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Prevalence of *Aspergillus flavus* Infection and Aflatoxin Contamination of Groundnut in Telangana and Andhra Pradesh

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ABSTRACT

Aflatoxin contamination is a qualitative problem in groundnut (Arachis hypogaea L.) occurring at both pre-and post-harvest stages. These aflatoxins are secondary metabolites produced by Aspergillus flavus and A. parasiticus and have carcinogenic, hepatotoxic, teratogenic and immuno-suppressive effects. To evaluate the prevalence of A. flavus infection and aflatoxin contamination in groundnut oil mills/traders' of Telangana and Andhra Pradesh (AP) pod samples were collected from eight selected oil mills/traders' in Mahaboobnagar, Rangareddy, Nizamabad, Karimnagar (Telangana); and Anantapur (AP) districts. A total of 24 pod samples were collected (Three samples from the each selected oil mill). Aflatoxin contamination in kernels was estimated by indirect competitive ELISA. In Telangana, kernel infection ranged from 42 (Mahaboobnagar) to 90.7% (Nizamabad). In AP, Tadimarri mandal recorded kernel infection up to 29.3% whereas Tadipatri recorded up to 59.3%. Aflatoxins in kernels from these mills in Telangana were highest in Rangareddy (1205.2 µg kg^{-1}) followed by Karimnagar (365.5 µg kg^{-1}). Oil mills of Nizamabad and Mahaboobnagar have recorded aflatoxins to a tune of 4.9 and 11.5 µg kg^{-1} in Telangana. In AP, aflatoxins in pod samples were 2.8 µg kg^{-1}(Tadipatri) and 6148.4 µg kg^{-1} (Tadimarri).

Key words: *Aflatoxin, Contamination, Groundnut, Infection,*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important grain legume and oilseed crop with huge revenue potential. The crop occupies about 25.4 Mha worldwide with an annual production of 45.2 Mt and a productivity of 1.77 tonnes ha^{-1}[6]. In India, the crop is grown to an extent of 5.25 Mha with a production of 9.47 Mt and productivity of 1.80 tonnes ha^{-1}[6]. Groundnut is a rich source of protein, dietary fiber, minerals, and vitamins[13]. Groundnut production all over the world is hampered by several biotic stresses that result in severe yield reduction[18,21]. Of different soilborne diseases affecting its production, collar rot (*Aspergillus niger*)[1]; stem rot (*Sclerotium rolfsii*)[11]; and bacterial wilt (*Ralstonia solanacearum*)[9] are the devastating ones in all crop growing areas of the world.

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Another important biotic stress in groundnut cultivation is aflatoxin contamination which occurs at both pre-and post-harvest stages of the crop. It is a qualitative problem affecting grain quality and trade[23]. Aflatoxins are a group of 20 secondary metabolites produced by *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare[10,17]. Major aflatoxins are categorized as B_1, B_2, G_1 and G_2 based on their fluorescence under UV light and their relative chromatographic mobility during thin layer chromatography (TLC).

In groundnut, environmental conditions play a major role in the attack of these molds, and the crop is affected at various stages such as pre, post-harvest and during storage[23]. Ascertaining the extent of aflatoxin contamination at groundnut oil mills/traders' level gives an indication on the prevalence of this qualitative problem. Several reports are available on the extent of *A. flavus* infection of pods and aflatoxin contamination at oil mills/traders' level[19]. Earlier studies have ascertained the aflatoxin contamination in groundnut at pre-and post-harvest stages[3]. However, a comprehensive mapping of the risk and sensitive areas with respect to toxin accumulation during storage at oil mills/traders' is not documented. Further, in several of the documented cases, there was no direct correlation between the quantum of *A. flavus* infection and kernel aflatoxin contamination[4].

MATERIAL AND METHODS

The present investigation was carried out with the facilities available at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India.

Selection of oil mills/Traders': Eight oil mills from different locations of Andhra Pradesh (Anantapur district) and Telangana were selected and three pod samples from each oil mill were collected. Among the eight oil mills, five were selected from Telangana comprising of four districts *viz*., Mahaboobnagar, Rangareddy, Nizamabad and Karimnagar. Three mills were selected in Andhra Pradesh from Anantapur district. All together 24 samples were collected from these selected sites. The pod sample size is approximately one kg. The details of the oil mills from where pod samples were collected were given in Table 1 & 2.

Enumeration of *Aspergillus flavus* infection in groundnut kernels

Extent of *A. flavus* infection in groundnut pods collected from oil mills/traders' was determined as follows. Pods were shelled and kernels were surface sterilized before plating them on Czapek dox agar (CDA) fortified with rose bengal. Fifty seeds per sample were plated to ascertain the *A. flavus* infection. For each sample, apparently healthy seeds were plated. The plates were incubated under dark at 28º C for seven days. Observations on number of seeds colonized by typical *A. flavus* colonies were counted and per cent seed infection was determined.

