

Original Research Article

Evaluation of groundnut varieties for resistance to seed infection by *Aspergillus flavus*

ABSTRACT

Groundnut is a legume crop an important food crop in the world and the third most important oil seed crop. However, groundnut crop suffers from major diseases such as flavus rot caused by *Aspergillus* which are soil-borne, they produce a potent toxin and carcinogenic substance called aflatoxin. This toxin has a great impact on human health. The resistant varieties can be the most viable and economical approach to reduce this problem. This research consisted of two experiments, the first was conducted at the Department of Plant Pathology Laboratory, while the second pot experiment was conducted in the green house at the Department of Plant Pathology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India during *Kharif*, 2020. The objective of the experiment was to evaluation of groundnut varieties for resistance to seed infection by *Aspergillus flavus*. Ten varieties were tested i.e. GJG 9, GJG 32, JL 501, TG 37A, KAUSHAL, GJG 17, GG 20, GJG 22, GJG 31 and KDG 128. A CRD design was used for this experiment, with three replications. Out of ten varieties tested against *Aspergillus flavus* in vitro, none of the varieties were found resistant. The minimum seed infection (20.48%) was recorded in JL 501 and KAUSHAL, whereas the maximum (80.46%) in GJG 31, maximum seed germination was recorded in variety JL 501 (99.99%), whereas the minimum recorded in GJG 31 (20.48%). The minimum mortality of germinated seeds (10.49%) was observed in the varieties GJG 9, GJG 32, JL 501, GG 20, GJG 22, and KDG 128, whereas the maximum in GJG 31 (30.48%). In the pot study, none of the varieties were found resistant or moderately resistant. The minimum seed infection (40.47%) was recorded with GJG 9, whereas the maximum (70.46%) in GJG 31. Maximum seed germination was recorded in GJG 9 (80.46%), whereas the minimum was recorded in GJG 31 (23.67%). The minimum seed mortality (10.49%) was observed in varieties GJG 9, TG 37A and KDG 128, whereas the maximum in GJG 31 (30.48%).

Keywords: Groundnut, *Aspergillus flavus*, seed infection, Selected varieties

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual legume known as peanut. It is the world's thirteenth most important food crop and third most important oil seed crop used for vegetable oil production. Groundnut is cultivated in the tropical and subtropical regions of the world. India stands first in the area with 4.7 million hectares, second in production (6.7 million ton) and productivity of 1422 kg ha⁻¹ (FAO, 2023). In Gujarat, groundnut is grown on about 1688 thousand hectares with a production of 4645 thousand ton and an average productivity of 2751 kg ha⁻¹. In North Gujarat, Banaskantha ranks first in groundnut production (3838 MT) followed by Aravlii (1178 MT) and Kachchh (610 MT) (DOA, 2023). Groundnut seeds (kernels) contain 35.8- 54.2 per cent oil (Jambunathan *et al.*, 1985), 16.2-36.0 per cent protein (Dwivedi *et al.*, 1990), and 10-20 per cent carbohydrate (Salunkhe *et al.*, 1992). Groundnut crop suffers from major diseases such as early leaf spot (*Cercospora arachidicola* Hori), late leaf spot (*Passalora personata* Berk. Curt. N. Arx), rust (*Puccinia arachidis* Speg), stem rot (*Sclerotium rolfsii* Sacc.), root rot [*Macrophomina phaseolina* (Tassi) Goid.], collar rot (*Aspergillus niger* Van Tieghem), afla rot (*Aspergillus flavus* Link Ex Fries), nematode disease like root knot and viral diseases like stem necrosis, bud necrosis, mottle and clum (Ghewande and Reddy, 1986). Among the soil borne diseases, afla rot caused by *Aspergillus flavus* is a one of the important diseases in the Gujarat region and Groundnut growing areas of the world (Klich, 2007). *Aspergillus flavus* is a pathogenic fungus in the phylum Ascomycota. *A. flavus* is most common in warm temperate zones and environment with low water level and higher temperature. *A. flavus* is found globally as a saprophyte in soils and causes diseases on many important agriculture crops including yellow mold in groundnut in the field, preharvest, postharvest, storage and during transit. *A. flavus* has the potential

to infect seedlings by sporulation of injured seeds. They produce a potent toxin and carcinogenic substance called aflatoxin. This toxin has great impact on human health. Therefore this research will look at the evaluation of groundnut varieties for resistance to seed infection by *Aspergillus flavus*.

