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Microscopic Studies of *Aspergillus flavus* and its Effect on Nutritional aspects in Groundnut

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ABSTRACT

The effect of *Aspergillus flavus* on groundnut seed and oil quality is estimated using both susceptible and resistant cultivars JL 24 and J 11 respectively. The mode of entry of fungi into seed were observed by incubating seeds for 1, 3, 5, 7 and 9 days along with untreated seeds both externally using seed colonization severity scale and internally using scanning electron microscopy (SEM). The percent surface area colonized due to *A. flavus* with a severity score of 1, 2, 3 and 4 were observed at 3, 5, 7 and 9 days of incubation in resistant cv J 11. Whereas, in the susceptible cv JL 24 recorded severity score of 2, 3, 4 and 4 at 3, 5, 7 and 9 days of incubation period indicating the differences in seed colonization in resistant and susceptible cultivars. In the present study the penetration and establishment of the fungi in case of cv J11 was slow as compared to cv JL 24. The results revealed that presence of pathogen mycelium in the damaged seed coat with fractured discontinuous epidermis with loose broken cell junctions between epidermal cells were observed. Intense hyphal branching with haustoria and abundant sporulation were observed in groundnut cv JL 24 as compared to resistant cv. J 11. The per cent reduction in oil content was high in susceptible groundnut cv JL 24 (18.3%) as compared to resistant groundnut cv J 11 (9 %). While the reduction in oil content was less in the untreated seeds of groundnut cv JL 24 and groundnut cv J 11 (13.7% and 6%). Overall the per cent reduction in the protein content was found high in susceptible groundnut cv. JL 24 (16.3 %) as compared to resistant groundnut cv. J 11 (6.5 %). While the reduction in protein content was less in the untreated seeds of groundnut cvs. JL 24 and J 11 (14.2 % and 5.1 %). The per cent reduction in the unsaturated fatty acids like linoleic and oleic acids were high in susceptible groundnut cv JL 24 (17.5 % and 16.6 %) as compared to resistant groundnut cv. J 11 (15 % and 14 %). Whereas in the untreated seeds, the per cent reduction in linoleic and oleic acids were found low (11.3 % and 6 %) in cv J 11 and 13.3 % and 8.2 % in cv JL 24, respectively. The increase levels of saturated fatty acids viz., palmitic and stearic acids were high in susceptible cv. JL 24 (4.5 % and 4.5 %) as compared to resistant cv J 11 (3.9 % and 2.93 %). Where as in untreated seeds, the increased levels in palmitic and stearic acids were found low (2.5 and 2 %) in cv J 11 and 2.9 % and 1.94 % in groundnut cv. JL 24.

Keywords Aflatoxin, *Aspergillus flavus*, J 11, JL 24, NIRS.

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the important oil seed crop grown all over the world. India stands first in production and area under legumes in the world. The major crops include groundnut, gram, pigeon pea, green gram and black gram. India is one of the largest producers of oilseeds in the world and occupies an important position in the Indian agricultural economy. Groundnut kernels contain 40-50 % fat, 20-50 % protein and 10-20 % carbohydrate and are rich in vitamin E, niacin, riboflavin, thiamine, falacin, calcium, phosphorus, magnesium, zinc, iron and potassium (USDA 2010).

In India, groundnut is cultivated during *kharif* under rainfed conditions and in *rabi* and summer seasons under irrigated conditions. 90 - 95 % of the total crop area is grown in *kharif* season. It is cultivated in an area of 25.4 M ha worldwide with an annual production of 45.20 M t and productivity of 3824 kg ha^{-1} (FAO 2013). In India, the crop is grown to an extent of 5.53 M ha with a production of 9.67 M t and productivity of 1750 kg ha^{-1} (INDIASTAT 2013). In united Andhra Pradesh, the crop is grown to an extent of 1.37 M ha with a production of 1.01 M t and productivity of 890 kg ha^{-1}. In Telangana state, the crop is grown to an extent of 0.21 M ha with a production of 0.35 M t and productivity of 1690 kg ha^{-1} (Directorate of Economics and Statistics 2015).

Cultivated groundnut originated from South America (Weiss, EA 2000). Its cultivation is mostly confined to the tropical, subtropical, and warm temperate (zones) countries between 40°N and 40°S latitude. In 2009, China, India and the United States were the three largest producers of groundnut (USDA-FAS 2010). Availability of good quality seeds of high yielding varieties is the key to increase the productivity. Groundnut production all over the world is limited by various biotic and abiotic constraints that results in severe yield reduction. Seed borne pathogens affect the seed quality and lower the yield. Knowledge on the type of the pathogen associated with farmers seed and their effect on seed quality helps in adopting suitable strategies to manage them.

