,28]. This test is based on the estimation of AfB_1-lysine, a metabolite of AfB_1, whose concentration in the blood albumin fraction has been shown to correlate with dietary aflatoxin intake over the previous 2 to 3 months. This test can detect levels of AfB_1-lysine in blood as low as 5 pg/mg albumin. The test is simple to perform, low-cost (US$ 2 per sample), and is effective for routine monitoring of human, as well as animal samples. Currently, this assay is being used to survey *Hepatitis B virus* positive serum samples in India to assess the risk of aflatoxins, so that appropriate interventions can be implemented to reduce the risk of liver cancer. In addition, a PCR-RFLP test is being developed to determine the aflatoxin induced DNA mutation at the codon 249^{ser} in Exon 7 of the p53 tumor suppressor gene in humans. These simple tests allow epidemiology studies to determine the level of human exposure and health risks, determine the source of contaminated food and stimulate appropriate interventions to limit dietary-aflatoxin exposure.

3.3. Participatory Knowledge Sharing

3.3.1. Farmer participatory varietal selection: Fourteen-short duration high yielding aflatoxin resistant varieties were evaluated in >50 farmer fields in Anantapur and Chittoor districts of Andhra Pradesh, India[29]. This activity initiated in 2003, in association with farmers, NGOs, traders, oil millers, scientists from NARES, over a 3 year period resulted in selection of 7 varieties (ICGV 91278, 91328, 94379, 94434, 91114, 91341, 93305). These were further widely disseminated in Anantapur and Chittoor districts. The elite varieties produced 15-34% higher pod yield over the local varieties. Similarly the varieties showed 41-73% reduction in aflatoxin level over the controls.

3.3.2. Capacity building and Awareness creation: Several training programs and demonstration visits were organized to NARES and farmers to promote the integrated aflatoxin management technologies, aflatoxin resistant varieties and application of ELISA for aflatoxin monitoring (see Annexure). Information bulletins and flyers in local languages were published to create awareness about the problem and management options. A website, www.aflatoxin.info has been established, which serves as repository of information on aflatoxin. Audio-visual programs were made on aflatoxin management aspects which were telecasted by *BBC World* and *Doordarshan India*. Technical panel comprising farmers, NGOs, officials from state health, agriculture and consumer forum divisions, were established to create awareness about aflatoxins and to muster favorable policy support. For instance, efforts of this kind have resulted in government subsidy for purchasing mechanical pod threshers in Andhra Pradesh State, India.

4. CONCLUSIONS

Several efforts are ongoing worldwide to find solutions for reducing the risk of aflatoxin contamination in staple foods. Our work suggests that a holistic approach based on IGNRM, combined with capacity development through participatory knowledge sharing are effective in reducing the risk of aflatoxin contamination in groundnut. This has resulted in several International Public Goods (listed below) that are providing much needed impetus to aflatoxin prevention strategies in the SAT.

- Aflatoxin resistant germplasm (varieties and breeding lines)
- Low-cost and convenient diagnostic tools for monitoring the toxins in commodities and biomarkers in humans
- Appropriate crop management practices [categorized based on the input costs as (i) no cost; (ii) low-cost; and (iii) high-input management strategies].
- Biocontrol microbes that counteract *A. flavus*, and procedures for bulk production
- Transgenic groundnut events for resistance to fungal invasion
- Protocols for screening for *A. flavus* resistance
- Publications (print, audio-visual, website), development of human skills and aflatoxin monitoring facilities.

