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# EFFECT OF SEED-BORNE MYCOFLORA ON GERMINATION AND FATTY ACID PROFILE OF PEANUTS

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**A B S T R A C T**

Peanut (*Arachis hypogaea* L.) occupies the second largest area among the oilseed crops after mustard and rapeseed in Pakistan. It is the main legume crop and primarily grown as oilseed crop. Due to the presence of high percentage of fatty acids in its oil, its nutritional properties are considered as favorable for human and animals. In the present study, seed-borne fungi were recovered from four different varieties of peanut with their incidence percentage. Data revealed that all the varieties were affected by the fungal species however, the variety, Golden showed maximum incidence of up to 70 % of *Aspergillus* spp. while the variety BARI-2011 had the least percent incidence of upto15 % of *Rhizopus* spp*.* Germination test was carried by Standard rolled paper method and minimum growth (8% as healthy seed germination) was observed in Golden and maximum growth in BARI-2011 of 22 % as healthy seed germination. Fatty acid profile determination was done by Gas Chromatography percentage of fatty acids were examined and evaluated that the fungal pathogen has an influence on the oil contents and fatty acids in the peanut seeds. The saturated fatty acids were increased and unsaturated fatty acids decreased due to an oxidation process and production of toxic metabolites in the seed which also reduced the germination percentage. It is concluded from the present study that the seed borne fungi are known to affect adversely seed germination and fatty acid composition due to the production of toxic metabolites in the peanut seeds.

**Keywords**: Fatty acid profile, seed germination, oxidation process, peanuts, seed born fungi.

## INRODUCTION

Peanut (*Arachis hypogaea* L.) is among the main food legume crop in the world and grown primarily as oilseed crop in the tropic and temperate zones (Bensal *et al*., 1993). It is very good source of edible oil and is also consumed as dried fruit (GOP, 1991).It is the sixth, most key oilseed crop in the world. Containing 48 to 50% of oil and 26 to 28% protein, also have high amount of nutritional fibers, minerals and vitamins. Grown globally on 26.3 million hac, total production of 37 million metric ton and standard productivity of 1.4 metric t/ha (FAO, 2003). The main countries producing peanut are China, India, Nigeria, United States, Indonesia and Sudan (ICRISAT, 2007). Asia has a 55.2% of total peanut production, China produces 37.5% of overall world production and is largest producer of peanut (Dutta *et al*., 2011).

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In Pakistan, mainly peanut is grown in rainfed areas and about 84 percent of the total area lies in Punjab, in Khyber Pakhtunkhwa 13 percent and 3 percent in Sindh (GOP, 2008).This crop is generally grown in drier southern part of the Pothwar (Ali *et al*., 2002). Out of the total area in Pakistan under peanut, 80% lies in Pothwar tract approximately, contributes 92% of the total production of the country (GOP, 2011).Peanut's cultivation is over an area of 99.4 hectares in Pakistan, with a production of about 1017 kg/ hectare or 101 tones during 2001-02(GOP, 2002).

Like other high value crops peanut is attacked by over 50 genera of fungi and 1 bacterium, 15 viruses, 16 nematodes, and 2 phanerogamic parasites (Naikoo *et al*., 2013). However, various microorganisms cause disease. *Aspergillus niger* and *A. flavus* are the most important pathogens of both temperate and tropical countries and causes the disease crown rot of peanut. Mostly diseases are caused by seed borne fungi that can survive in

infected seeds of peanut (Magnoli *et al*., 2006). Fungi are one of the factors in storage seeds which reduce seed viability (Olkowski *et al*., 1995). Among different disease causing fungi, *F solani* and *F. oxysporum* causes damping off of the peanut seedlings (Reddy and Rao, 1980). *Aspergillus flavus* attacks on the germinating peanut seeds. Different mold fungi and their toxin producing ability in stored grains deteriorate the products, which is detected by any workers (Vedahayagam *et al*., 1989).