Groundnut is affected by several diseases, such as late leaf spot (*Phaeoisariopsis personate* Berk and Curt), early leaf spot (*Cercospora arachidicola* Hori), collar rot (*Aspergillus niger*), rust (*Puccinia arachidis* Speg) and bud necrosis (bud necrosis virus (BNV). Apart from these, deterioration in seed quality of groundnut is mainly due to *A. flavus* which makes the product unfit for marketing and consumption. In groundnut, seed and seedling decay and aflarot diseases were caused due to *A. flavus* pathogen. In addition to this, environmental conditions also play a major role in the attack of these molds and the crop is affected at various stages such as pre, post-harvest and storage conditions (Waliyar *et al.* 2008).

In agriculture, seeds play very important role for the production of healthy crop. About 90% of the crops all over the world are produced by using seeds. Seeds in the field as well as in ill storage conditions interact with several microbes which deteriorate the seeds, both qualitatively and quantitatively. The microorganisms grow on the seeds by the consumption of easily digestible components. The successful invasion or colonization, however, depends largely upon the efficiency of microorganisms to degrade complex molecules into simpler forms.

Fungi growing on stored grains reduce the germination rate, carbohydrate, protein, total oil content, increase moisture content and also enhancing other biochemical changes of grains (Bhattacharya 2002). Such seeds are unfit for human consumption and are also rejected at the industrial level. Like other plant products, oil seeds also carry a variety of microorganisms which originate from soil, air and plant source. However, presence of these microorganisms in or on products of human interest is not harmful. But under certain conditions they may start consuming such products for growth and reproduction and their activity is likely to cause undesirable change of varied nature in the product concerned including quality of nutrients, loss of constituents nutrients, poisoning of the products by mycotoxins, loss of germinability. The seeds are also found to be responsible for disease transmission because they carry number of pathogens, which get associated either in the field or in the post harvest storage condition. The associated moulds spoil the seeds, the process is known as seed biodeterioration.

Many developing countries including India have been trying to increase seed production in recent years. Unfortunately, due to lack of improved post-harvest preservation technique, a large portion of annual yield gets lost in storage, and these losses have been attributed partly to the microbial action in storehouses. Fungi growing on stored grains, can reduce the germination rate along with loss in the quantum of carbohydrate, protein and total oil content, induces increased moisture content, free fatty acid content enhancing other biochemical changes.

Groundnut being an oil seed, it contains lesser amount of carbohydrates than cereals but more amount of oil and protein and they break down into simple sugars and amino acids which is essential for germinating seed as an energy source. Reduction in oil and protein content and increased levels of free fatty acids were noticed in the stored kernels than in the pods due to invasion of storage fungi (Ramamoorthy and Karivaratharaju 1989). Presently, there are no tools that would measure the total oil content of groundnut seeds, economically and non-destructively. Groundnut pods have to be shelled and cleaned before oil content is measured which takes time and resources. Near-infrared reflectance spectroscopy (NIRS) is effectively utilized for analysis of chemical and physical properties without sample preparation and applied for the analysis of quality characteristics in food and agricultural commodities (Williams and Norris 2001).

Ultrastructural studies in understanding the mode of entry of *A. flavus* pathogen in groundnut resistant and susceptible cultivars through scanning electron microscopy was useful to identify the fungal structures and facilities correct diagnosis and detailed examination of taxonomic characters of seed borne fungi. Observation of microorganisms with light microscope and stereomicroscopy can be supplemented by alternative methods with greater precision, such as electron microscopy, often employed to study plants, algae, fungi, soil micro-organisms, seeds, fruits, pollens, spores (Heywood 1969), among other applications. This may be justified, because the introduction of scanning electron microscopy (SEM) has caused a revolution in the study of the microscopic world, considering advantages such as high depth of field giving three-dimensional aspect to the images, large magnitude of increase from 10 to 1,000,000 times, rapid processes of image digitalization and acquisition, easiness to prepare and operate samples, as well as accessible costs (Bozzola and Russell 1999)The images generated by SEM were also used to enlarge the possibilities of teaching-learning interaction based on the application of virtual reality techniques for microorganisms' visualization (Sforza and Eisenback 2001) and to study details of microorganisms' taxonomy (Alves and Pozza 2009). According to Machado *et al.* (2002), detection of various formae specialis of fungi is a challenge in Seed Pathology. In this context, the objective of the present study was to evaluate the application of standard SEM methodology as an alternative to identify seed-borne fungi.

MATERIALS AND METHODS

Groundnut cultivars of both susceptible (JL 24) and resistant (J 11) were used in the study.

Agar plate method (ISTA 1996)

PDA medium was prepared by using the following components for isolation of the seed mycoflora in the laboratory. Potato-200 g, Dextrose-20 g, Agar-20 g, Water-100 ml, pH-6.8.

