Kasetsart J. (Nat. Sci.) Vol. 23 : 417 - 423 (1989)

Yield Performance, Seed Colonization and Aflatoxin Contamination of Selected Peanut Lines

Aree Wanayuwat and Porntip Pratoomsiri[1]

ABSTRACT

A number of peanut genotypes newly developed by Kasetsart University were yield tested in the field and evaluated in the laboratory for resistance to *Aspergillus* spp., the aflatoxin - producing fungi. The performance in yielding ability was presented and the seed colonization and aflatoxin production were discussed.

A few resistant lines performed as well as the susceptible Tainan 9 check in yield but showed less seed colonization when inoculated with various strains of *Aspergillus* as compared to the check. Only two *Aspergillus* strains were found to be most potent. Tainan 9 showed highest fungal infection at all storage times. However, when averaged across peanut lines it was found that the seeds kept 50 days after harvest (DAH) had lower infection than those of other storage times.

The seeds stored 50 DAH had lower aflatoxin content while those of other storage times were similar in aflatoxin contamination. Tainan 9 contained on average higher aflatoxin than all other resistant lines when kept 50 DAH. No difference was observed at all other storage times. Among the 4 selected lines Asp. 220 had lowest aflatoxin content but only when kept 50 DAH.

A genotype designated as Asp. 220 derived from a cross of PI 337394 F x Tainan 9 appeared to be promising because it yielded similar to Tainan 9 check but showed less seed colonization and aflatoxin contamination.

INTRODUCTION

The presence of aflatoxin in the seed of peanut is a worldwide problem particularly in the hot and humid tropics. This is also the case in Thailand in which the contamination from the fungi *Aspergillus* has caused the aflatoxin levels to offen far exceed safety standards (Karunyavanich, 1978). As a consequence the export of peanut from Thailand to some countries is prohibited. This restriction may have some effects on the production countrywide. In addition, the low quality peanuts which are contaminated with aflatoxin would be harmful to local consumers. One way to minimize the aflatoxin contamination problem is to use a resistant crop cultivar that inhibits the fungal colonization on the seed.

Two sources of Aspergillus resistance have been used in breeding programs to improve the resistance of peanut to this fungus (Mixon and Rogers, 1973; Waranyuwat, 1986). The selected lines have been evaluated both in the field and laboratory prior to this study. (Waranyuwat, 1986). This paper reports the yield performance, the resistance to fungal colonization and level of aflatoxin contamination of some selected peanut genotypes.

[1] Dept. of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

MATERIALS AND METHODS

Field Study

Twelve peanut lines derived from previous study (Waranyuwat, 1986) and 4 check lines (Table 1) were yield compared in a randomized complete block design in the rainy season (July - November) of 1985 and dry season (January - May) of 1986 at Suwan Farm, Pak Chong, Nakhon Ratchasima. Each treatment was replicated 4 times. A treatment consisted of four, 5 m rows for the first trial and four 8.4 m rows for the second trial. Prior to sowing 50 kg/rai (1 rai = 0.16 ha) of 12 - 24 - 12 fertilizer was broadcasted and disked into the soil. Sowing was done on the slightly - raised ridges that were 50 cm apart. The seeding rate was 2 seeds per hill spaced 20 cm apart. After planting, a pre - emergence herbicide (alachlor) at a rate of 750 ml/rai was applied and then sprinkler irrigated. Supplemental irrigation was applied as needed during the dry season. Pest control and cultural management were done as necessary. At harvest the end plants of two middle rows were trimmed and hand pulling done. The yield of pods and seeds, 100 - seed weight and shelling percentage were measured.

The third yield trial was conducted in the rainy season of 1987 using similar field procedure as previously described but only 4 selected lines (Asp. 220, 229, 243 and 533) and the 4 check lines were used (Tainan 9, SK 38, PI 337394F and PI 337409).

Table 1 Pod and seed yield and 100 - seed weight of 16 peanut lines conducted at Suwan Farm in rainy season (Trial 1) and dry season (Trial 2)

Line	Trial 1			Trial 2			Mean seed yield (kg/rai)
	Pod (kg/rai)[1]	Seed	100 - seed wt. (g)	Pod (kg/rai)	Seed	100 - seed wt. (g)	
Asp. 220	405	304	43	265	171	50	237.5
Asp. 533	402	275	46	376	258	51	266.5
Tainan 9(ck)	379	279	47	317	226	58	252.5
J11 (ck)	379	279	37	168	107	41	193.0
PI 337394F (ck)	361	265	38	340	268	43	266.5
PI 337409 (ck)	343	254	38	307	230	41	242.0
Asp. 187	326	226	42	143	84	44	155.5
Asp. 303	312	211	43	224	180	46	195.5
Asp. 243	300	213	44	342	232	49	222.5
Asp. 229	293	209	46	339	228	53	218.5
Asp. 180	265	185	44	216	137	51	161.0
Asp. 308	257	175	43	273	153	49	164.0
Asp. 422	251	180	42	162	105	48	142.5
Asp. 255	198	128	38	156	100	44	114.0
Asp. 425	172	125	42	154	100	57	112.5
Asp. 97	149	94	41	209	131	64	112.5
LSD (5%)	71	52	3.6	104	61	4.6	
CV (%)	16.7	17.3	6.0	24.9	24.0	5.6	

