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Dynamics of soil population of *Aspergillus flavus* and aflatoxin contamination in groundnut based production system in Gujarat

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ABSTRACT: Studies were conducted at NRCG, Junagadh during 2005 and 2006 to determine soil population of *Aspergillus* spp. with special reference to *A. flavus* in major groundnut growing districts of Gujarat and the level of aflatoxin contamination in groundnut kernels collected from different locations. The population of *A. flavus* was low in summer crop (< 4.0 x 10^3 c.f.u./g soil) than in rainy season crop (10-44 x 10^3 c.f.u./g soil) and the population increased towards pod development stage. Bhuj and Bhavnagar districts showed lowest population in both the crop seasons. Among the species of *Aspergillus*, *A. flavus* was dominant in Junagadh and Amreli whereas *A. terreus* was dominant in Bhuj and Bhavnagar during summer however, during rainy season *A. flavus* was dominated in most of the districts. In majority of the samples, positive correlation was found with the population of *A. flavus* and level of aflatoxin contamination. Cropping system also influenced the level of population.

Key words: Groundnut, *Aspergillus flavus*, population dynamics, aflatoxins, cropping system

Aspergillus flavus Link ex Fries is a soil-inhabiting fungus that frequently produces secondary metabolites called aflatoxins in several commodities under certain conditions and groundnut is one of the high-risk commodities. Aflatoxins are carcinogenic and if present in food commodities, reduce their quality and hence the market value. Occurrence of aflatoxins has become the major impediment in the export of groundnut**.** Aflatoxin is primarily produced by some strains of *A. flavus* and *A. parasiticus* (Raper and Fennell, 1965) however, it is also produced by some other fungi *viz*., *A. ruber, Penicillium citrinum, P. puberalum* (Hodges *et al*., 1964), *A. terreus* (El-Fouly *et al,* 1989), *A. glaucus*, *A. fumigatus* (Tildan *et al*., 1968), *A. ochraceus* and *A. nomius* (Kurtzman *et al*., 1987). The most significant contamination usually occurs prior to harvest during the periods of late season drought stress. The entry of *A. flavus* to the developing groundnut pods is directly from the soil. Soil population of toxigenic isolates of *A. flavus* is one of the risk factors in pre-harvest aflatoxin contamination of groundnut. The distribution of *Aspergillus* is influenced significantly by agro-ecological locations and cropping system. The present study was conducted at NRCG, Junagadh to understand the prevalence and variability of soil population of aspergilli specifically *A. flavus* across geographic locations in major groundnut growing areas of Gujarat, which may be helpful in developing location specific strategy for aflatoxin management.

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MATERIAL AND METHODS

Soil samples were collected from different locations across 27 *taluka* of ten districts *viz.* Amreli, Anand, Bhavnagar, Bhuj, Jamnagar, Junagadh, Porbandar, Rajkot, S.K. Nagar and Surendranagar of Gujarat State during summer and rainy seasons of 2005 and 2006. The crop was grown under irrigated condition during summer and under rainfed condition during rainy season. Soil samples were collected from groundnut fields shortly after sowing and two weeks before harvesting from the same soil sites. At each sampling, soil samples were collected from five randomly selected

spots at 0-10 cm depth from the region between the plants; individual samples were pooled for each plot. The fungus was isolated using A. flavus and parasiticus agar (AFPA) medium by dilution plating (Horn and Dorner, 1998) and the cultures were purified using single spore isolation technique. A total of 262 isolates of *Aspergillus* spp. mostly *A. flavus* (71 %) were isolated, purified and maintained as single spore culture on agar slants. The morphological and growth characteristics of all the isolates were studied on solid medium (Czapek's Dox Agar medium). At harvest, pods were collected from three randomly selected spots in each plot. Seed infection and colonization by *A. flavus* was recorded after seed plating onto moistened filter paper in sterilized 9 cm diameter Petri dishes and incubated at 28±1 °C for 7 days. The level of aflatoxin contamination in kernel was analyzed through indirect competitive ELISA (Ajitkumar *et al.,* 2004; Heather and Michael, 2006). To know the dynamics of population, 20 samples from each district from randomly selected farmers' field were collected at each stage both during summer and rainy from the same plot and the crop history, soil type and cultural practices were noted. Temporal and spatial analysis of variability in population of *A. flavus* and correlation of level of aflatoxin with soil population were done.