Peeled potato pieces were boiled in 500 ml of distilled water in a 1000 ml beaker till the pieces got softened and the extract were collected in a beaker by sieving through a double layered muslin cloth. Agar - agar was melted in another 500 ml of distilled water in 1000 ml beaker into which 20 g dextrose was added. The final volume of the medium was made up to 1000 ml by adding sterile distilled water. The pH of the medium was adjusted to 6.8 with 0.1 N NaOH or 0.1 N HCl as the case may be with the pH meter. The medium was sterilized in an autoclave at 15 psi for 15 minutes.

Preparation of *A. flavus* culture

Toxin producing aflatoxigenic strain of *A. flavus* (Af 11 - 4) fungal culture was grown on potato dextrose agar (PDA) media. PDA plates were kept in BOD incubator for 7 days at 25 ± 20C. Greenish fungal sporulation was observed at 7 days after incubation.

Table 1. *Aspergillus flavus* seed colonization in groundnut cv J 11 and JL 24 at different days of incubation period

Interval (Days)	J 11 (Resistant cultivar)	JL 24 (Susceptible cultivar)
1	-	-
3	1	2
5	2	3
7	3	4
9	4	4

External seed colonization by *A. flavus* in groundnut cvs J 11 and JL 24

Seeds of groundnut cvs J 11 and JL 24 were artificially inoculated with toxigenic strains of *A. flavus* (isolate of Af 11 - 4) @ 10^9 conidia/ml were placed on sterilized petri plates and incubated at 1, 3, 5, 7 and 9 days (Table 1). Seeds of both the cvs J 11 and JL 24 were assessed for surface seed colonization by pathogen. The seed was colonised with 5% scanty mycelial growth and scanty sporulation it can be considered at scale 1 and the fungal growth with 5-25 %, 26-50 % and more than 50 % can be considered as scale 2,3 and 4 respectively (Thakur *et al.* 2000).

Scanning electron microscopic studies

Groundnut seeds of cv JL 24 (susceptible) and cv J 11 (resistant) were artificially inoculated with *A. flavus* @ 10^9 conidia/ml and placed on sterilized blotter papers and maintained at 25 ± 20 C in a BOD incubator. Further seed samples were prepared with an interval of 48 h i.e., 1, 3, 5, 7 and 9 days after incubation. Ultra-structural mycelial characters in the infected groundnut seeds were analyzed through Scanning Electron Microscopy (SEM) RUSKA laboratory, College of Veterinary Science, SPVN-RTSUVAFS, Rajendranagar, Hyderabad, Telangana state. Infected groundnut seeds were cut into sections measuring not more than 1-2 mm with a razor blade. Healthy groundnut seeds were aseptically washed and sectioned similarly to serve as control treatments. Samples were fixed in 2.5 % glutaraldehyde in 0.1M phosphate buffer (pH 7.2) for 24 h at 4^0C and post fixed in 2 % aqueous osmium tetroxide for 4 h. Dehydrated in series of graded alcohols and dried to critical point drying with CPD unit. The processed samples were mounted over the stubs with double - sided carbo conductivity tape and a thin layer of gold coat over the samples were done by using an automated sputter coater (Model – JEOL JFC-1600) for 3 min and observed under Scanning Electron Microscope (SEM-Model: JEOLJFC-1600) at required magnifications as per the standard procedures. The basic steps involved in SEM sample preparation was surface cleaning, stabilizing the sample with a fixative, rinsing, dehydrating, drying, mounting the specimen on a metal holder and coating the sample with a layer of material that is electrically conductive.

Effect of *A. flavus* infection on oil quality

Toxin producing aflatoxigenic strain of *A. flavus* (*Af* 11–4) was grown on potato dextrose agar (PDA) and plates were kept in BOD incubator for 7 days at 25 ± 2^0C. Seeds of groundnut cv JL 24 (susceptible) and cv J11 (resistant) were surface sterilized using 0.01 % clorax solution for one min. Seeds were washed in three times with sterile water and placed in dry blotter paper to remove the excess moisture. Seeds of both the cultivars were artificially inoculated with *A. flavus* @ 10^9 conidia/ml and kept on sterilized blotter paper discs of 9 cm diameter and moistened with sterile distilled water. The excess water was drained off from the plates. Groundnut seeds in four replications were placed equidistantly on sterile blotter paper and incubated at 25 ± 2^0C for a period of two months with an interval of 3, 7, 14, 21, 28, 35, 42, 49 and 56 days. After incubation at different intervals groundnut seeds of both the cultivars (infected and healthy seeds) were assessed for oil quality (per cent oil, protein and fatty acids i.e., saturated and unsaturated) by using near infrared reflectance spectroscopy (NIRS-model XDS RCA, FOSS Analytical AB, Sweden, Denmark). Non-destructive method of estimation was used in NIRS. Approximately 70 - 100 g of each intact groundnut sample was kept in rectangular cup in the NIR machine and readings were taken at different days of incubation.