[1] Note : To convert yield to kg/ha multiply by 6.25.

Laboratory Studies

Four selected lines designated as Asp. 220, Asp. 229, Asp. 243 and Asp. 533 plus 3 checks which were yield tested in the first two trials were used in three laboratory studies for the assessment of fungal colonization and aflatoxin production.

In the natural infection study hand - shelled seed samples kept 30 days after digging were sorted to obtain 160 sound, mature kernels of each line. The seeds were immersed in sterilized water for 30 minutes and then surface - disinfected by immersion in a 20% Clorox solution for another 30 minutes, followed by 2 rinses in sterilized water. The seeds of each peanut line were placed on 7.5% malt salt agar petri plates as described by Christensen (1973). Each plate contained 10 seeds. Each treatment consisted of 4 duplicate plates replicated 4 times. All the plates were incubated at room temperature for 5 days. The percentage infection data were transformed using arcsine formula, prior to analysis according to the completely randomized design.

The inoculation study on 5 strains of Aspergillus was conducted using the same seed lots of the same peanut genotypes. The seed preparation for this experiment was similar to that previously described, but each treatment consisted of 25 seeds per plate replicated 4 times. A spore suspension of each fungal strain was prepared from a 7 - day - old subculture in sterilized water mixed with Tween - 80 for uniform distribution. One ml of spore suspension at a similar concentration was thoroughly mixed with the seeds on each plate and then incubated at 25°C for 10 days. The transformed data, as described above, were analyzed by using nested classification method.

The study on the effect of storage time on per cent contamination and aflatoxin content in the seeds was undertaken using a similar procedure. However, the experiment could not begin at 30 days after harvest; therefore, this seed lot was kept in a refrigerator until the inoculation began at 50 days and the rest of the seed samples were stored at ambient conditions for 65, 80 and 95 days. For each storage time 2 samples of 20 g of seed were inoculated with 1 ml of spore suspension of *A. parasiticus* and two other samples of the same amount were mixed with sterilized water as a control. The plates were incubated for 10 days in a controlled environment.

Per cent seed infection was recorded and the samples analyzed for aflatoxin B_1 content by the Plant Pathology and Microbiology Division, Department of Agriculture. An analysis of variance was performed for aflatoxin B_1 content by using log transformed values.

RESULTS AND DISCUSSION

Yield Trial

The pod and seed yields and seed size of 16 peanut genotypes is given in Table 1. Based on mean yields the discussion will be placed on only 4 selected lines, i.e. Asp. 220, Asp. 229, Asp 243 and Asp. 533. It should be noted that seed yield will be mentioned in most cases as both pod and seed yields are highly correlated in peanuts.

Asp. 220 gave highest pod and seed yields of 405 and 304 kg/rai respectively, in the rainy season, but was not significantly different from other checks. The seed size was comparable to that of Tainan 9 and larger than the other resistant checks.

In the dry season the top yielding lines were Asp. 533 and Asp. 243, which were not different from Tainan 9 and PI 337409, a resistant check, with seed yield ranging from 230 to 258 kg/rai. Although the seed size of the resistant lines were different, several selected lines had larger seed than the resistant checks.

On the basis of their good yielding ability, seed size and plant characteristics (not shown)

the lines Asp. 220, Asp. 229, Asp. 243 and Asp. 533 were selected for further evaluation in the following wet season. It is shown in Table 2 that Asp. 220 and Asp. 243 gave comparable yields to the Tainan 9 check. However, these selected lines had larger seed size than the resistant checks (PI 337394 F and PI 337409), although the yields were not different. It was also observed in the field that the growth type and maturity of these new lines was similar to that of Tainan 9.

Table 2 Pod and seed yields, 100 - seed weight, shelling percentage and percent natural seed infection of 8 peanut lines conducted during July - November, 1987 at Suwan Farm.