Peanut seeds and seedlings are highly susceptible to several fungal pathogens; e.g, *Rhizoctonia* spp., *Aspergillus* spp., *Fussarium* spp., *Alternaria* spp., *Pythium* spp., *Cladosporium* spp., *Penicillium* spp. (Mohamed and El-Metwally, 2001). Following fungi, *Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp., *Rhizopus* spp., *Penicillium* spp., *Aspergillus* spp., *Trichothecium*, *Macrophomina phaseolina*, *Alternaria* spp., *Botrytis cinerea*, *Helminthosporoium* spp., *Mucor* spp., *Curvularia* spp., *Cladosporium* spp., *Botryodiplodia theobromae*, *Chaetomium* spp. from peanut pods, shells and seeds were isolated (Porter *et al*., 1990; Shim-Hyeongkwon *et al*., 1996).

Fatty acids can be classified in classes as saturated, mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, the unsaturated fatty acids are classified into sequence known as omega (Assiesa *et al.*, 2004; Ristic and Ristic, 2003). An important feature common to mostly plant source oils and fats is the high percentage of nonsaturated fatty acids in triacylglycerols. In common, higher degree of un-saturation of vegetable oils fatty acids, the susceptible they are to oxidative deterioration (Bradley and Min, 1992; St. Angelo, 1996; Zambiazi and Zambiazi, 2000). Peanut oil showed the highest long chain fatty acid content, comprising 6, 18% of arachidic, behenic and lignoceric fatty acids.

Mycoflora and its effect on fatty acid composition of peanut seeds have also been studied in the world (Anderson *et al*., 1998). But information about this on peanut seeds in Pakistan is lacking. Keeping view on the importance of the peanut as an oilseed crop, present study was planned to determine the effect on oil and fatty acid composition.

## MATERIALS AND METHODS

**Collection of Seed Samples:** Seed samples of Peanut were collected from BARI Chakwal and NARC, Islamabad varieties included BARI-2000, BARI-2011, ZAKI and Golden. These seed samples were investigated at Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi and Central seed health test Laboratory of Seed Certification and Registration Department, Islamabad and National Agriculture Research Centre, Islamabad.

**Detection of Seed Borne Mycoflora:** Peanut seed samples were analyzed for their association of seed mycoflora by using standard blotter paper method (ISTA, 1985). A working sample of 400 seeds were taken at random. At the time of isolation, the samples were surface sterilized with 1% Chlorox aqueous solution for approximately one minute. These seeds were rinsed in sterilized distilled water then blot dried. Seeds plated in the each petridish were 5 seeds per plate. The plated seeds were incubated at 25 ± $2^0$C for 8 days. Petri plates having diseased specimens were observed to identify the seed borne fungi on the basis of colony color. Further confirmation was made by preparing the slides and observed under microscope with low as well as high magnifications (10, 20, 40, 60 and 100X). The seeds will be examined under stereoscope based on morphological habit characteristics (Malone and masket, 1964).

Disease Incidence was recorded by the following formula:

$$\text{Incidencde \%} = \frac{\text{No. of infected seeds}}{Total\ No.of\ Planted\ seeds} \times 100$$

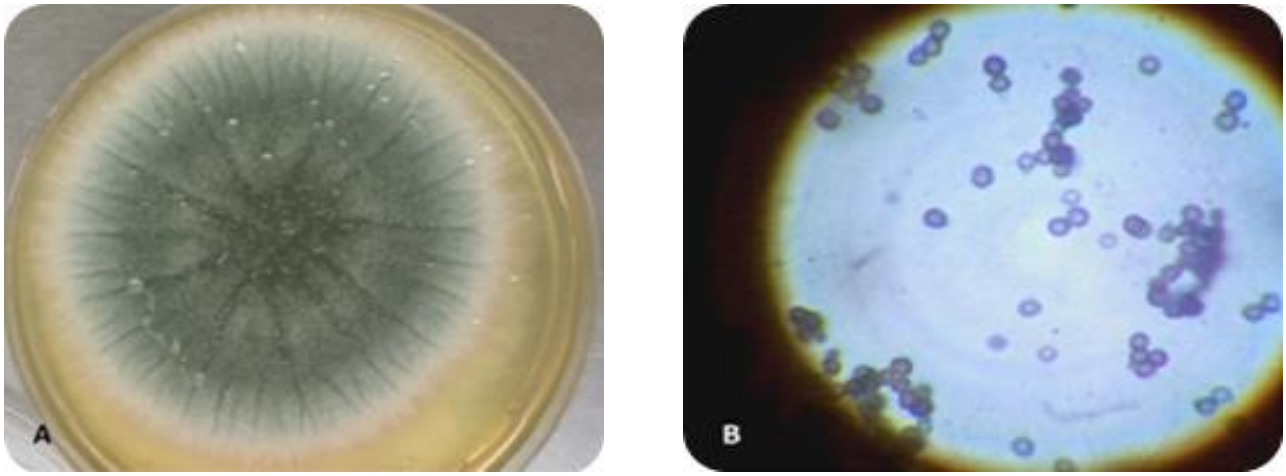
**Effect of Mycoflora on Germination:** Germination test was carried out by using standard rolled paper method (ISTA, 1985). Two hundred untreated seeds were randomly selected and allowed to germinate between two blotter paper layers at 25 ± $2^0$C. After 12 days of incubation, seeds were counted. The emerged seedlings were graded as normal and abnormal as defined by (Anwer *et al*., 1994).