Statistical analysis

The data obtained in various laboratory/glasshouse experiments were statistically analyzed by using Completely Randomized Design (CRD) as suggested

by Gomez and Gomez (1984). The data pertaining to percentage were angular transformed wherever necessary.

RESULTS AND DISCUSSION

External seed colonization by *Aspergillus flavus* in groundnut cvs J 11 and JL 24

Differences in seed colonization were observed in groundnut seeds of resistant cv J 11 and susceptible cv. JL 24 which were artificially inoculated with *A. flavus* toxigenic strain (*Af* 11 - 4) @ 10^9 conidia/ml and incubated for 1, 3, 5, 7 and 9 days along with untreated seeds were externally examined for seed colonization. The percent surface area colonized due to *A. flavus* with a severity score of 1, 2, 3 and 4 were observed at 3, 5, 7 and 9 days of incubation in resistant cv J 11. Whereas, in the susceptible cv. JL 24 recorded severity score of 2, 3, 4 and 4 at 3, 5, 7 and 9 days of incubation period indicating the differences in seed colonization in resistant and susceptible cultivars (Table 1) (Fig. 1a, 1b). Our findings stated that the cultivar J 11 is showing resistant mechanism against fungus compared to susceptible cultivar JL 24.

Similar variation in seed colonization due to *A. flavus* pathogen in groundnut accessions was reported earlier by Deshpande and Pancholi (1979) found that colonization of groundnut samples by the fungus *A. flavus*, was visible after 4 days of incubation. Thakur *et al.* (2000) and Nakai *et al.* (2008) also recorded the susceptibility of groundnuts to colonization of *A. flavus* especially during storage.

Scanning electron microscopic studies

Groundnut seeds of resistant cv J 11 and susceptible cv JL 24 were artificially inoculated with *A. flavus* @ 10^9 conidia/ml and incubated at 1, 3, 5, 7 and 9 days along with untreated seeds. Nature of seed colonization by *A. flavus* and entry of the pathogen into the groundnut seeds was observed with Scanning Electron Microscopy (SEM). The results revealed

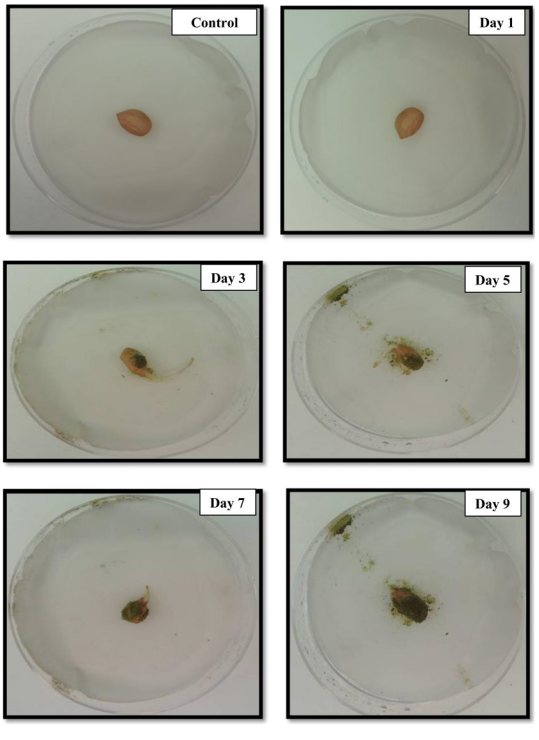

Fig 1a. External seed colonization of *A. flavus* in groundnut cv J 11 at different days of incubation period.

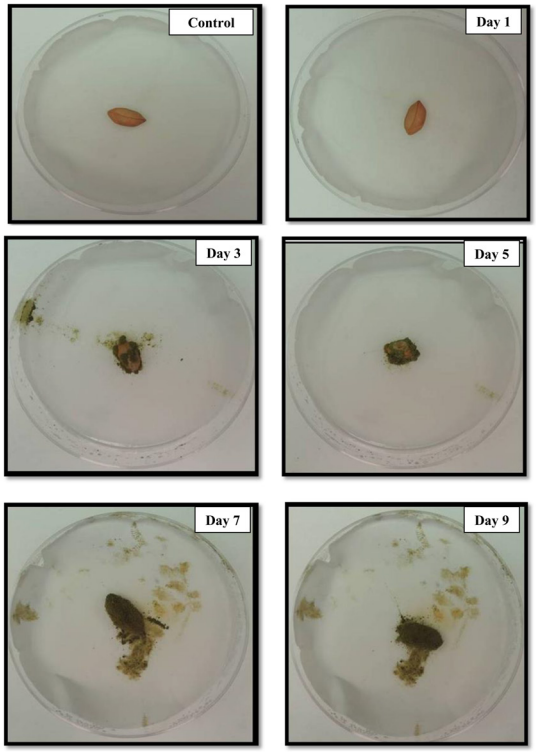

Fig 1b. External seed colonization of *A. flavus* in groundnut cv JL 24 at different days of incubation period.

