The catalysates were analysed by t.l.c., g.l.c., n.m.r. and chemical methods (Table I).

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EFFECT OF CROP SEQUENCE ON *ASPERGILLUS FLAVUS* INFESTATION AND AFLATOXIN ACCUMULATION IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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ASPERGILLUS FLAVUS is an ubiquitous fungus known to invade a variety of agricultural and food commodities and produce a group of toxic metabolites known as aflatoxins, which are highly carcinogenic. Only a few strains are toxin producing and groundnut is said to be one of the best substrates for aflatoxin production[14]. This fungus may invade groundnut pods while developing in the soil or after the harvest and subsequent storage[1,7,8].

Crop rotation is said to be one of the most beneficial agronomic practices to control certain diseases and also to regain soil fertility. Pettit and Taber (1968) found that groundnut harvested from lands planted with the same crop during the previous season also was more highly infested with fungi and contained more aflatoxins than groundnut raised on lands planted with rye, oats, melons or potatoes as the previous crop. Joffe and Lisker (1970) also found high fungal infestation to groundnut kernels in fields previously sown with groundnut than non-groundnut soils.

The objective of present investigation is to examine the effect of crop sequence on *A. flavus* and other fungal populations in soil, rhizosphere and geocarposphere, shells and kernels at various stages of crop development and also aflatoxin content at harvesting period.

Field plots (approx. 7×12 m in size) with different crop sequence history were selected near S.V. University Campus, Tirupati (A.P.). The plots had the following crop sequence in *Kharif* and *Rabi* since three years. Plots with vegetable crop (brinjal, chilli, tomato and mesta as mixed crops) in *Kharif* and groundnut in *Rabi*; rice in *Kharif* and groundnut in *Rabi* and groundnut in both seasons. In each case three replicated plots were maintained and cultivated under similar agronomic practices. Fungal populations, pod infestation and aflatoxin accumulation were studied in late *Rabi* season (February–May) of 1972 and 1973. The soils are of red sandy type, low in organic matter content and colloidal content[12].

For the estimation of fungal populations, ten plants were pulled up from different regions of each replicated plot. The plants and also the soil in between the rows (control soil) were brought to the laboratory in polythene bags. The rhizosphere and geocarposphere mycofloras were estimated according to the method of Rao (1962) and Joffe (1969) respectively. Shell and kernel infestation was examined by the method of Mc-Donald (1970). At the time of harvest about 15,000 pods from each replicated plot were collected and graded into (1) "Undamaged pods" (without any kind of damage) and (2) "damaged pods" (rotted, insect-bored, injured, etc.). Aflatoxins were estimated in both undamaged pods and damaged pods by the method of Pons *et al.* (1966) and confirmed by bioassay using *Bacillus megeterium* (NRRL. 1368) and *B. brevis* (NRRL. 1874) following the method of Burmeister and Hesseltine (1966).

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There was a positive rhizosphere and geocarposphere response on fungal populations as reported by the previous workers[4,5,11]. This is mostly due to exudation of roots and pods[13,15] and also due to sloughed-off root and shell tissue at maturing stage. In both years high *A. flavus* population was seen in soil rhizosphere and geocarposphere in plots with groundnut as previous crop (Table I). This may be due to left-over pods and other plant waste like senesced leaves of groundnut in soil on which *A. flavus* may colonize and servive well than on paddy and vegetable waste. High *A. flavus* infestation to shells and kernels and also high aflatoxin accumulation was noticed in both undamaged and damaged pods in plots previously sown with groundnut. In all cases aflatoxin accumulation is

TABLE I

Aspergillus flavus and other fungal populations in soil (S), rhizosphere (R) and geocarposphere (G) of groundnut (Thousands/gram soil)