Table 1: Details of oil mills/traders' surveyed in Telangana during November 2014 to assess the post-harvest aflatoxin contamination of groundnut induced by *Aspergillus flavus*

Groundnut pod sample	District	Mandal/Village	Source
T-1a	Mahaboobnagar	Mahaboobnagar Town	M/s Sri Laxmi Oil Mill, Shed No. 12, Ind. Mahaboobnagar
T-1b			
T-1c			
T-2a	RangaReddy	Mankhal	Lohiya edible oils Pvt Ltd. Plot No. 4, PH11, IDA, Mankhal, Maheswaram
T-2b			
T-2c			
T-3a	Nizamabad	Khanapur (v) &Nizamabad (M)	Agarwal Agro Industries, Sy. No. 186/AA/1, Kaloor Road, H. No. 7-8-710, Nizamabad-503002
T-3b			
T-3c			
T-4a	Karimnagar	Jammikunta (V) &Jammikunta (M)	Sandhya Traders,H.No. 4-1-23, Market road,Jammikunta
T-4b			
T-4c			
T-5a	Karimnagar	Jammikunta (V) &Jammikunta (M)	Bachu Veera Mallaiah & Sons Oil Mill, H.No. 4-4-60, Market road, Jammikunta
T-5b			
T-5c			

Table 2: Details of oil mills/traders' surveyed in Anantapur district of Andhra Pradesh during November 2014 to assess the post-harvest aflatoxin contamination of groundnut induced by *Aspergillus flavus*

Groundnut pod sample	Mandal/Village	Source
A-6a	Terannapalli (V) &Tadipatri (M)	M/S. Sri Buggaramalingeshwara Oil Mills, Sy. No. 184-3
A-6b		
A-6c		
A-7a	Terannapalli (V) &Tadipatri (M)	Sri Sai Ram Oil Mills, S. No. 233/1 & 239/A
A-7b		
A-7c		
A-8a	Tadimarri (V) &Tadimarri (M)	M/S New Sreenivasa Baby decorticators 595/1, Main Road, Tadimarri, Narayan Reddy Palli-515631
A-8b		
A-8c		

Aflatoxin estimation:

Aflatoxin contamination in the pod samples were estimated by following standard protocols of indirect competitive enzyme linked immunosorbent assay (indirect competitive ELISA)[15].

The data pertaining to the *A. flavus* infection and aflatoxin contamination were analyzed using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) and the treatment means were differentiated by a least significant difference (LSD) at P=0.05 using PROC- GLM. Data on extent of kernel infection by *A. flavus* were square root transformed, whereas, the data on aflatoxin contamination were log transformed.

Mapping of risk and sensitive areas with respect to kernel infection by *A. flavus* and aflatoxin contamination

The mean kernel infection by *A. flavus* and aflatoxin levels in the pod samples collected at oil mills/traders' in Telangana and Andhra Pradesh were mapped using Geographical Information System (GIS) at ICRISAT, Patancheru, India. Accordingly, the surveyed areas were categorized as follows.

Areas with kernel aflatoxins (µg kg^{-1}): ≤30=safe zone; and >30=risk zone

Kernel infection by *A. flavus* (%): ≤30= safe zone; and >30= risk zone

RESULTS

Kernel infection in surveyed oil mills

Of different oil mills surveyed in Telangana and Andhra Pradesh, there was a significant difference with respect to kernel infection by *A. flavus* (P<0.0001). District-wise, the kernel infection in groundnut was highest (96%) in samples (A-7) collected from Tadipatri mandal of Anantapur district (Table 3) (Fig 1). This was followed by kernel infections in samples collected from Nizamabad (T-3) (90.7%) and Rangareddy (T-2) (90%) of Telangana. No significant differences in kernel infection were found among these samples. For the remaining samples, the kernel infections ranged from 22.7 to 54.0%. The kernel infections in Karimnagar (T-4 & T-5) and Mahaboobnagar (T-1) were significantly inferior with samples from Nizamabad (T-3) and one sample from Anantapur district (A-7). The other two samples from Anantapur district, A-6 (Tadipatri) and A-8 (Tadimarri) have recorded least *A. flavus* infection levels of 22.7% and 29.3% respectively (Table 3).