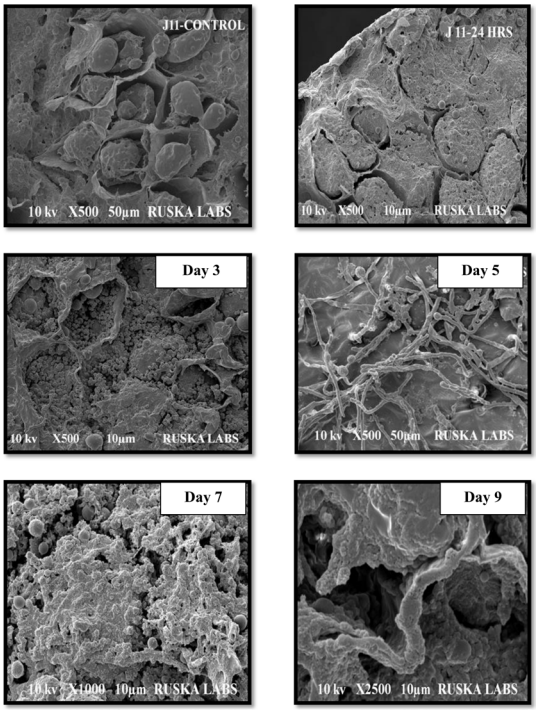

Fig 2a. Scanning electron micrographs of *A. flavus* growth in treated seeds of groundnut resistant cv J 11 at different days of incubation period.

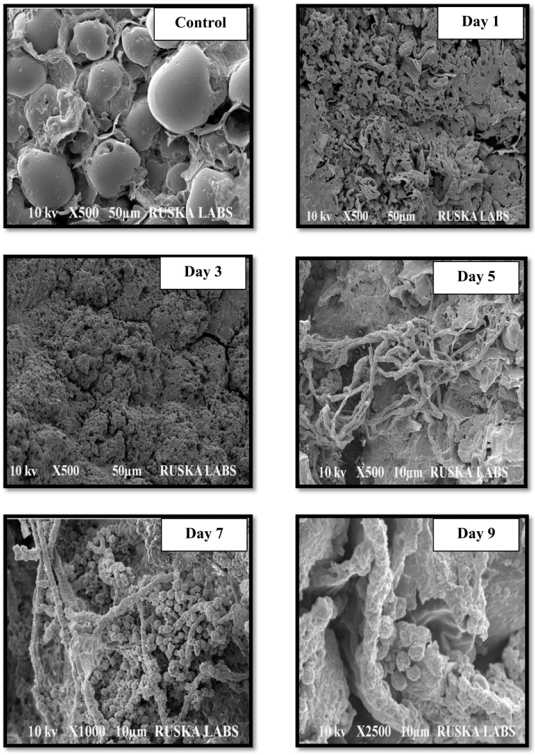

Fig 2b. Scanning electron micrographs of *A. flavus* growth in treated seeds of groundnut susceptible cv JL 24 at different days of incubation period.

that presence of pathogen mycelium in the damaged seed coat with fractured discontinuous epidermis with loose broken cell junctions between epidermal cells were observed. In addition to this rough, discontinuous, disorganized parenchyma, broken and missing cell walls and almost with total depletion of storage proteins were observed. Similarly, cells of healthy embryos of both cultures were well organized, unruptured cell walls with minimal intercellular space and abundant storage proteins. Hyphae penetrated into embryonic tissues and established intercellularly and intracellularly leading to an overall depletion of storage proteins. Intense hyphal branching with haustoria and abundant sporulation were observed in groundnut cv JL 24 as compared to resistant cv J 11 (Fig. 2a, 2b). Seed coat responsiveness is a major key factor in establishing the pathogen when infection occurs. In the present study when the seeds of both the cultivars of groundnut were inoculated with *A. flavus* toxigenic strain, the penetration and establishment of the fungi in case of cv J11 was slow as compared to cv JL 24. Cell wall fortifications such as deposition of callose, cellulose, lignin and structural proteins directly below the point of attempted penetration to prevent the pathogen infection (ElAe gendy *et al.* 2001). A thorough understanding of the host pathogen interaction between groundnut and *A. flavus* may provide information that might be used to develop novel detection and screening methods. The toxic properties of the aflatoxins produced by *A. flavus* are a major concern for growers and consumers of groundnut. Elimination of the threat of infection due to *A. flavus* is important before groundnut seeds goes into the storage. The present investigation reveals that *A. flavus* was seed borne in nature and contaminated seeds were important source of inoculum for seed infection and spread of the fungus from one seed to another during storage. SEM studies proved to be a reliable method to detect the intercellular and intracellular hyphae of *A. flavus* which is undetectable with the naked eye or by conventional light microscopy.