5. FUTURE PROSPECTS

Although aflatoxin *per se* is not a new issue, it requires new strategies to address it effectively within developing countries where aflatoxin exposure is intertwined with the issues of food insufficiency and inadequate emphasis on/ lack of facilities for food safety monitoring. Emerging evidence on the causal role of aflatoxins in heightening liver cancer and other health consequences is leading to a paradigm shift in focus on aflatoxin as a serious health hazard in the developing countries, particularly when 95-100% of the produce is used for domestic consumption. Moreover, with the stringent food safety regulations in place, in order for smallholder farmers to participate in global markets requires greater capacity for monitoring aflatoxins to implement food safety protection standards as per the *Codex Alimentarius* guidelines followed by the FAO, WHO and WTO. Efforts to mitigate aflatoxin contamination will also addresses the Millennium Development Goals (MDGs) on eradicating extreme poverty and hunger (MDG-1); reducing childhood mortality (MDG-4); improving maternal health (MDG-5); and combat HIV/AIDS, malaria and other diseases (MDG-6), because aflatoxin-free food is key for better health, especially to improve the health of post-weaning children, and to cope with malnutrition and HIV/AIDS and to overcome trade barriers. Sensing these needs and to augment our own ongoing efforts on aflatoxin mitigation, our future research will focus on:

- Genetic enhancement using modern biotechnological applications (inter-specific hybrids, transgenics, in combination with conventional breeding).
- Capacity building for monitoring aflatoxins in SSA and South Asia (SA), and development of simple on-site aflatoxin monitoring tests.
- Establishment of a prediction system to forecast likely risk of aflatoxin contamination during the cropping season (work initiated in Mali).
- Develop international partnerships involving government and non-governmental agencies, together with the human health research community, to strengthen the regional research efforts in the SAT to pursue adoption of IGNRM technologies to deal with aflatoxins for enhancing food safety and trade.
- Monitoring human exposure to aflatoxins, and examine quality of household diets, the impact of household incomes on dietary choices and evaluate the effects of aflatoxins on the HIV/AIDS.

For further information/clarification please contact Dr Farid Waliyar. F.waliyar@cgiar.org