2. MATERIAL AND METHODS

This research consisted of two experiments, the first was conducted at the Department of Plant Pathology Laboratory, while the second pot experiment was conducted in the green house at the Department of Plant Pathology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India during Kharif, 2020. The objective of the experiment was to evaluation of groundnut varieties for resistance to seed infection by *Aspergillus flavus*. Ten varieties were tested i.e. GJG 9, GJG 32, JL 501, TG 37A, KAUSHAL, GJG 17, GG 20, GJG 22, GJG 31 and KDG 128. A CRD design was used for this experiment, with three replications.

The first experiment in vitro:

2.1 Seed preparation in vitro

Screened ten varieties of groundnut against *A. flavus* (Table 1). Sound, healthy and mature 100 g seeds of each variety were surface sterilized with 0.1 percent mercuric chloride solution for one minute and washed three times with sterilized distilled water. The seeds of each variety were decanted and aseptically placed in sterilized Petri dish. The seeds were uniformly inoculated with spore suspension of the seven days old culture of *A. flavus* (10^6 spores ml^{-1} @ 1 ml per 10 g seeds), the seeds were rolled gently for evenly spread of inoculum. Ten seeds were placed aseptically in each sterile 15 cm diameter Petri dish with three replications and incubated under standard conditions of temperature and humidity. After 10 days of inoculation observations were recorded for seed infection, the seed of germination, and mortality of germinated seed.

Table 1: Varieties of groundnut tested against *A. flavus* in vitro and pot conditions

Sr.no	Varieties	Sr.no	Varieties
1	GJG 9	6	GJG 17
2	GJG 32	7	GG 20
3	JL 501	8	GJG 22
4	TG 37A	9	GJG 31
5	KAUSHAL	10	KDG 128

The second experiment in the poly house:

2.2 Seed preparation in pot conditions

Ten groundnut varieties were screened against *A. flavus* (Table 1) as the material and method described above, ten seeds of each variety were sown in sterilized pots filled with sterilized soil and watering was done regularly in the glass house. After 10 days of inoculation, observations were recorded for seed infection, the seed of germination, and mortality of germinated seed.

The varieties were classified as below (Ghewande *et al.*, 1993).

Level of resistance	Criteria
Resistant	Sporulating growth of <i>A. flavus</i> present on less than 15 percent of seed growth and sporulation sparse
Moderately resistant	Sporulating growth of <i>A. flavus</i> present on 16 to 30 percent of seeds sporulating moderate to dense
Susceptible	Sporulating growth of <i>A. flavus</i> present on 31 to 50 percent of seeds sporulating dense
Highly susceptible	Sporulating growth of <i>A. flavus</i> present on over 50 percent of seeds with dense growth and sporulation

3. RESULTS AND DISCUSSION

The first experiment in vitro:

3.1 Seed preparation in vitro

3.1.1 Seed infection (%)

Among ten varieties tested, the seed infection rate ranged from 20.48 to 80.46 percent. The minimum seed infection (20.48%) was recorded with JL501 and KAUSHAL (Table 2), whereas the maximum seed infection (80.46%) was recorded in GJG31. Categorization of different varieties based

on seed infection (Table 3) revealed that in ten varieties tested, none of the varieties were found resistant against most virulent isolates of *A. flavus* (AF-6).

Two varieties viz., JL501, KAUSHAL were found moderately resistant, whereas three variety viz., GJG9, TG37A and GJG22 were found susceptible and the remaining five varieties were highly susceptible to *A. flavus* invasion.