Table 2. Effect of *A. flavus* infection on oil and protein content (%) in treated and untreated seeds of groundnut cvs J 11 and JL 24.

Sl. No	Interval (Days)	Oil content (%)				Protein (%)			
		cv J 11		cv JL 24		cv J 11		cv.JL 24	
		Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
1	1	50.3	50.6	50.6	50.6	33.6	33.2	41.2	40.1
2	3	47.2	48.4	47.9	47.9	33.2	31.0	41.0	39.9
3	7	47.1	47.5	46.8	47.8	33.0	31.0	39.8	37.1
4	14	47.0	47.5	46.8	47.5	32.4	30.2	39.8	36.4
5	21	45.8	47.4	46.4	47.3	31.4	29.7	38.9	36.4
6	28	45.8	47.4	46.3	47.0	28.9	29.2	34.9	36.3
7	35	45.5	47.2	43.5	42.5	28.8	29.2	33.0	35.7
8	42	45.5	46.1	38.3	39.3	28.5	28.8	29.6	29.9
9	49	44.8	45.8	34.9	38.0	27.6	28.1	29.4	29.4
10	56	41.3	44.6	32.3	36.9	27.1	28.1	24.9	25.9
	SE (m) ±	1.29	0.91	0.73	0.93	1.19	0.68	1.62	1.57
	CD at 5 %	3.80	2.70	2.17	2.76	3.52	2.03	4.80	4.65

Mycelium of *A. flavus* was seen established in the host tissues both intercellularly and intracellularly and continuous branching of young hyphae was seen in the groundnut seed (Achar *et al*. 2009). Structure of aflatoxigenic molds and their identification up to species level and characterization (Rodriguez *et al.* 2007). Hermetz *et al.* (2014) observed that establishment of seed borne nature of *A. flavus* and its significance in seed and seedling infection using light microscopy, Scanning Electron Microscopy and Transmission Electron Microscopy. Scanning Electronic Microscopic (SEM) methodology enabled to observe the interaction of fungi on surface of seeds and potential to increase the opportunities for teaching and learning in Seed Pathology depending on the level of detail of observed structures. The adoption of this technique in the future seed health analysis could be useful to identify fungal structures that enable ensuring the implementation of correct diagnoses as well as to facilitate conduction of more detailed taxonomic classification of seed-borne fungi.

Effect of *A. flavus* infection on oil content in groundnut resistant cv J 11 and cv JL 24

The effect of *A. flavus* infection on oil content in groundnut seeds were differed in treated and untreated seed samples analyzed. The oil content was gradually reduced at 1 to 56 days after incubation to an extent of 50.3 % to 41.3 % in the seeds treated with *A. flavus.* In the untreated seeds (control) there was less reduction in oil content (50.6 % to 44.6 %) (Table 2). In cv JL 24 the reduction in oil content was high in treated seeds (50.6 % to 32.3 %) as compared with untreated seeds (50.6 % to 36.9 %).

The per cent reduction in oil content was high in susceptible groundnut cv JL 24 (18.3%) as compared to resistant groundnut cv J 11 (9 %). While the reduction in oil content was low in the untreated seeds of groundnut cv JL 24 and groundnut cv J 11 (13.7 % and 6 %).

The reduction in oil content might be attributed to lipids present in the seeds were primarily neutral triglycerides and their hydrolysis to free fatty acids and glycerol were catalyzed by seed borne fungi which caused the oxidation of fatty acids and inactivation of enzymes. This might be one of the reasons for the reduction in oil content in the groundnut cultivars. The present results are in agreement with Deshpande and Pancholy (1979), Bhattacharya and Raha (2002) and Narayanswamy (2003) who reported that significant changes in oil content in the inoculated groundnut seed samples with the advancement in the storage period.