Kernel infection in Telangana

District-wise, kernel infection ranged from 42 to 90.7% in samples collected from oil mills of Telangana. District-wise, mean kernel infection by *A. flavus* was highest in Nizamabad (90.7%). This was followed by samples from Rangareddy district that recorded 90% kernel infection. No significant differences were noticed between the kernel infections for samples from Nizamabad and Rangareddy (Fig 2). Further, the kernel infection in samples from Nizamabad and Rangareddy were significantly superior over that of Mahaboobnagar (42%) and Karimnagar (54%). Further, no significant differences were noticed between samples of Mahaboobnagar

and Karimnagar with respect to kernel infection. Overall, our results indicated higher *A. flavus* infections at oil mills of Nizamabad and Rangareddy (Table 3).

Kernel infection in Andhra Pradesh

In Andhra Pradesh, in the surveyed mandals of Anantapur district, there were marked differences in the kernel infections (Fig 3). In the two mandals surveyed, Tadimarri recorded a mean kernel infection of 29.3%. However, in the other mandal, Tadipatri, kernel infections were up to 59.3%. Though, the mean kernel infection in Tadipatri was 59.3%, individually, the two locations in this mandal have shown *A. flavus* infection levels as 22.7% (A-6) and 96% (A-7) recorded kernel infections (Table 3).

Table 3: Extent of kernel infection by *Aspergillus flavus* at selected groundnut oil mills/traders' level in surveyed districts of Telangana and Andhra Pradesh, India during 2014

State	District	Kernel sample Identity	Kernel infection by *A. flavus* (%)
Telangana	Mahaboobnagar	T-1	42(6.38)^{bc}
	Rangareddy	T-2	90(9.49)^{a}
	Nizamabad	T-3	90.7 (9.52)^{a}
	Karimnagar	T-4	54(7.30)^{b}
	Karimnagar	T-5	54(7.35)^{b}
Andhra Pradesh	Anantapur(Tadipatri)	A-6	22.7(4.75)^{d}
	Anantapur(Tadipatri)	A-7	96(9.80)^{a}
	Anantapur (Tadimarri)	A-8	29.33(5.38)^{cd}
LSD (0.05%)			15.3(1.18)
CV			26.02 (9.15)

Values are means of three replications

Means with the same letter are not significantly different

Square root transformed values are parenthesized

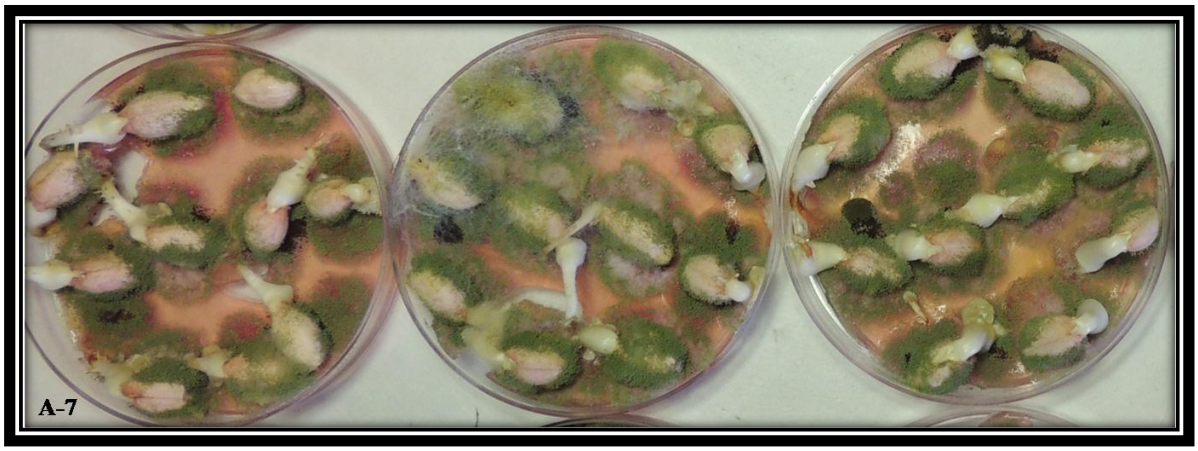

Fig. 1: Highest kernel *A. flavus* infection of groundnut in the samples (A-7) collected from Tadipatri mandal of Anantapur district