3.1.2 Seed germination (%)

99.99 percent of seed germination was recorded in variety JL 501 followed by varieties GJG 32 (90.47%), KDG 128 (90.47%) and TG 37A (87.47%) (Table 2). The minimum seed germination was recorded in variety GJG 31 (20.48%).

3.1.3 Mortality of germinated seed (%)

The mortality of germinated seeds ranged from 10.49 to 30.48 percent (Table 2). The minimum mortality of germinated seeds (10.49%) was noted in varieties GJG 9, GJG 32, JL 501, GG 20, GJG 22 and KDG 128, whereas the maximum seed mortality was recorded in GJG 31 (30.48%).

Table 2: Seed infection, seed germination, and mortality of germinated seed of different groundnut varieties after 10 days of with *A. flavus* inoculation in vitro conditions

Sr. No.	Name of Varieties	Seed infection (%)	Seed germination (%)	Mortality of germinated seed (%)	Level of resistance
1	GJG9	43.35 ^b (47.12)	51.04 ^c (60.46)	18.90 ^a (10.49)	S
2	GJG32	51.04 ^d (60.46)	72.02 ^b (90.47)	18.90 ^a (10.49)	HS
3	JL501	26.91 ^a (20.48)	89.39 ^a (99.99)	18.90 ^a (10.49)	MR
4	TG37A	43.35 ^b (47.12)	69.27 ^b (87.47)	21.57 ^a (13.52)	S
5	KAUSHAL	26.91 ^a (20.48)	51.04 ^c (60.46)	26.91 ^b (20.48)	MR
6	GJG17	47.19 ^{bcd} (53.82)	43.35 ^d (47.12)	26.91 ^b (20.48)	HS
7	GG20	49.12 ^{cd} (57.16)	47.19 ^{cd} (53.82)	18.90 ^a (10.49)	HS
8	GJG22	45.27 ^{bc} (50.47)	43.35 ^d (47.12)	18.90 ^a (10.49)	S
9	GJG31	63.77 ^e (80.46)	26.91 ^e (20.48)	33.51 ^c (30.48)	HS
10	KDG128	51.04 ^d (60.46)	72.02 ^b (90.47)	18.90 ^a (10.49)	HS
	S.Em. ±	1.22	1.37	0.84	
	C.D. at 5%	3.59	4.03	2.49	
	C.V.%	4.70	4.18	6.58	

Numbers in parentheses are retransformed values of arc sine transformed values.

Treatment means with the letter(s) in common are not significant by DNMR at 5% level significance.

Level of resistance based on seed infection:

< 15 percent – Resistant (R), 16-30 percent – Moderately Resistance (MR), 31- 50 percent – Susceptible (S), > 51 percent – Highly Susceptible (HS)

Table 3: Categorization of different groundnut varieties based on seed infection in vitro condition

Sr. No.	Categories	No. of varieties	Percent Varieties	Name of Varieties
1	Resistant < 15%	00	0.0	-
2	Moderately Resistance 16-30%	02	20.0	JL501 and KAUSHAL
3	Susceptible 31-50%	03	30.0	GJG9, TG37A and GJG22
4	Highly Susceptible > 51%	05	50.0	GJG32, GJG17, GG20, GJG31 and KDG128

The second experiment in the poly house:

3.2 Seed preparation in pot conditions

3.2.1 Seed infection (%)

Among ten varieties tested, having the seed infection ranged from 40.47 to 70.46 percent. The minimum seed infection of 40.47 percent was recorded with GJG9 (Table 4), whereas the maximum seed infection (70.46%) was recorded in GJG 31 which was at par with GJG 17 (67.21%). Categorization of different varieties based on seed infection (Table 5) revealed that out of ten varieties tested, none of the varieties was found resistant and moderately resistant against most virulent isolates of *A. flavus* (AF-6), whereas six varieties viz., GJG 9, GJG 32, JL 501, KAUSHAL, GJG 22, KDG 128 were found susceptible and the remaining four varieties were highly susceptible against *A. flavus* invasion.