Year	Previous crops in the field		*A. flavus*				Other fungi			
			20*	45	75	105	20	45	75	105
1972	Vegetables	S	0	2·2± 0·46	4·1± 0·75	5·5± 0·80	5·1± 0·82	9·5± 0·64	8·5± 0·56	15·1± 1·28
		R	1·2± 0·21	0	12·1± 0·82	10·2± 0·68	12·0± 1·96	74·1± 10·42	60·4± 8·96	64·0± 9·52
		G	..	..	4·2± 0·71	10·0± 0·71	..	..	32·5± 6·21	28·2± 5·10
	Rice	S	0	0	2·1± 0·42	2·0± 0·42	3·0± 0·22	12·0± 2·10	4·2± 0·36	10·2± 0·68
		R	0	8·5± 0·61	7·2± 0·58	8·0± 0·75	16·2± 1·42	76·2± 10·10	45·5± 6·10	52·1± 10·0
		G	..	..	5·2± 0·35	6·1± 0·40	..	..	22·3± 4·54	25·5± 4·0
	Groundnut	S	12·1± 0·92	8·9± 0·71	16·0± 1·21	18·0± 1·84	8·0± 0·58	15·2± 0·98	12·0± 1·98	14·2± 2·10
		R	16·1± 2·00	34·0± 2·98	12·0± 1·50	15·0± 1·10	24·8± 3·92	64·5± 8·86	68·0± 8·58	70·1± 9·28
		G	..	..	16·6± 2·80	18·8± 2·22	..	..	45·4± 7·0	40·0± 3·82
1973	Vegetables	S	1·2± 0·1	0	2·1± 0·21	5·5± 0·65	3·1± 0·52	7·8± 0·92	3·0± 0·6	15·2± 2·0
		R	5·1± 0·42	12·1± 1·75	0	15·1± 1·56	15·2± 2·1	78·2± 6·88	52·2± 5·42	60·0± 5·58
		G	..	..	5·1± 0·64	12·1± 1·82	..	..	40·0± 5·00	25·3± 2·92
	Rice	S	5·3± 0·30	0	0	4·0± 0·50	3·2± 0·42	9·2± 1·10	4·2± 0·52	10·2± 1·68
		R	3·0± 0·42	0	5·0± 0·62	10·0± 2·22	10·5± 1·50	69·0± 16·48	35·1± 4·82	53·6± 15·10
		G	..	..	3·2± 0·56	7·0± 2·0	..	..	34·5± 4·0	38·0± 4·0
	Groundnut	S	3·1± 0·50	2·2± 0·32	12·5± 1·92	8·8± 1·66	5·1± 0·66	11·2± 1·0	14·4± 1·82	18·8± 2·0
		R	4·5± 0·62	14·8± 2·5	27·0± 5·0	18·2± 2·92	18·6± 1·98	92·5± 12·52	54·2± 5·42	60·1± 6·42
		G	..	..	18·8± 2·86	25·5± 4·46	..	..	45·2± 5·82	53·2± 4·98

* Number of days after planting.

TABLE II

Isolation frequency of **A.** flavus *and other fungi from groundnut shells and kernels and aflatoxin content in kernels at the time of harvest*

Year	Previous crop in the field	Shells						Kernels						Pod condition		Aflatoxin content[b] (μg/kg)	
		A. flavus			Other fungi			*A. flavus*			Other fungi			% undamaged pods	% damaged pods	undamaged pods	damaged pods
		60[a]	85	105	60	85	105	60	85	105	60	85	105				
1972	Vegetables	1[d]	10	25	12	34	38	0	0	16	0	15	45	92·1	7·9	Tr[c]	2761±455·42
	Rice	0	0	20	6	52	25	0	5	5	0	12	31	87·0	13·0	Tr	2676±450·56
	Groundnut	5	12	52	10	45	92	0	5	53	5	48	48	90·8	9·2	140±18·5	3736±586·38
1973	Vegetables	2	20	54	15	38	68	0	2	35	0	5	65	93·0	7·0	Tr	3608±320·42
	Rice	0	25	30	10	22	53	0	0	17	12	18	24	84·5	15·5	0	3696±758·82
	Groundnut	0	32	65	18	54	60	0	15	52	15	24	42	91·4	8·6	854±105·50	6180±1028·98

[a]=Number of days after planting. [b]=Average of five replications. [c]=Traces (less than 1 μg). [d]=Values expressed as percentage of shells or kernels from which *A. flavus* or other fungi grew out onto agar.

more in damaged pods, than in undamaged pods. The kernel surface of the damaged pods will be exposed to the surrounding soil and becomes more susceptible for fungal invasion. But in undamaged pods the intact shell and seed coat act as a natural barrier for fungal invasion.

The results relating to the counts of *A. flavus* (actual values as percentage on totals) on shells and kernels at 105 days have been subjected to statistical analysis according to the analysis of variance method on factorial basis. The mean actual values of *A. flavus* counts at 105 days for the three main factors: (1) 1972 *vs.* 1973 (years), (2) shells *vs.* kernels (pod parts) and (3) Vegetables *vs.* rice *vs.* groundnut (previous crop) have become statistically significant even at 1% probability. Considering the percentage values, it is seen that the effects of the main factors have not become statistically significant even at 5% probability. However, it is noted that the effects of the previous crops are significant at 10% probability.

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