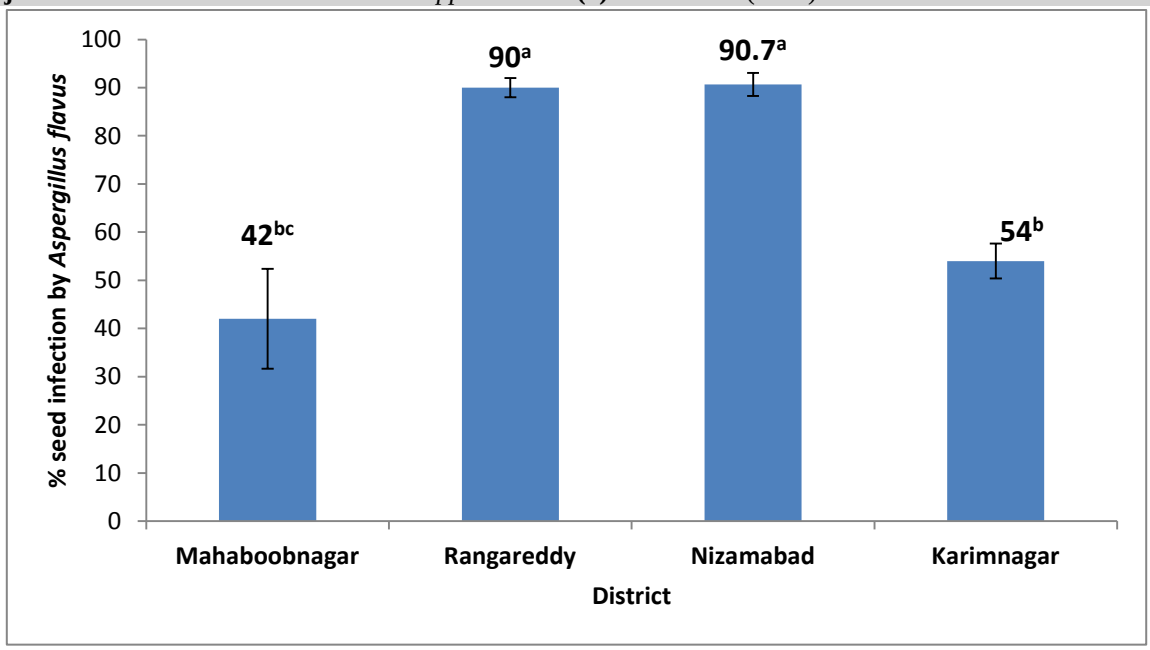

Fig. 2: Extent of groundnut kernel infection by *Aspergillus flavus* at oil mills/traders' in surveyed districts of Telangana during 2014

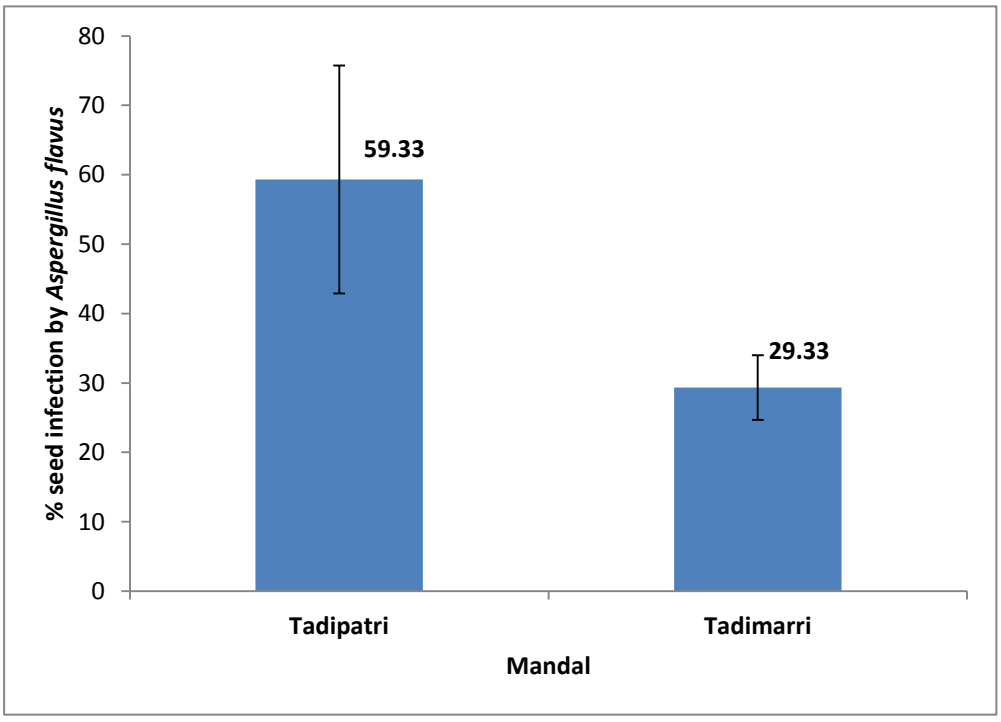

Fig. 3: Extent of groundnut pod infection by *Aspergillus flavus* at oil mills/traders' in surveyed mandals of Anantapur district of Andhra Pradesh during 2014