3.2.2 Seed germination (%)

80.46% seed germination was recorded in variety GJG 9 followed by varieties KDG 128 (67.21%), TG 37A (63.87%), GG 20 (63.87%) and GJG 32 (60.46%) (Table 4). The minimum seed germination was recorded in GJG 31 (23.67%).

3.2.3 Mortality of germinated seed (%)

Mortality of germinated seeds was recorded in the range of 10.49 to 30.48 percent (Table 4). The minimum mortality of germinated seeds (10.49%) was noted in varieties GJG 9, TG 37A and KDG 128, whereas the maximum seed mortality was recorded in GJG 31 (30.48%).

The present results are new as the latest varieties adopted by farmers of North Gujarat were not tested earlier. The investigation on seed infection, seed germination, and mortality of germinated seed *in vitro*, pot condition, and field condition done by earlier workers Mixon and Rogers (1973) developed a new *in vitro* seed colonization procedure for screening the groundnut genotypes against *A. flavus*, indicated that valencia type genotypes viz., PI337394F and PI337409 were resistant to two toxin-producing strains of the fungus. Mehan *et al.* (1981) reported that inoculation of seeds of seven groundnut cultivars with three different toxigenic strains of *A. flavus* showed marked differences in invasion potential between cultivars. Among them J-11, PI 337409, and PI 337394 were found to be resistant to invasion and colonization by all three strains and the strain NRRL 3000 was less virulent than the other two on all the cultivars. Mehan *et al.* (1987) used a modified method to screen 850 germplasm accessions for their reaction to seed invasion and colonization by *A. flavus*. Of which, the resistance of the three genotypes viz., PI-337394F, PI-337409, and UF-71513 was confirmed and six new sources of resistance (Ah 7223, J-11, U-4-47-7, var. 27, Faizpur and Monir 240-30) were identified. Mehan *et al.* (1988) evaluated the 11 peanut genotypes by IVSCAF and showed that six were resistant and five susceptible, further evaluated under field conditions in seven environments in South India and found that five of the IVSCAF resistant genotypes had significantly greater resistance to infection of seed by *A. flavus* and had lower aflatoxin contamination than the susceptible genotypes.

Kiran *et al.* (1988) evaluated 53 groundnut cultivars and found high-yielding lines were susceptible to invasion by *A. flavus* and aflatoxin contamination and indicated that line Oh 53-1 showed the highest resistance with low yield potential and J-11 showed resistance to aflatoxin production and moderately susceptible reaction to *A. flavus* invasion. Mehan *et al.* (1991) screened out the 34 genotypes and reported varied levels of seed colonization severity (1.0 to 4.0) on different genotypes viz., ICG-1126, 1323, 1122, 1859, 3263, 3267, 3336, 3700, 4749, 4888, 7412, 7633, 9610, 10020. Collison *et al.* (1992) examined four groundnut genotypes for mold flora and reported that the degree of contamination ranged from 68 to 76 percent, with *A. flavus* as the predominant mold on groundnut.

Waliyaret *et al.* (1994) tested 25 lines, including germplasm, advanced breeding lines, and cultivars found that out of these, cultivars 55-437, J11, and PI 337394 F were the least infected, whereas among the ICRISAT advanced breeding lines involving parents resistant to *A. flavus*, ICGV 87084, ICGV 87094, and ICGV 87110 were resistant. Rao *et al.* (1995) assessed the Spanish groundnut germplasms ICGV-88145 and ICGV-89104 for seed colonization by *A. flavus* under artificial inoculation conditions and it averaged 22.2 and 24.0 percent compared with 15.6 percent in the best resistant control (J-11).

Upadhyay *et al.* (2001) have released three lines viz., ICGVs 91278, 91283, and 91284 as improved germplasm for resistance to natural seed infection and *in vitro* seed colonization by the aflatoxin-producing fungus *Aspergillus flavus* in groundnut and they also found J-11 as resistant and JL-24 as susceptible varieties. Varma *et al.* (2001) evaluated 14 different groundnut cultivars for

resistance to *in vitro* seed colonization with toxigenic strains of *A. flavus*. Among them, three genotypes S 206, KRG 1, and GPBD 4 recorded relatively low levels of colonization, whereas cultivars JL-24, TAG-24, and TMV-2 were reported as susceptible.

