Original Research Article

Screening of Popular Groundnut Varieties Grown in North Gujarat Agroclimatic Zone against *Aspergillusflavus In Vitro* and Pot Condition

ABSTRACT

Out of ten varieties tested against *Aspergillus flavus in vitro*, none of the variety was found resistant. The minimum seed infection (20.48%) was recorded in JL501 and KAUSHAL, whereas the maximum (80.46%) in GJG31, maximum seed germination was recorded in variety JL501 (99.99%), whereas the minimum recorded in GJG31(20.48%). The minimum mortality of germinated seeds (10.49%) observed in the varieties GJG9, GJG32, JL501,GG20, GJG22 and KDG128, whereas maximum in GJG31 (30.48%). In pot study, none of the variety was found resistant or moderately resistant. The minimum seed infection (40.47%) was recorded with GJG9, whereas the maximum (70.46%) in GJG31. Maximum seed germination was recorded in variety GJG9 (80.46%), whereas the minimum recorded inGJG31 (23.67%).The minimum seed mortality (10.49%) observed in varieties GJG9, TG37A and KDG128, whereas maximum in GJG31 (30.48%).

Keywords:Aspergillus flavus, Groundnut, Screening

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual legume and also known as peanut. It is the thirteenth most important food crop of the world and third most important oil seed crop usedfor vegetable oil production. Groundnut is cultivated in the tropical and subtropical regionsof the world. India stands first in the area with 4.7 million hectares, second in production(6.7 million ton) and productivity of 1422 $kgha^{-1}$ (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 $kgha^{-1}$. 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 (*Cercosporaarachidicola* Hori), late leaf spot (*Passalorapersonata* Berk. Curt. N. Arx), rust (*Puccinia arachidis*Speg), stem rot (*Sclerotium rolfsii*Sacc.), root rot [*Macrophominaphaseolina*(Tassi)Goid.],collar rot (*Aspergillus niger* Van Tieghem), afla rot(*Aspergillus flavus* Link Ex Fries),nematodedisease like root knot and viral diseases like stem necrosis, bud necrosis, mottle andclum(Ghewande and Reddy, 1986). Among the soil borne diseases, afla rot caused by *Aspergillus flavus* is a one of the important diseases in Gujarat region and also in Groundnut growingareas of the world (Klich, 2007). *Aspergillus flavus* is a pathogenic fungus in thephylum 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 on injured seeds. They produce a potent toxin and carcinogenic substance called aflatoxin. This toxin has great impact on human health.

2. MATERIAL AND METHODS

2.1 Screening *in vitro* conditions

Ten varieties of groundnut (Table 1) werescreen against *A. flavus.*Sound, healthy and mature 100 g kernels of each variety were surface sterilized with 0.1 per cent mercuric chloride solution for one minute and wash three times with sterilized distilled water. The seeds of each variety were decanted and were aseptically placed in sterilized Petri plates. The kernels were uniformly inoculated with spore

suspension of seven days old culture of *A. flavus* (10^6 spores ml^{-1} @ 1 ml per 10 g kernels), the seeds were rolled gently for evenly spread of inoculum. Ten seeds were placed aseptically in each sterile 15 cm diameter Petri plates with three replications andincubated under standard conditions of temperature and humidity. After 10 daysof incubation observations were record for seed infection, seed germination, mortality of germinated seed.

Table 1: Varieties of groundnut tested against *A. flavusin 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

2.2 Screeningin pot conditions

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

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

Level of resistance	Criteria
Resistant	Sporulating growth of *A. flavus* present on less than 15 per cent of seeds growth and sporulation sparse
Moderately resistant	Sporulating growth of *A. flavus* present on 16 to 30 per cent of seeds sporulating moderate to dense
Susceptible	Sporulating growth of *A. flavus* present on 31 to 50 per cent of seeds sporulating dense
Highly susceptible	Sporulating growth of *A. flavus* present on over 50 per cent of seeds with dense growth and sporulation

3. RESULTS AND DISCUSSION

The use of resistant varietycan be the most viable and economic approach to reduce*A. flavus* and aflatoxin problem. Hence, this study was undertaken to screen the groundnut varieties of North Gujarat Agroclimatic Zone for resistance to *in vitro* and pot condition seed infection by toxigenic isolates of *A. flavus* (AF-6). The seed infection, seed germination and mortality of germinated seeds are recorded after 10 days of incubation.

3.1 Screening *in vitro* conditions

3.1.1 Seed infection (%)

Among ten varieties tested, having seed infection ranged from 20.48 to 80.46 per cent.The minimum seed infection (20.48%) was recorded with JL501 and KAUSHAL(Table2), whereas the maximum seed infection (80.46%) was recorded in GJG31.Categorization of different varieties based on seed infection (Table 3) revealed that out of ten varieties tested, none of the variety was 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 against *A. flavus* invasion.