Aflatoxin contamination in the surveyed oil mills

Of different oil mills surveyed in Telangana and Andhra Pradesh, there was a significant difference with respect to kernel aflatoxin content ($P<0.0035$). District-wise, aflatoxin contamination was highest (6148.4 µg kg^{-1}) in kernels collected from Anantapur (Tadimarri) (A-8), followed by T-2 sample from Rangareddy (1205.5 µg kg^{-1}) and T-5 sample from Karimnagar (724.03 µg kg^{-1}) (Table 4). Overall, the aflatoxin levels in surveyed oil

mills in Telangana and Andhra Pradesh ranged from 2.16 µg kg^{-1} to 6148.4 µg kg^{-1}. The pod samples from Tadipatri of Anantapur had recorded low aflatoxin levels (up to 2.2 µg kg^{-1}). Aflatoxin levels were also less in pod samples collected from Nizamabad (4.9 µg kg^{-1}), T-4 sample of Karimnagar (7.06 µg kg^{-1}) and Mahaboobnagar (11.5 µg kg^{-1}).

Aflatoxin contamination in Telangana

District-wise in Telangana, pod samples from oil mills of Rangareddy recorded highest mean aflatoxin levels (1205.2 µg kg^{-1}) (Fig 4). This was followed by oil mills from Karimnagar with mean kernel aflatoxin levels up to 365.55 µg kg^{-1}. Samples from the oil mills of Nizamabad and Mahaboobnagar have recorded aflatoxins to a tune of 4.9 and 11.5 µg kg^{-1}.

Aflatoxin contamination in Andhra Pradesh

In the surveyed mandals of Anantapur district of Andhra Pradesh, oil mills of Tadipatri have recorded mean aflatoxin levels of 2.8 µg kg^{-1}. However, pod samples from Tadimarri mandal have shown highest aflatoxin levels (6148.4 µg kg^{-1}) (Fig 5).

Table 4: Extent of aflatoxin contamination by *Aspergillus flavus* at selected groundnut oil mills/traders' level in surveyed districts of Telangana and Andhra Pradesh, India during 2014

State	District	Kernel sample Identity	Aflatoxin content (µg kg^{-1})
Telangana	Mahaboobnagar	T-1	11.5 (0.97)^{bc}
	Rangareddy	T-2	1205.5 (2.84)^{ab}
	Nizamabad	T-3	4.9 (0.50)^{c}
	Karimnagar	T-4	7.06 (0.80)^{b}
	Karimnagar	T-5	724.03 (2.04)^{ab}
Andhra Pradesh	Anantapur(Tadipatri)	A-6	2.2 (0.27)^{c}
	Anantapur(Tadipatri)	A-7	2.16 (-0.12)^{c}
	Anantapur (Tadimarri)	A-8	6148.4 (2.95)^{a}
LSD (0.05%)			(1.57)

Values are means of three replications

Means with the same letter are not significantly different

Log transformed values are parenthesized

Aflatoxins were estimated by indirect competitive ELISA

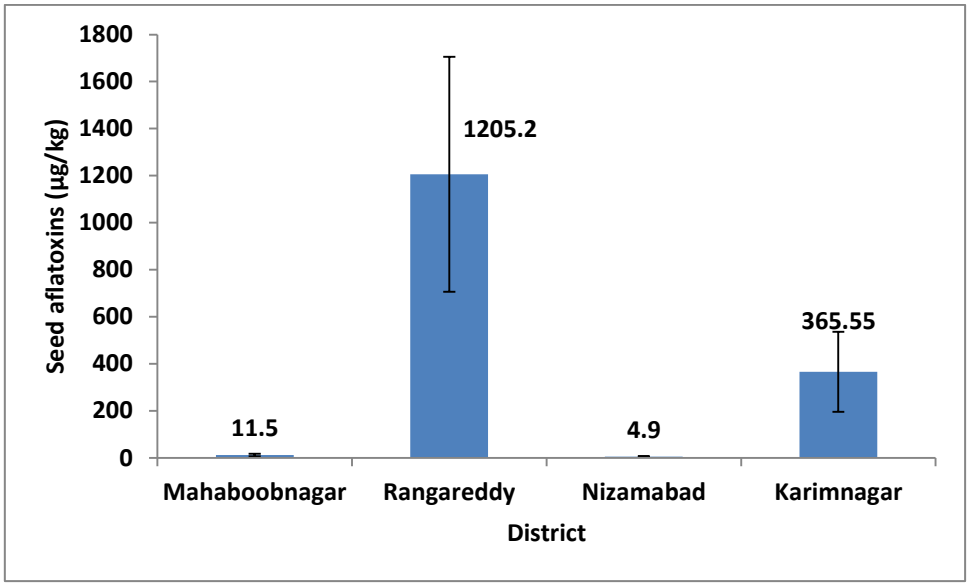

Fig. 4: Extent of groundnut aflatoxin contamination in pods by *Aspergillus flavus* at oil mills/traders in surveyed districts of Telangana during 2014