Gowda *et al.* (2002) studied mutant 28-2 a bold seeded groundnut genotype for disease resistance and noted colonization severity of 3.0 for *A. flavus* compared to 3.7 for JL-24 on a 1-4 scale. Babu *et al.* (2004) reported that ICGV 86155, ICGV 86699 and ICGV 96266 were significantly more resistant to *A. flavus* compared to resistance check J-11.

Babu *et al.* (2005) reported that Trombay groundnut genotypes TG-19, TG-49, TG-18A, and TG-18 showed a high level of resistance to very low seed colonization by *A. flavus* compared to resistant check J-11.

Kumar (2005) evaluated fifteen genotypes in the laboratory for seed coat resistance against *A. flavus* reported Dh-86 as moderately resistant, three as susceptible (GPBD-4, Dh-102 and R-8808), 11 as highly susceptible (TGLPS-3, TKG-19A, R-9251, Dh-40, J-11, JL-24, ICGV- 92242, TAG-24, Dh-3-30, Dh-54, TMV-2) and none of the genotype was found resistant.

Waliyaret *et al.* (2006) evaluated 14 new varieties for *in vitro* seed colonization with *A. flavus* reported that new varieties (ICGV 91278, 91279, 91283, 91284, 91315, 91317, 91324, 91328, 91341, 92302, 93305, 93328, 93379 and 94434) consistently showed < 10 percent seed colonization as compared with susceptible variety TMV-2 (> 50-90 percent colonization). Dube and Maphosa (2014) tested 11 genotypes for seed resistance status only three genotypes (Falcon, CG 7 and Nyanda) were found to be moderately resistant to infection by *A. flavus* and the remaining eight genotypes (Makulu Red, Tern, Teal, Mwenjere, SC Orion, Flamingo and SC GV 00004) were susceptible in the laboratory tests. Ranganathswamy *et al.* (2017) carried out *in vitro* study to identify a resistant source against *A. flavus* causing aflatoxin contamination in groundnut by spore spray method and among 70 genotypes tested 7 were moderately resistant, 21 were susceptible and 42 were highly susceptible in spore spray method. Commey *et al.* (2021) investigate the role of seed coat against *A. flavus* infection. *In vitro* seed colonization with and without seed coat showed that the seed coat acts as a physical barrier, and the developmental series of peanut seed coat showed the formation of a robust multilayered protective seed coat. Radial growth bioassay revealed that both insoluble and soluble seed coat extracts from 55-437 line (resistant) showed higher *A. flavus* inhibition compared to the TMV-2 line (susceptible). Nakrani and Sevak (2021) screened the twenty varieties of groundnut for *A. flavus* invasion and aflatoxin production by dry seed resistance test, among them none of the varieties was found immune as well as resistant against most virulent isolates. Furthermore TMV-2 showed maximum seed infection (83.33%), TMV-7 showed cent percent seed germination while the maximum seed mortality was recorded in ICGV- 89280 (73.89%), whereas minimum seed infection (16.67%) and mortality of germinated seeds (10.74%) in the J-11.

Salunke *et al.* (2021) conducted a screening trial of groundnut genotypes for resistance to *in vitro* seed colonization and infection by *A. flavus*, comprising 9 local cultivars of Karnataka, 34 germplasm collections and 25 advanced breeding lines from ICRISAT along with popular tolerant variety J-11 used as check and the results showed that ICGV-02207 and ICGV-02266 exhibited both seed coat and cotyledon resistance. From time to time after development of different genotypes, cultivars, varieties *etc.* and found the different levels of resistant which are important tools in breeding works and also helpful to the farmers to grow resistant varieties against *A. flavus* and that is a way aflatoxin reduction which was hurdles for groundnut export.