Line	Yield kg/rai		100 - seed wt. (g)	% Shelling	% Infection after 30 days storage
	Pod	Seed			
Tainan 9 (ck)	338	255	51	75	2.5
Asp. 243	258	193	45	79	10.0
Asp. 533	256	181	45	70	8.1
Asp. 220	255	188	44	74	6.9
SK 38 (ck)	239	168	45	72	—
PI 337394 F (ck)	219	166	37	71	5.0
Asp. 229	211	149	48	73	5.0
PI 337409 (ck)	210	153	37	77	7.5
LSD (5%)	98	69	2.5	3.9	NS
CV (%)	24.9	24.0	3.8	7.4	23.9

Laboratory Evaluation

The natural infection by *A. flavus* on the seed of 7 peanut lines is shown in Table 3. All the lines had a low percentage of seed colonization, demonstrating that the infection could have occurred some time during storage. These results reflect the possibility of obtaining clean seed without fungal infection if the post - harvest handling is appropriate. This view is accentuated by the inoculation study, where the control treatment showed no colonization on the seed of all the lines used (Table 3). It must be noted, however, that other fungal genera such as *A. niger, Fusarium, Penicillium* and *Rhizopus* were also observed.

The inoculation study showed that some fungal strains were more potent in colonizing than others (Table 3). A local strain of *A. parasiticus* was more efficient in infection of all the peanut lines tested, with Tainan 9 the most susceptible. Mixon and Rogers (1973) observed the seed infection by *A. flavus* of PI 337394 F and PI 337409 being 5 and 9%, respectively, as compared to 1 and 3% and 2 and 5% by two *A. flavus* strains respectively from the present study. Again Tainan 9 had the most seed infection and Asp. 533 showed no infection. Of all the peanut genotypes studied it would appear that both species of *Aspergillus* could cause infection with varying degrees and thus subsequent aflatoxin contamination.

Table 3 Percent seed colonization as inoculated with 5 strains of *Aspergillus* spp.

Line	Avg. of 4 replications						
	Control	*A. flavus*	*A. flavus* NRRL 840	*A. parasiticus*	*A. parasiticus* NRRL 300	*A. parasiticus* NRRL 482	Means
Tainan 9	0	14.0	6.0	34.5	1.3	0	11.16
PI 337394 F	0	1.0	2.0	9.0	0	0	2.40
PI 337409	0	3.0	5.0	7.0	0	0	3.00
Asp. 220	0	6.0	1.0	6.0	5.0	0	2.16
Asp. 229	0	1.0	4.0	3.0	3.8	0	4.76
Asp. 243	0	4.0	2.0	9.0	7.5	1.3	2.20
Asp. 533	0	0	0	6.0	5.0	0	4.21
Avg.	0	4.14b*	2.86b	10.64a	3.23b	0.19c	

Note : F - test indicates significant difference both among lines and *Aspergillus* strains, CV 54%.
* Means followed by different letters are significantly different at 5% level.

In order to study the effects of storage time on the percent infection of seeds kept under ambient conditions, selected lines were inoculated with *A. parasiticus*. It was found that the infection percentage increased as the storage time was increased up to 80 days (Table 4) with variable reaction in different lines. At 50 days after harvest (DAH) Tainan 9 showed highest seed infection (21.5%), while the other lines on average had 2.3% infection. The resistant checks in most cases were least infected as compared to other lines, irrespective of storage times. The analysis of variance (not shown) showed that at all the storage times studied Tainan 9 had significantly higher seed colonization than the other 6 lines. Asp. 220 also had significantly lower infection than other selected lines at all the storage regimes.

Results of the analysis of aflatoxin B_1 at the 4 storage times (Table 5) showed that the seeds kept for 50 DAH on average contained lower aflatoxin than those stored for longer periods with considerable variation in aflatoxin content at other times. Tainan 9 was contaminated with aflatoxin in higher quantity than most other lines at 50 DAH. Asp. 220 had much lower aflatoxin content than Asp. 229, Asp. 243 and Asp. 533, but higher than the resistant checks. At all other storage times all the peanut lines, in most instances, were not different in aflatoxin content.

Note that there appeared to have low correlations between percent seed infection and aflatoxin content as shown in Tables 4 and 5. Such observation was also reported in several studies (Mehan *et al.*, 1982; Priyadarshini and Tulpule, 1978).

Note that the uninoculated samples were contaminated with aflatoxin to some extent (figures in brackets), even though in some cases no fungal growth was observed on the seed. If this was the case it could be that natural infection took place without visible growth on the seed surface at time of counts. Subsequently, aflatoxin in the uninoculated samples was accumulated with time until seed analysis.

CONCLUSION

The results of this study illustrate the possibility of using peanut varieties which are resistant to *Aspergillus* spp. infection for minimizing the aflatoxon problem, in addition to the requirement

Table 4 Percent colonization of seed at 4 storage times as inoculated with *Aspergillus parasiticus*.