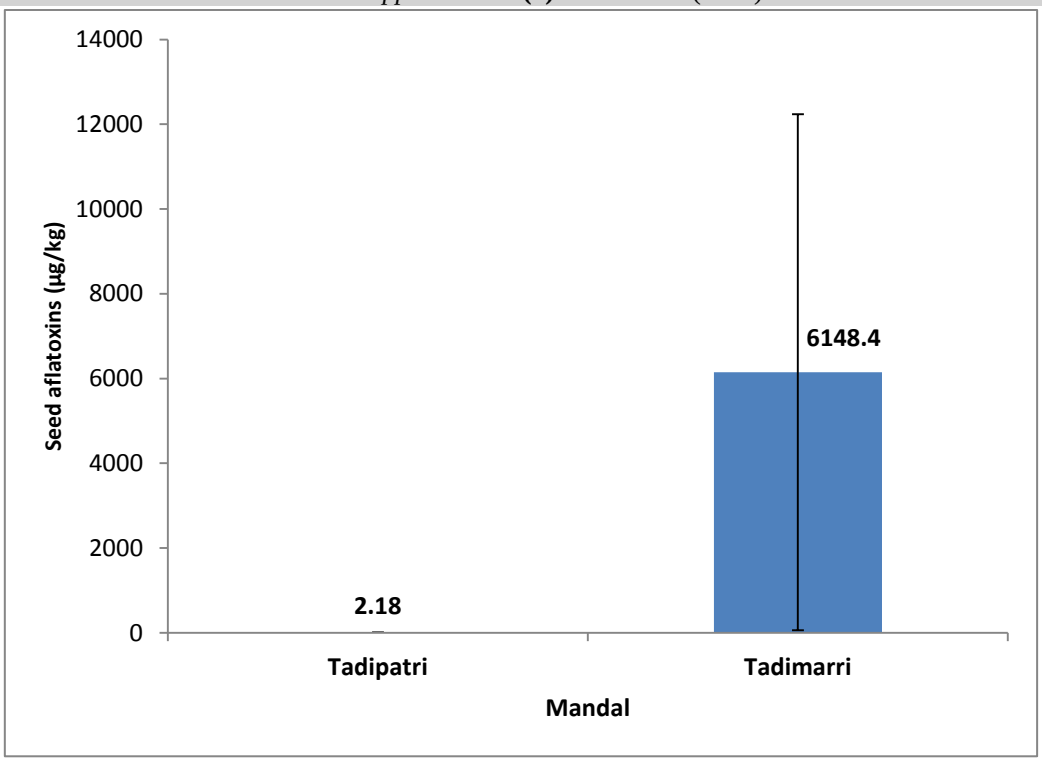

Fig. 5: Extent of groundnut aflatoxin contamination in pods by *Aspergillus flavus* at oil mills/traders in surveyed mandals of Anantapur district in Andhra Pradesh during 2014

Mapping of risk and sensitive areas with respect to kernel infection and aflatoxin contamination of groundnut by *Aspergillus flavus*

Based on the results obtained in the kernel infection and aflatoxin contamination in pods at selected groundnut mills of Andhra Pradesh and Telangana, GIS mapping was carried out to depict the risk and sensitive areas.

Risk and sensitive areas in Telangana

In Telangana, all the surveyed oil mills were categorized under risk zone for kernel infection (%) by *A. flavus*. Based on the aflatoxin contamination in the pod samples, oil mills sampled in Mahaboobnagar and Nizamabad districts were categorized as safe zone. The other oil mills that fall in Rangareddy and Karimnagar districts were categorized as sensitive areas (Fig 6).

Risk and sensitive areas in Andhra Pradesh

In Anantapur district of Andhra Pradesh, based on kernel infection by *A. flavus*, Tadipatri mandal was categorized as risk zone. However, based on aflatoxin levels in pod samples, oil mills from Tadipatri mandal was categorized as safe zone (Fig 9). In Tadimarri mandal, oil mills were categorized as safe zone based on *A. flavus* infection and as risk zone based on aflatoxin contamination (Fig 7).