Table 4: Seed infection, seed germination and mortality of germinated Seed of different groundnut varieties after 10 days of inoculation with *A. flavus* in pot conditions

Sr. No.	Name of Varieties	Seed infection (%)	Seed germination (%)	Mortality of germinated seed (%)	Level of resistance
1	GJG9	39.51 ^a (40.47)	63.77 ^a (80.46)	18.90 ^a (10.49)	S
2	GJG32	45.27 ^{bc} (50.47)	51.04 ^b (60.46)	24.24 ^b (16.86)	S
3	JL501	41.43 ^{ab} (43.78)	45.27 ^c (50.47)	26.91 ^{bc} (20.48)	S
4	TG37A	47.19 ^c (53.82)	53.05 ^b (63.87)	18.90 ^a (10.49)	HS
5	KAUSHAL	41.43 ^{ab} (43.78)	45.27 ^c (50.47)	26.91 ^{bc} (20.48)	S
6	GJG17	55.07 ^d (67.21)	41.43 ^{cd} (43.78)	31.31 ^{cd} (27.01)	HS

7	GG20	47.19 ^c (53.82)	53.05 ^b (63.87)	24.24 ^b (16.86)	HS
8	GJG22	45.27 ^{bc} (50.47)	37.51 ^d (37.07)	29.11 ^{bcd} (23.67)	S
9	GJG31	57.08 ^d (70.46)	29.11 ^e (23.67)	33.51 ^d (30.48)	HS
10	KDG128	45.27 ^{bc} (50.47)	55.07 ^b (67.21)	18.90 ^a (10.49)	S
	S.Em. ±	1.37	1.57	1.55	
	C.D. at 5%	4.05	4.63	4.56	
	C.V.%	5.11	5.73	10.59	

Numbers in parentheses are retransformed values of arc sine transformed values.

Treatment means with the letter(s) in common are not significant by DNMRT at 5% level significance.

Level of resistance based on seed infection:

< 15 percent – Resistant (R), 16-30 percent – Moderately Resistance (MR),
31- 50 percent – Susceptible (S), > 51 percent – Highly Susceptible (HS)

Table 5: Categorization of different groundnut varieties based on seed infection in pot conditions

Sr. No.	Categories	No. of varieties	Per cent Varieties	Name of Varieties
1	Resistant < 15%	00	0.0	-
2	Moderately Resistance 16-30%	00	0.0	-
3	Susceptible 31-50%	06	60.0	GJG9, GJG32, JL501, KAUSHAL, GJG22 and KDG128
4	Highly Susceptible > 51%	04	40.0	TG37A, GJG17, GG20 and GJG31

4. CONCLUSION:

This research consisted of two experiments first *in vitro* condition in the laboratory and the second in the poly house in the pot condition while the percent seed infection, percent seed germination and percent mortality of germinated seed were observed during the experiment. Out of ten varieties tested against *Aspergillus flavus* *in vitro*, none of the varieties were found resistant. The minimum seed infection (20.48%) was recorded in JL 501 and KAUSHAL, whereas the maximum (80.46%) in GJG 31, maximum seed germination was recorded in variety JL 501 (99.99%), whereas the minimum recorded in GJG 31(20.48%). The minimum mortality of germinated seeds (10.49%) was observed in the varieties GJG 9, GJG 32, JL 501, GG 20, GJG 22, and KDG 128, whereas the maximum in GJG 31 (30.48%). In the pot study, none of the varieties were found resistant or moderately resistant. The minimum seed infection (40.47%) was recorded with GJG 9, whereas the maximum (70.46%) in GJG 31. Maximum seed germination was recorded in GJG 9 (80.46%), whereas the minimum was recorded in GJG 31 (23.67%). The minimum seed mortality (10.49%) was observed in varieties GJG 9, TG 37A and KDG 128, whereas the maximum in GJG 31 (30.48%). So, the recommendation from this experiment was that there is a urgent need for to find the resistance sources of groundnut varieties that can resist the *Aspergillus flavus*, that in fact is the major factor constrain in the groundnut growing areas,

CONFLICT OF INTEREST: None

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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