3.1.2 Seed germination (%)

In case of seed germination, 99.99 per cent seed germination was recorded in variety JL501. Which were followed by GJG32 (90.47%), KDG128 (90.47%) and TG37A (87.47%) (Table 2). The minimum seed germination was recorded in GJG31(20.48%).

3.1.3 Mortality of germinated seed (%)

Per cent mortality of germinated seed were recorded in the range from 10.49 to 30.48per cent (Table 2). The minimum mortality of germinated seeds (10.49%) was noted in varieties GJG9, GJG32, JL501, GG20, GJG22 and KDG128, whereas the maximum seed mortality was recorded in GJG31 (30.48%).

Table 2: Seed infection, seed germinationand mortality ofgerminated seedofdifferent groundnut varieties after 10 days of incubation with *A. flavus 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	

Figures 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 of significance.

Level of resistance on the basis of seed infection:
< 15 per cent – Resistant (R), 16-30 per cent – Moderately Resistance (MR),
31- 50 per cent – Susceptible (S), > 51 per cent – Highly Susceptible (HS)

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

Sr. No.	Categories	No. of varieties	Per cent 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 andGJG22
4	Highly Susceptible > 51%	05	50.0	GJG32, GJG17, GG20, GJG31 and KDG128

3.2 Screening in pot conditions

3.2.1 Seed infection (%)

Among ten varieties tested, having seed infection ranged from 40.47 to 70.46per cent.The minimum seed infection of 40.47 per cent was recorded with GJG9 (Table 4), whereas the maximum seed infection(70.46%) was recorded in GJG31 which was at par with GJG17 (67.21%). Categorization of different varieties based on seed infection (Table 5) revealed that out of ten varieties tested, none of thevariety was found resistant and moderately resistant against most virulent isolates of *A. flavus* (AF-6), whereas six variety *viz*., GJG9, GJG32,JL501, KAUSHAL, GJG22, KDG128 were found susceptible and the remaining four varieties were highly susceptible against *A. flavus* invasion.

3.2.2 Seed germination (%)

In case of seed germination, (80.46%) seed germination was recorded invariety GJG9. Which was followed by KDG128 (67.21%), TG37A (63.87%),GG20 (63.87%) andGJG32 (60.46%) (Table 4). The minimum seed germination was recorded inGJG31(23.67%).

3.2.3 Mortality of germinated seed (%)

Per cent mortality of germinated seed were recorded in the rangefrom 10.49 to30.48per cent (Table 4). The minimummortality of germinated seeds (10.49%) was noted in varieties GJG9, TG37Aand KDG128, whereas the maximum seed mortality was recorded in GJG31 (30.48%).

The present results are new as latest varieties adopted by farmers of North Gujarat which was not tested earlier. The investigation on seed infection, seed germination and mortality of germinated seed in *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 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, 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 that, high yielding lines were susceptible to invasion by *A. flavus* and aflatoxin contamination and indicated that, line Oh 53-1 showed highest resistance with low yield potential and J-11 showed resistanceto aflatoxin production and moderately susceptible reaction to *A. flavus* invasion.Mehan *et al.* (1991) screened out the 34 genotypes and reported varied level 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 per cent, with *A. flavus* as the predominant mold on groundnut. Waliyar*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 per cent compared with 15.6 per cent 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 level 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 that 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 high level of resistance with very low seed colonization by *A. flavus* compared to resistant check J-11.

Kumar (2005) evaluated fifteen genotypes in laboratory for seed coat resistance against *A. flavus* and 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. Waliyar*et al.* (2006) evaluated 14 new varieties for *in vitro* seed colonization with *A. flavus* and reported that new varieties (ICGV 91278,

91279, 91283, 91284, 91315, 91317, 91324, 91328,91341, 92302, 93305, 93328, 93379 and 94434) consistently showed < 10 per cent seed colonization as compared with susceptible variety TMV-2 (>50-90 per cent 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, Mwenje, 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. *Invitro* 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 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 variety was found immune as well as resistant against most virulent isolates. Further more TMV-2 showed maximum seed infection (83.33%), TMV-7 showed cent per cent 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*, comprised 9 local cultivars of Karnataka, 34 germplasm collections and 25 advance 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.Time to time after development of different genotypes, cultivars, varieties *etc.* and found the different levels of resistant which is important tools in breeding works and also helpful to the farmers to grow resistant variety against*A. flavus* and that's way aflatoxin reduction which was hurdles for groundnut export.

Table 4: Seed infection, seed germination and mortality of germinated Seedofdifferent groundnut varieties after 10 days of incubation with *A. flavus* in potconditions

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	

Figures 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 of significance.

Level of resistance on the basis of seed infection:

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

Table 5: Categorization of different groundnut varieties based on seed infectionin 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

CONFLICT OF INTEREST: None

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