Effect of *A. flavus* on protein content in groundnut resistant cv J 11 and cv JL 24

The effect of *A. flavus* infection on protein content in groundnut cv J 11and JL 24 was recorded. The

protein content was gradually reduced at 1 to 56 days from 33.6 % to 27.1 % in the treated seeds and untreated seeds (33.2 % to 28.1 %). In cv JL 24 the protein ranging from 41.2 % to 24.9 % in the seeds treated with *A. flavus* where-as in the untreated seeds (control) recorded less reduction in protein content 40.1 % to 25.9 % (Table 2).

Overall the per cent reduction in the protein content was found high in susceptible groundnut cv JL 24 (16.3 %) as compared to resistant groundnut cv J 11 (6.5 %). While the reduction in protein content was low in the untreated seeds of groundnut cvs JL 24 and J 11 (14.2 % and 5.1%). The present results revealed that the rate of depletion in total protein was significantly differed. It might be attributed that protein served as a primary source of readily available carbon and nitrogen for growth and metabolism of the invading fungi. Loss in protein content during the early phase of invasion and incubation indicated that proteolysis and formation of simpler compounds such as amino acids which were utilized by the fungi. Similar trend of reduction in protein content in the groundnut due to storage fungi was reported earlier by Narayanswamy (2003), Ushamalini *et al.* (1998), Rammorthy and Karivarataraju (1989) noticed that there was a progressive decrease in oil and protein content and an increase in free fatty acids in stored kernels because invasion of storage fungi to kernels. Reduction in oil content in *A. flavus* inoculated seeds compared to apparently healthy ones. Invading fungus causes the oxidation of fatty acids and inactivation of enzymes (Adiver *et al*. 2015). Braccini *et al.* (2000) observed that, reduction in protein, lipid content during storage. Kakde and Chavan (2011) also found that, storage fungi are responsible for reduction in oil content in oil seeds. Bilgrami *et al.* (1976) studied that, infesting seeds with *A. flavus* results in significant decrease in seed protein.

Effect of *A. flavus* on saturated fatty acids (palmitic and stearic acid) in groundnut resistant cv J 11 and susceptible cv JL 24

The effects of *A. flavus* infection on palmitic acid content of groundnut seeds were recorded. The palmitic acid content in resistant cv J 11 was gradually increased from 1 to 56 days after incubation. In the treated seeds the increase in palmitic acid content was high (11 % to 14.9 %) as compared with untreated seeds (control) (11 % to 13.9 %). Where as in susceptible cv JL 24 the increase in palmitic acid content of 11 % to 15.5 % in the treated seeds where as in the untreated seeds there was slow increase of 11.0 % to 13.5 %.

The effects of *A. flavus* on stearic acid content of groundnut seeds were also recorded. The increased levels of stearic acid content in resistant cv J 11 was observed at 1 to 56 days after incubation in treated

Table 3. Effect of *A. flavus* infection on saturated fatty acids (Palmitic and Stearic acid) content (%) in treated and untreated seeds of groundnut cvs J 11 and JL 24.

Sl. No,	Interval (Days)	Palmitic acid (%)				Stearic acid (%)			
		cv J 11		cv JL 24		cv J 11		cv JL 24	
		Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
1	1	11.0	11.0	11.0	11.0	0.72	0.74	0.72	0.71
2	3	11.4	11.5	11.5	11.3	0.79	0.83	0.96	0.72
3	7	11.6	11.6	11.5	11.5	1.36	0.95	0.95	1.36
4	14	12.5	11.7	11.8	11.5	1.67	0.96	1.19	1.67
5	21	12.5	11.7	11.9	11.9	1.91	1.68	2.48	1.91
6	28	12.9	12.5	11.9	11.9	2.20	2.17	2.84	2.30
7	35	13.5	12.5	12.3	13.0	2.37	2.30	3.35	2.31
8	42	13.5	12.5	12.8	13.0	2.40	2.48	3.45	2.40
9	49	14.3	13.8	13.0	13.5	2.63	2.56	4.89	2.63
10	56	14.9	13.9	15.5	13.5	3.65	2.68	5.31	2.71
	SE (m) ±	0.31	0.39	0.56	0.55	0.20	0.30	0.39	0.19
	CD at 5 %	0.92	1.17	1.66	1.64	0.61	0.90	1.16	0.57

Table 4. Effect of *A. flavus* infection on unsaturated fatty acids (Linoleic acid and Oleic acid) content (%) in treated and untreated seeds of groundnut cvs J 11 and JL 24.