Figure 1: Aflatoxin affects on trade and health

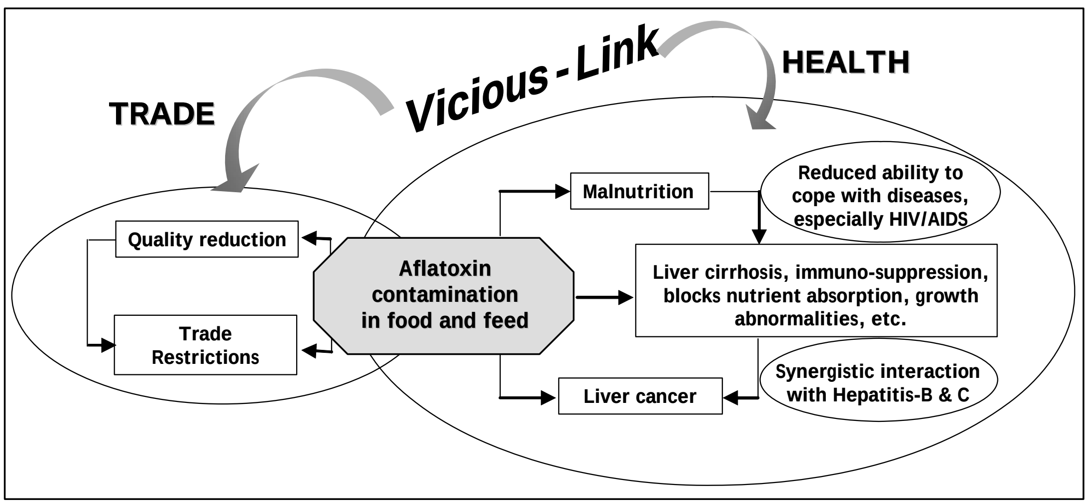
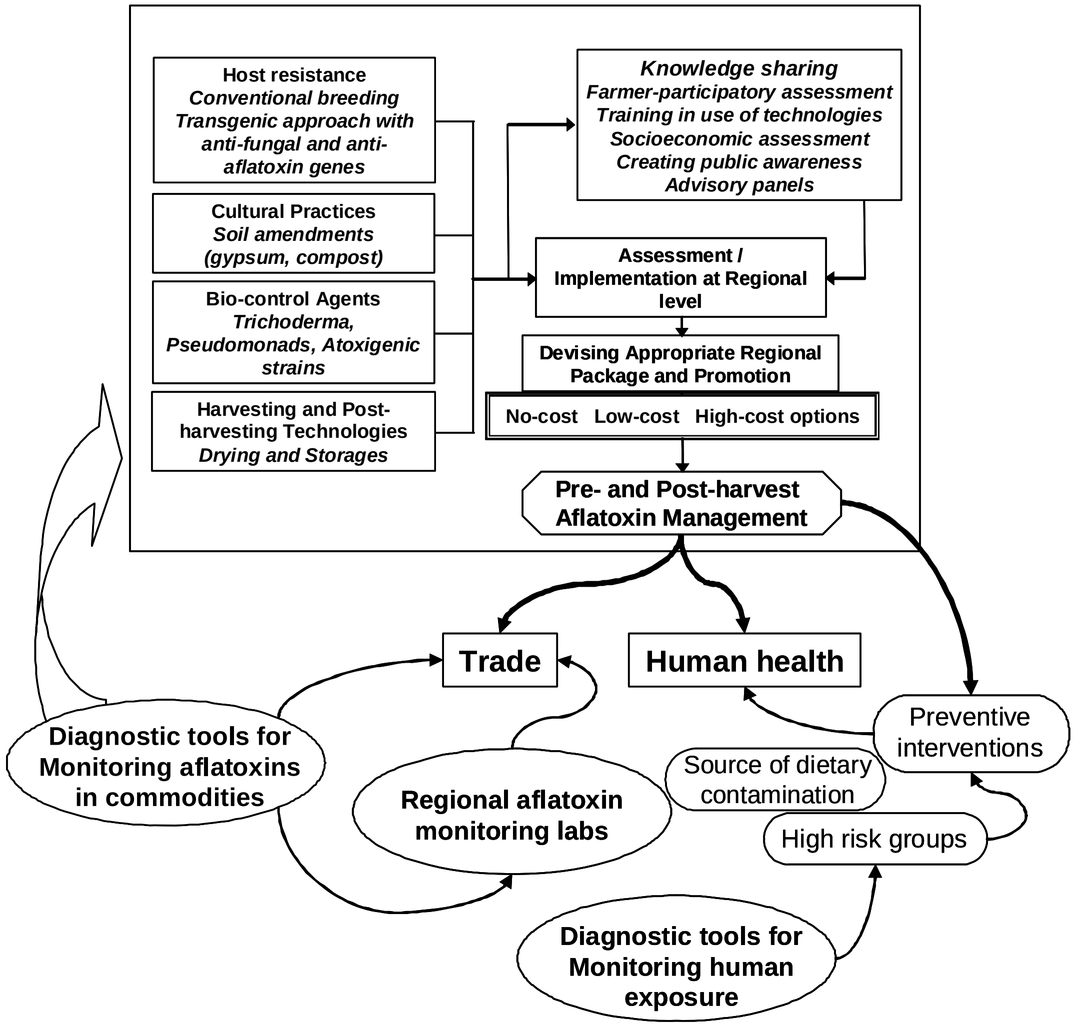


Figure 2: Pictorial representation of ICRISAT's integrated approach for aflatoxin management in groundnut for enhancing trade and health.



Annexure 1: Partners and Collaborators

ICRISAT Centers in SSA:

- ICRISAT, Mali (Dr BR Ntare)
- ICRISAT, Malawi (E.S Monyo and Dr Moses Siambi)
- ICRISAT, Nairobi (Dr R.B. Jones)

Other Centers (NARS and IARCs)

- The National Smallholder Farmers' Association of Malawi (NASFAM),
- Institut d'Economie Rurale (IER), Mali
- National Research Center on Groundnut, India
- Acharya NG Ranga Agriculture University, India
- Indian Council of Agriculture, India
- Scottish Crop Research Institute, UK
- University of Reading, UK
- University of Wisconsin, Madison, USA
- Institute for Agriculture Research, Nigeria
- Institut sénégalais de recherches agricoles (isra), Sengal
- Institut National de la Recherche Agronomique du, Niger

Annexure 2: Literature Cited

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Annexure 3: Participatory Knowledge Sharing Activities since 2002 onwards

TRAINING COURSES TO NARES

Training courses (6 in Asia and 2 in Africa) were conducted on "*Aspergillus flavus* seed infection and aflatoxin estimation by ELISA and aflatoxin management options in groundnut". Training courses schedule was as follow.