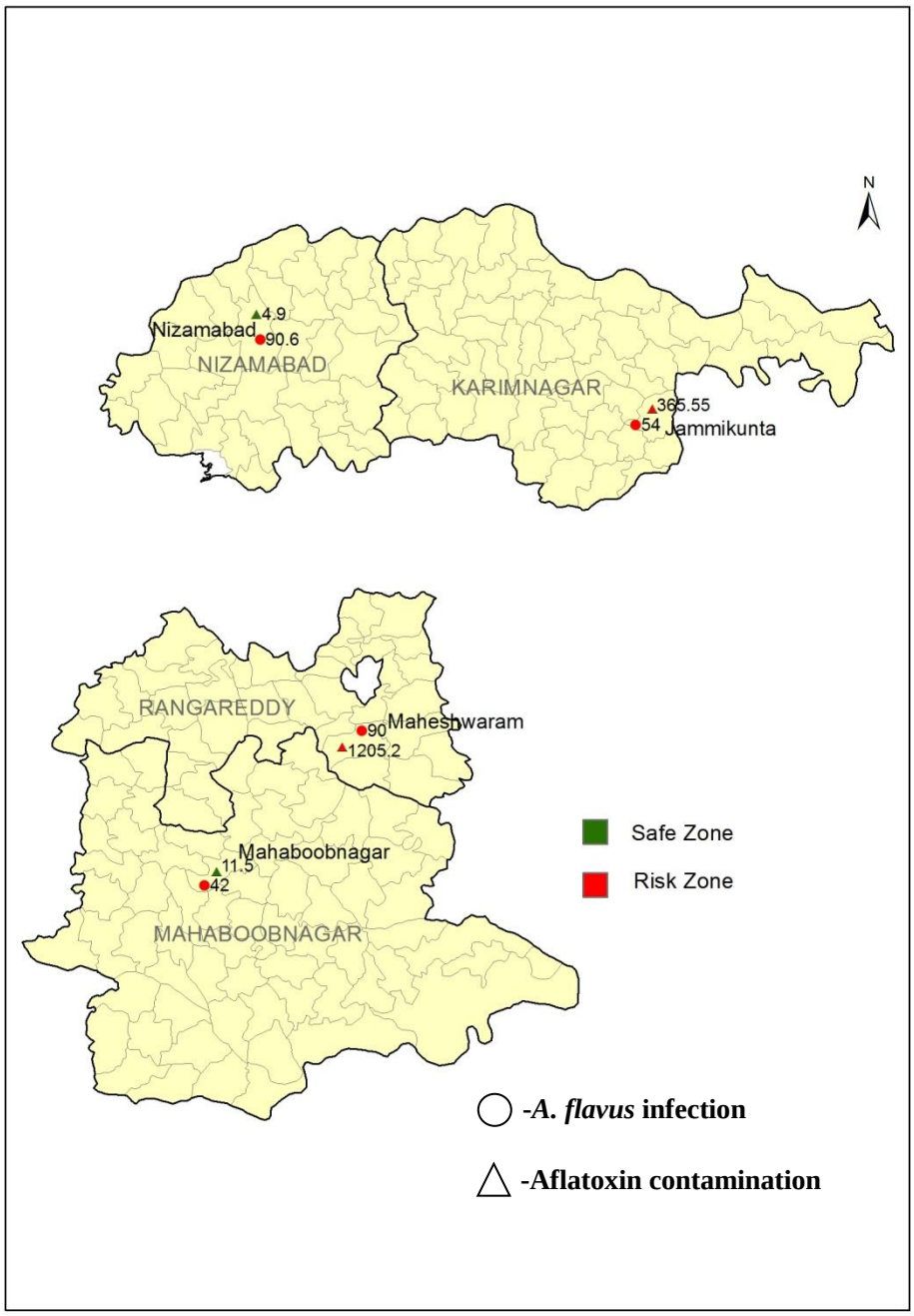

Fig. 6: Risk and sensitive areas with respect to groundnut kernel *Aspergillus flavus* infection and aflatoxin contamination at oil mills/traders' level in surveyed districts of Telangana, India (surveyed during rainy season/Kharif 2014)

Fig. 7: Risk and sensitive areas with respect to groundnut kernel *Aspergillus flavus* infection and aflatoxin contamination at oil mills/traders' level in surveyed mandals of Anantapur district of Andhra Pradesh, India (surveyed during rainy season/Kharif 2014)

DISCUSSION

Our studies indicated that both Telangana and Andhra Pradesh have significant levels of *A. flavus* infection and aflatoxin contamination in kernels at oil mills. Post-harvest contamination of groundnut pods during storage at traders'/markets/oil mills in the present study is attributed to either the improper storage conditions or the carry over inoculum of *A. flavus* from field to farmers' storage and ultimately to markets/oil mills. Improper storage practices are the major factors for aflatoxin build up during storage. Pod storage at high moisture levels increases post-harvest molding and aflatoxin contamination[7]. Besides, storing the pods at optimum moisture levels, pod drying by farmers immediately after harvest also helps in reducing the *A. flavus* infection and aflatoxin contamination during storage. Inverted windrowing is an ideal drying procedure of pods after harvest. It helps in proper drying of pods with adequate

exposure to sunlight. Dick[5] (1987) reported that ≥7% moisture levels in grains are ideal for preventing mold growth including aflatoxigenic strains. Proper drying practices such as windrowing and immediate stripping of pods is recommended for minimizing aflatoxin contamination during storage below acceptable limits[16]. Based on our results, we can assume that groundnut pods/Kernels from oil mills of Telangana and Andhra Pradesh are at increased risk to aflatoxin contamination.