RESULTS AND DISCUSSION

A total of 185 soil samples and 74 pod samples were collected during summer season. The soil population of *A. flavus* in samples just after sowing and two weeks before harvest varied from 0 to 10 x 10^3 and 1.0 to 14.3 x 10^3 c.f.u./g soil. In the majority of the samples the population was below 4.0 x 10^3 c.f.u./g soil. The districts that recorded low soil population (< 4 x 10^3 c.f.u./g soil) during summer were Bhuj and Bhavnagar. A total of 306 soil samples and 160 pod samples were collected from farmers' fields during rainy season from the same fields from which the samples were collected during summer. The population of *A. flavus* in samples taken just after sowing and two week before harvest varied between 0.00-29.33 x 10^3 and 3.00-44.00 x 10^3 c.f.u./g soil, respectively (Table 1). The districts that recorded low soil population (below 10 x 10^3 c.f.u./g soil) during rainy season were Junagadh, Bhavnagar, and Bhuj. The soil population of *A. flavus* was higher in the samples collected during rainy season from the same plots as compared to the summer. The population increased towards pod development stage of the crop which is evident by higher soil population in samples that were taken two weeks before harvest of the crop during both seasons.

Table 1. Soil population of *Aspergillus flavus* in major groundnut growing areas of Gujarat during 2005-06

District	Population of *A. flavus* (x 10^3 c.f.u./g)*			
	Summer season		Rainy season	
	After sowing	Before harvesting	After sowing	Before harvesting
Bhavnagar	0.67-5.00 (17)	1.00-7.33 (25)	0.00-17.67 (42)	10.25-21.50 (65)
Bhuj	0.33-5.00 (19)	1.00-8.00 (30)	0.33-9.33 (63)	3.00-14.75 (100)
Amreli	0.67-10.00 (4)	2.00-14.30 (5)	4.33-12.33 (6)	22.75-36.25 (0)
Junagadh	0.00-7.00 (26)	1.00-9.89 (30)	0.67-16.67 (33)	9.75-23.00 (45)
Jamnagar	-	-	0.67-21.67 (21)	11.25-31.00 (30)
Rajkot	-	-	2.00-14.00 (4)	18.75-34.75 (0)
Surendranagar	-	-	4.00-29.33 (34)	9.25-44.00 (40)

(-) = Samples were not taken for observation as in these districts summer crop is not grown
Figures in parenthesis are percentage of samples sowing £ 2 x 10^3 c.f.u./g soil
*average of two years

During rainy season, the majority of the samples collected from Bhuj, Bhavnagar and Junagadh recorded lowest population both after sowing and before harvest as compared to Amreli, Jamnagar, Rajkot and Surendranagar. The population of *A. flavus* two week before harvest *i.e.* the pod development stage is very critical for infection and aflatoxin contamination of pods. In Amreli, though the population was low shortly after sowing (<10 x 10^3 c.f.u./g in 77% samples) but it increased to ³ 22 x10^3 c.f.u./g soil before harvest. The possible reasons for low soil population in Bhuj and Bhavnagar may be attributed to soil and water salinity and intercropping with pearl millet and sorghum. El-Mougith (1993) observed reduction in fungal growth as the NaCl concentrations increased above 5%. Increased salinity of the culture medium inhibited the production of aflatoxins by *A. flavus* (Singh *et al.*, 1987). The soil types in various districts may have differential influence on the population of *A. flavus.* In Bhuj and Bhavnagar the soil type is sandy to sandy loam whereas in other districts, majority of soil is black calcareous. Ahmad and Singh (1994) also found that soil types differed in their capability for harbouring *A. flavus* and sandy soils of reduced organic carbon were less favourable than loamy soils. Extensive use of biocontrol agent, *Trichoderma* in Bhuj and Junagadh is also attributed for low soil population of *A. flavus* in these districts.

Among the different species of *Aspergillus*, *A. flavus* was dominant in Junagadh (39.3%) and Amreli (37.8%) districts whereas, the other species like *A. terreus* was dominant in Bhuj, Bhavnagar and Anand (Table 2) during summer. During rainy season, however in most of the districts *A. flavus* dominated over other species. Over the seasons it was evident that during rainy season *A. flavus* population increased and become the dominant species leading to higher aflatoxin contamination of groundnuts.

A direct relationship between soil population and the extent of seed infection and aflatoxin contamination at pre-harvest stage were observed except in some cases where high population at pod development stages was not associated with high aflatoxin contamination. Since, not only the population of *A. flavus* but also the proportion of toxigenic and non-toxigenic isolates influences the level of contamination. Among the summer crop, maximum samples were free from infection (>70%) and colonization (>80%). The ranges of infection, colonization and aflatoxin content in samples were 0-10%, 0-6% and 0.00-99.36 ppb (µg kg^{-1}), respectively. Though, among the four major districts, *Aspergillus* propagules were highest in Amreli during summer the level of contamination was not the highest. This may be due to presence of higher proportion of non-toxigenic isolates (Table 3).