**Fatty Acid Profile Determination:** Eleven naturally infected and four uninfected peanut seed samples comprising four varieties were used to study fatty acid profile in relation to seed mycoflora using gas chromatography which determines the fatty acid acylesters into oil. Oil from peanut seeds were extracted with Raney oil seed crusher. One ml of methylating solution was dispensed into test tubes in which 0.5ml petroleum ether was added. A loop full of oil extracted from sample was added to each test tube. Oil loop was wirled in solution to disperse for methylation. One µl of the upper layer of these samples was injected into gas chromatographer (Raney, 1987; Hougenand-Bado, 1973). Results were recorded by G.C. data recorded/analyzer and concentration of each fatty acid in percentage was measured.

**RESULTS AND DISCUSSION**

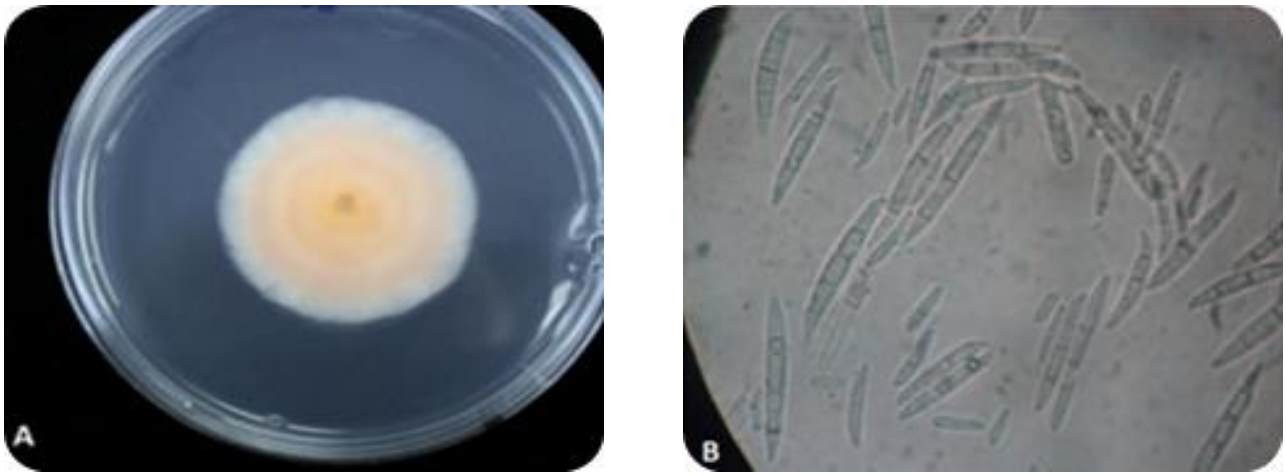
**Detection of Seed- Borne Fungi:** Fungal colonies were observed and identified as following:

***Aspergillus* spp.:** Septatic and hyaline hyphae. At apex, conidiophores were terminated in vesicle. Round conidia having radial chains over the phialides. Conidial heads of Specie have conidial head and loosely columnar. Conidiophores of the pathogen's dimensions 800 x 15-20, 20-45 µm. Sutton *et al.* (1998) and De-Hoog (2000) also observed these characteristics.




Macroscopic (A) and Microscopic (B) view of *Aspergillus* spp.