In our present study, there seemed to be no correlation between kernel *A. flavus* infection and aflatoxin contamination for the pods sampled from oil mills. Our studies reported that safe zones with respect to *A. flavus* infection had higher levels of aflatoxin contamination. For example, pod samples from oil mills of Nizamabad (T-3) district of Telangana had higher *A. flavus* infection (90.7%), whereas the aflatoxin content was at significantly lower levels (4.9 µg kg^{-1}). Similarly, pod samples from oil mills in Mahaboobnagar (Telangana) (T-1) also had higher kernel *A. flavus* infection (42%) and low aflatoxin levels (11.5 µg kg^{-1}). In Tadipatri mandal, (Anantapur district) of Andhra Pradesh also, the pod samples from oil mills had mean higher *A. flavus* infections (59.3%) and mean low aflatoxin levels (2.18 µg kg^{-1}) (Fig 4 & 7). Higher *A. flavus* infections and low aflatoxin levels in these samples is attributed to either the co-existence toxigenic and atoxigenic *A. flavus* strains. Another reason could be the storage practices at these oil mills could be relatively safer that prevent aflatoxin production by the contaminated toxigenic molds.

In our present study, pod samples from from oil mills of Tadimarri (A-8) of Anantapur district in Andhra Pradesh had higher aflatoxin levels (6148.4 µg kg^{-1}), but with lower *A. flavus* infection (29.3%).Higher toxin levels with low mold infections in Tadimarri samples is attributed to the fact that the infection of pods by potent toxigenic strains. Another important reason could be that pre-harvest aflatoxin contamination at farmers' fields in Tadimarri mandal could be at higher levels. However, proper drying practices in these areas by the farmers before selling them to traders/oil mills might have prevented the mold growth at oil mills during storage. In our studies, the pod samples from oil mills/traders of Rangareddy and Karimnagar districts had higher aflatoxin levels and *A. flavus* infections. Thus there is a potential threat to human and animal health in areas with more aflatoxin contamination and *A. flavus* infections.

Aflatoxin contamination in groundnut and peanut butter is a major post-harvest problem in Kenya. Earlier studies on the prevalence and potential of aflatoxin contamination in Nairobi and Nyanza provinces of Kenya indicated that sources of groundnut and presence of defective nuts were the major factors influencing increased aflatoxin contamination in these food commodities. Further, the predominant fungal species in the groundnut samples collected from farmers and traders in these areas were *A. flavus* (L and S strains), *A. parasiticus*, *A. niger*, *A. tamari*, *A. alliaceus*, *A. caeletus* and *Penicillium* spp[12]. In another study at Eldoret and Kericho towns of Kenya, it was observed that the levels of total aflatoxins were higher in groundnut samples from informal (97.1 µg kg^{-1}) than formal (55.5 µg kg^{-1}) market outlets[14].

Studies in Andhra Pradesh, India have reported alarming levels of post-harvest aflatoxins in Anantapur district. Aflatoxin levels in insect damaged samples collected from farmers of Andhra Pradesh were above 500 µg kg^{-1}[24]. Significant measures at both pre-harvest and post-harvest stages are to be advocated to farmers in these areas to prevent pre-harvest *A. flavus* infection and aflatoxin contamination. Further, importance of ideal grain storage practices are to be explained to farmers as well as traders to curb the aflatoxin problem from reaching alarming levels. Since, groundnut and oil cakes are the major products from oil mills, constant monitoring at these places has to be taken up to prevent the aflatoxins from reaching the food chain. Aflatoxin contamination in oilcake is a major problem[2], and is potentially hazardous to animal health. Further, groundnut oil

(unrefined) from aflatoxin contaminated kernels also has significant levels of aflatoxins[8].

Good agricultural practices at both pre-and post-harvest stages of groundnut including storage are available to prevent aflatoxin build up in groundnut. Ascertaining the actual reasons for post-harvest build up of aflatoxins will therefore help in identifying areas of intervention to overcome the problem. Based on this, different management tools for reducing this aflatoxin contamination such as proper post-harvest grain handling, role of post-harvest machinery, physical separation of grains, proper storage methods and conditions, disinfestation, detoxification, inactivation, filtration, use of binding agents and antifungal compounds can be advocated situation-wise to overcome this problem[22]. Adequate drying of pods to safe moisture levels and proper storing in containers of jute bags is therefore essential for stored groundnut that prevent critical increases in fungal populations and thereby aflatoxin contamination[20].

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