During rainy season, 45-90 % samples were free from infection and 50-90% samples free from colonization. The ranges of infection, colonization

Table 2. Distribution pattern of *Aspergillus* and *Penicillium* spp. in the soil of different districts of Gujarat during 2005-06

District	Percent distribution*									
	A. flavus		*A. ochraceus*		*A. terreus*		*A. nidulans*		*Penicillium* sp.	
	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy
Junagadh	39.29	51.35	5.95	2.70	23.81	10.81	10.71	21.62	20.24	13.51
Amreli	37.78	48.28	11.11	6.90	17.78	13.79	2.22	17.24	31.11	13.79
Bhuj	22.86	41.38	2.86	0.00	48.57	0.00	8.57	44.83	17.14	13.79
Bhavnagar	13.79	68.97	13.79	0.00	24.14	10.34	6.90	6.90	41.38	17.24
Anand	18.18	-	9.09	-	54.55	-	9.09	-	9.09	-
Rajkot	-	20.83	-	0.00	-	16.67	-	25.00	-	37.50
Jamnagar	-	21.05	-	0.00	-	21.05	-	36.84	-	21.05
Surendranagar	-	56.41	-	0.00	-	2.56	-	23.08	-	17.95
Porbandar	-	52.94	-	0.00	-	5.88	-	11.76	-	29.41

* Average of two years

(-) = Samples were not taken for observation

Table 3. Frequency of kernel infection and colonization by *A. flavus* and the level of aflatoxin contamination in Gujarat during 2005-06

District	Infection (%)		Colonization (%)		Aflatoxin B_1 (ppb)	
	Summer	Rainy	Summer	Rainy	Summer	Rainy
Bhavnagar	0.00-2.00 (92)*	0.00-13.33 (70)*	0.00-2.00 (92)*	0.00-6.00 (85)*	0.00-0.68 (81.25)**	10.40-82.89 (40)**
Bhuj	0.00-4.50 (90)	0.00-2.22 (90)	0.00-2.00 (95)	0.00-2.22 (90)	0.00-19.66 (88.88)	2.21-17.69 (100)
Amreli	0.00-10.00 (70)	0.00-16.22 (70)	0.00-6.00 (80)	0.00-10.00 (80)	0.00-4.78 (60)	4.43-52.68 (10)
Junagadh	0.00-8.00 (85)	0.00-11.11 (80)	0.00-3.33 (90)	0.00-9.33 (80)	0.00-99.36 (81.25)	18.83-93.70 (5)
Jamnagar	-	0.00-21.44 (45)	-	0.00-13.33 (60)	-	10.65-278.03 (10)
Rajkot	-	0.00-29.00 (45)	-	0.00-26.00 (50)	-	0.97- 421.12 (30)
Surendranagar	-	0.00-33.00 (65)	-	0.00-21.00 (75)	-	2.59-229.49 (25)

* Data in parenthesis are percentage of samples sowing zero infection/colonization

** Percent sample having aflatoxin B_1 zero ppb (µg kg^{-1})

and aflatoxin content in samples were 0-33%, 0-26% and 0.97-421.12 ppb (µg kg^{-1}), respectively (Table 3). Lowest soil population and aflatoxin content in samples were in Bhuj followed by Bhavnagar and Junagadh districts. The samples from Amreli had higher population level but the infection, colonization and aflatoxins in sample were not in proportionate due to presence of non-toxigenic isolates, which was confirmed in later studies. Various workers also reported that all strains of species of *Aspergillus* are not toxigenic and among aflatoxigenic strains also, the capability to produce aflatoxins vary across the strains (Davis and Diener 1970; Chourasia and Sinha, 1994).

In addition to other factors, cropping system also influenced the survival of the fungus. It was observed that the soil population was highest in monocroped areas where groundnut was followed by groundnut every year in rainy season (only one crop was taken as rainfed) in Jamnagar, Rajkot, Surendranagar and Amreli districts. In Bhavnagar, the farmers commonly followed groundnut-onion-groundnut rotation. The root exudates of onion may have the adverse effect on the population of *A. flavus* in soil as it was observed by earlier workers (Sharma *et al*., 1979; Bilgrami *et al*., 1992). The onion and garlic extracts proved inhibitory to *A. flavus* under *in vitro* conditions. Intercropping of groundnut with pearl millet and sorghum in Bhuj and Bhavnagar may also be attributed for low soil population of *A. flavus*.

Keeping in view the risk factors of soil population of *A. flavus* and the dynamics and distribution of different species of aspergilli it is concluded that summer groundnuts have less risk of aflatoxins. Also, during rainy season, groundnuts from Bhuj, Bhavnagar and Junagadh districts of Gujarat have less risk of aflatoxins. In these regions groundnut could be targeted for direct consumption with low aflatoxins risk.

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