Line	Avg. of 2 replications				Means
	50 DAH[1/]	65 DAH	80 DAH	95 DAH	
Tainan 9	21.5	40.5	34.0	37.5	33.37
PI 337394 F	2.0	7.5	32.0	14.5	14.00
PI 337409	0	4.0	0	4.0	2.00
Asp. 220	2.0	14.0	11.0	21.5	12.12
Asp. 229	1.5	25.0	29.5	39.5	23.87
Asp. 243	5.5	38.5	31.0	27.5	25.62
Asp. 533	3.0	39.5	60.5	48.5	37.87
Avg.	5.1	24.1	28.3	27.6	21.27
F - test Tainan 9 vs Others	**	**	*	*	
Asp. 220 vs Other Asp. lines	NS	**	*	**	

Note : F - test indicates significant difference both among storage times and peanut lines, CV 15.0%. *, ** indicate significance at 5 and 1% level.

1/ Number of days after harvest.

Table 5 Aflatoxin B_1 in part per billion in the seed at 4 storage times when inoculated with *A. parasiticus* as compared to uninoculated (in bracket).

Line	Time of storage				Mean
	50 DAH	65 DAH	80 DAH	95 DAH	
	Aflatoxon B_1 Content (ppb)				
Tainan 9	2,504 (0)	23,212 (0)	3,736 (0)	14,568 (90)	11,005.0 (22.5)
PI 337394 F	401 (0)	38 (14)	7,994 (0)	3,784 (0)	3,054.2 (3.5)
PI 337409	0 (0)	1,463 (0)	209 (0)	3,181 (0)	1,213.2 (0)
Asp. 220	591 (0)	5,356 (0)	1,945 (0)	9,081 (0)	4,243.2 (0)
Asp. 229	751 (0)	4,122 (0)	5,460 (0)	11,033 (120)	5,341.5 (30)
Asp. 243	4,405 (0)	33,197 (0)	6,109 (0)	13,382 (15)	14,273.2 (3.7)
Asp. 533	2,500 (0)	20,078 (0)	10,840 (0)	10,775 (2,294)	11,048.2 (573.5)
Avg.	1,593 (0)	10,903 (2)	5,185 (0)	9,401 (360)	6,770.5 (90.5)
F - test Tainan 9 vs Others	**	ns	ns	ns	
Asp. 220 vs Other Asp. lines	*	ns	ns	ns	

Note : F - test indicates significant difference both among storage times and peanut lines, CV 21.2% See asterisk denotation in Table 4.

for proper post harvest handling of the crop. The resistant lines that had been developed and evaluated in the present study should be tested more widely to determine whether the resistance exists under varying growing conditions. For the time being, line Asp. 220 has proved to be resistant to infection by *A. flavus* and *A. parasiticus* in terms of low seed colonization and low aflatoxin contamination, as compared with Tainan 9 and therefore seems to be promising. This peanut genotype has acceptable seed size in comparison with the resistant checks and seed yield comparable to Tainan 9. In minimizing the risk of aflatoxin contamination the resistant peanut line has some value and therefore should be further evaluated.

ACKNOWLEDGEMENT

The authors are grateful to the Division of Microbiology, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, for the assistance in aflatoxin determination. We greatly appreciate the assistance given by Dr. Orapin Bhumibhamon of the Department of Biotechnology, Faculty of Agro - industry, Kasetsart University for laboratory facilities and Dr. Surapol Oopadissakoon for the statistical advice.

LITERATURE CITED

Christensen, C.M. 1973. Loss of viability in storage microflora. Seed Sci. Technol. 1 : 547 - 562.

Karunyavanich, S. 1978. Aflatoxin in peanut food stuff. A paper presented at the seminar on peanuts and other legumes held at Khon Kaen University, 2-4 March 1978, p. 186 - 191 (in Thai).

Mehan, V.R., D. McDonald and R.W. Gibbons. 1982. Seed colonization and aflatoxin production in groundnut genotypes inoculated with different strains of *Aspergillus flavus*. Oleagineu 37 : 185 - 189.

Mixon, A.C. and K.M. Rogers. 1973. Peanut accessions resistant to seed infection by *Aspergillus flavus*. Agron. J. 65 : 560 - 562.

Priyadarshini, F. and P.G. Tulpule. 1978. Relationship between fungal growth and aflatoxin production in varieties of maize and groundnut. J. Agric. Food Chem. 26 : 247 - 252.

Waranyuwat, A. 1986. Peanut breeding. A report submitted to IDRC by Faculty of Agriculture, Kasetsart University, p. 7 - 43.