- 10-20 Jan 2001: Held at ICRISAT-Patancheru center, 7 participants from India and Thailand 26 Feb. – 8 March 2001: Held at ICRISAT-Bamako center, Mali, 11 participants from 11 West African countries
- 24-28 March 2003: Held at ICRISAT-Patancheru center, 9 participants (2 from Kenya, 1 Indonesia, 6 India)
- 20-25 Sept 2004: Held at ICRISAT-Lilongwe center, Malawi, 22 participants from Malawi Kenya
- 4-15 April 2005: Held at ICRISAT-Patancheru center, 6 participants from 3 east African countries
- 16-27 May 2005: Held at ICRISAT-Patancheru center, 15 Scientists/technicians from University/ICAR, India
- 13-17 June 2005: Held at ICRISAT-Patancheru center, 8 Scientists from University/ICAR, India.
- 28 Aug.-15 Sept. 2006: Held at ICRISAT-Patancheru center, 4 participants (3 from Iran and 1 France)

FARMER FIELD DAYS

- 3-10 Nov. 2003-05: Field days organized in West Narsapuram in Anantapur district, Ontillu in Chittoor district (Pileru area) in which 100-200 farmers were participated. During the periods participatory varietal evaluations was also carried out, in which farmers, NGOs, traders, oil millers, Scientists from ICRISAT, University of Reading, UK, ANGRAU were involved for the selection of high yielding groundnut with low aflatoxin risk
- 8 Nov. 2003: Kissan mela held at Agricultural Research Station, Anantapur, briefed to >500 farmers about aflatoxin in groundnut and it management.
- 28 Oct. 2004: Field day at ICRISAT center, >100 farmers from Anantapur were participated and explained about "Aflatoxins in groundnut, health as well as implications and management"
- 2-5 Jan. 2005: Held at ARS, Anantapur on " Integrated crop and disease management in groundnut for more pod, nutritious haulms and healthy milk" more than 50 farmers participated
- 7 Jan 2005: Held at Ramayampet, Medak district, Andhra Pradesh on "Aflatoxins in maize and its management" and about 100 farmers participated
- 16 Sept. 2005: Held at ICRISAT center on " Sorghum and pearl millet sampling methodology for aflatoxin analysis" and 8 field staff and NGOs were participated
- 28-29 Oct. 2005: Held at RDT conference hall, Anantapur on "Participatory varietal evaluation for selecting high yielding groundnut with low aflatoxin risk" and about 20 farmers were participated
- 22 Feb. 2006: Held at Jagdevpur, Medak district, Andhra Pradesh on "Creating awareness and training farmers in harvest and post-harvest methods to mitigate aflatoxin contamination" and about 60 farmers were participated
- 25 Aug. 2006: Held at Palvai, Mahboobnagar district, Andhra Pradesh on "Mycotoxin management in sorghum and pearl millet" and about 200 farmers were participated

Flyers / Extension bulletins / Audio-visual

- BBC TV Program 2006. BBC World Telecast on "Aflatoxin detection by ELISA and its management in groundnut and maize" the program was covered under earth watch program, telecasted globally in Feb-Mar 06./www.handsontv.info/series7/03_plague_to_plenty_reports/03_plague_to_plenty_mm/dried_and_tested_popup.html
- Doordarshan 2006. Doordarshan India TV. Two Programs on aflatoxin management and aflatoxin affects on human and animal health telecasted in Telugu and English during 2005-2006.

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