Sl. No,	Interval (Days)	Linoleic acid (%)				Oleic acid (%)			
		cv J 11		cv JL 24		cv J 11		cv JL 24	
		Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
1	1	42.1	43.0	40.9	40.8	43.9	43.9	41.7	42.0
2	3	41.4	41.5	39.3	37.9	42.8	42.9	37.8	41.1
3	7	40.6	38.9	37.3	37.8	40.3	41.3	36.9	40.6
4	14	36.6	36.9	32.6	33.8	40.2	41.2	33.6	38.0
5	21	36.1	36.8	30.9	30.3	40.1	40.6	30.3	36.9
6	28	34.4	33.8	30.2	29.4	40.1	40.2	29.4	36.8
7	35	33.9	33.6	29.8	29.3	39.7	40.1	29.0	36.8
8	42	32.3	33.2	25.4	29.3	38.9	39.7	27.7	35.9
9	49	31.3	32.1	24.1	28.1	38.6	38.7	27.5	34.6
10	56	27.1	31.7	23.4	27.5	29.9	37.9	25.1	33.8
	SE (m) ±	2.15	1.70	4.04	2.43	1.70	1.02	1.98	1.82
	CD at 5 %	6.34	5.03	11.9	7.18	5.01	3.03	5.85	5.38

seeds (0.72 % to 3.65 %) and untreated seeds (0.74 % to 2.68 %). Whereas, in susceptible cv JL 24 the stearic acid content was gradually increased from 1 to 56 days after incubation. The per cent increase in stearic acid content was high (0.72 % to 5.31 %) in the treated seeds as compared with the untreated seeds (0.71 % to 2.71%) (Table 3).

Many of the fungi have been reported to cause physical and biochemical changes in crops during storage as well as in releasing toxic substances which tend to limit their use and general acceptability. In general, when the seeds were stored at higher moisture content the activity of Aspergilli were found high which releases toxic metabolites into seeds. Presence of these toxic substances in the seeds mainly affects seed quality and adversely making the seeds unfit for consumption. An increased levels of free fatty acid contents in the groundnut cultivars over a period of storage indicates the breakdown of triglycerides in groundnut oil leading to an eventual deterioration of the seed quality and production of hydrolytic rancidity.

The present results are in conformity with Rammorthy and Karivaratataraju (1989) who reported a progressive increase in free fatty acid levels in the stored kernels than pods because there was invasion of storage fungi. The increased levels of free fatty acid content in the damaged seeds by fungal invasion (Jain 2008). Rancidity was significantly increased during storage. Storage fungi can change fat quality of groundnuts by hydrolytic enzymes producing free fatty acids and glycerol. There was decrease in crude fat because fungi might have degraded the lipids by lipase enzyme (Mutegi *et al*. 2013).

Effect of *A. flavus* infection on unsaturated fatty acids (linoleic acid and oleic acid) in groundnut resistant cv J 11 and susceptible cv JL 24

The effect of *A. flavus* infection on linoleic acid content of groundnut seeds were recorded. The linoleic acid content was gradually reduced at 1 to 56 days after incubation. The reduction in linoleic acid was high in the treated seeds (42.1 % - 27.1 %) as compared with untreated seeds (43 % to 31.7 %). Whereas, in susceptible cv JL 24 the linoleic acid content was gradually reduced at 1 to 56 days after incubation. The rate of decrease was high in the treated seeds (40.9 % - 23.4 %) as compared with untreated seeds (40.8 % - 27.5 %) (Table 4).

The effect of *A. flavus* infection on oleic acid content in groundnut cv J 11 was also recorded. The reduction in oleic acid content was observed in the treated seeds (43.9 % to 29.9 %) as compared with untreated seeds (43.9 % to 37.9 %). Similar trend of reduction in oleic acid content were observed in treated seeds of susceptible cv JL 24 (41.7 % to 25.1 %) and untreated seeds (42.0 % to 33.8 %) (Table 4).

The reduction in linoleic and oleic acid contents were high in the treated seeds as compared to the untreated seeds.

The per cent reduction in the unsaturated fatty acids like linoleic and oleic acids were high in susceptible groundnut cv JL 24 (17.5 % and 16.6 %) as compared to resistant groundnut cv J 11 (15 % and 14 %). Whereas, in the untreated seeds, the per cent reduction in linoleic and oleic acids were found low (11.3 % and 6 %) in groundnut cv J 11 and 13.3 % and 8.2 % in groundnut cv JL 24, respectively.

These results are in conformity with Braccini *et al.* (2000) who reported that reduction in unsaturated fatty acids, protein and lipid content of soybean.

CONCLUSION

Present investigation reveals that *A. flavus* was important source of inoculum for seed infection and spread of the fungus during storage. SEM studies proved to be a reliable method to detect mode of entry of *A. flavus* which is undetectable with the naked eye. The present investigation shows that during incubation or storage seeds were easily invaded by storage fungi. By understanding the effect of fungi in storage in seeds especially in oil seeds should be taken with much care. Awareness should be create among farmers to store the seeds in a scientific conditions with a suitable practices. The implementation of predictive models like NIRS and SEM support to find applications in field, laboratory and processing plants.

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