***Fusarium* spp.:** Colony contain woollen to cottony, flat and spreading having whitish, creamy, yellowish, and surface is pinkish, colorless to purple and brown on reverse. Colonies of *Fussarium oxysporum* are whitish to ting with the salmon on the surface and purple on reverse. Similar findings were recorded by Hennequin, (1999).

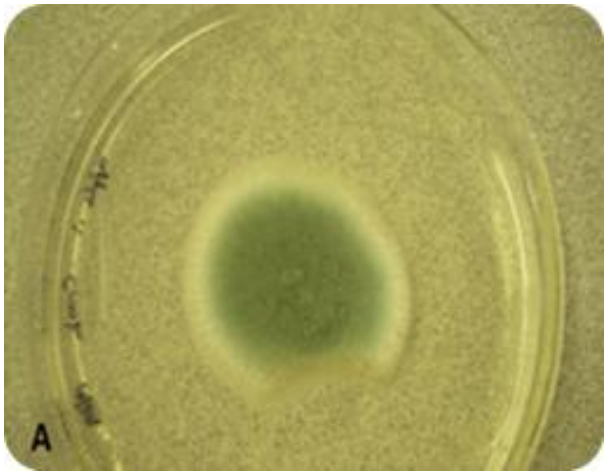


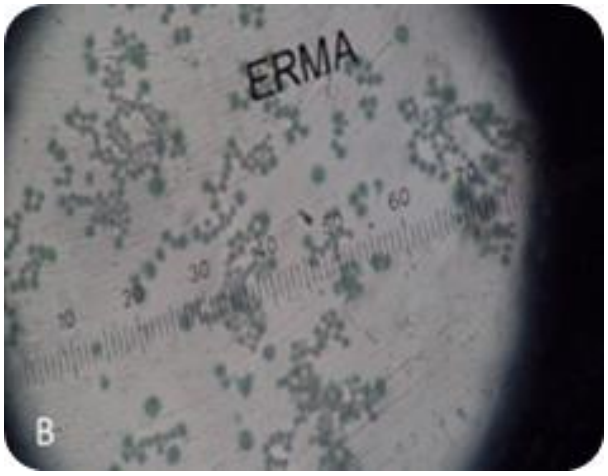

Macro scopic (A) and Microscopic (B) view of *Fusarium* spp.

***Penicillium* spp.:** Colony shows the growth rapidly, and is flat and filamentous, have cottony textured. Colonies are blue green from white, turning from pinkish to yellowish with age. Pale at the back, the conidia round, single celled, un-branched at apix of the Phialides. Larone (1995) and Sutton *et al.* (1998) described the similar features.

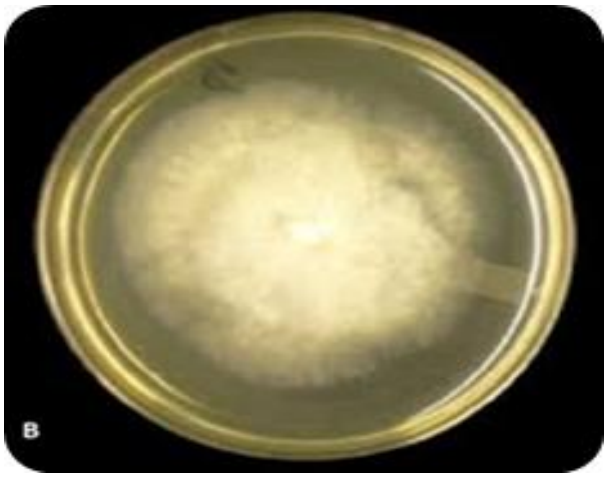
***Mucor* spp.:** *Mucor* colonies were found fast growing at temperature of 25-30 $^0$C and covered the growing media's surface rapidly. Appeared fluffy having color initially white, then become grayish brown. Hyphae 6-15 µm broad. Rhizoid, stolon both were not present. Sporangiophores were small and erected.

***Rhizopus* spp.:** The texture of the *Rhizopus* spp colonies were cottony candy like and it was white initially from the front and then it turned from grey to brown color upon maturity. Firstly whitish and then pale from back (Sutton *et al.,* 1998). Broad hyphae with the diameter 6-15 µm, non-septate. Similar finding were recorded by Larone (1995) and Sutton *et al.* (1998).

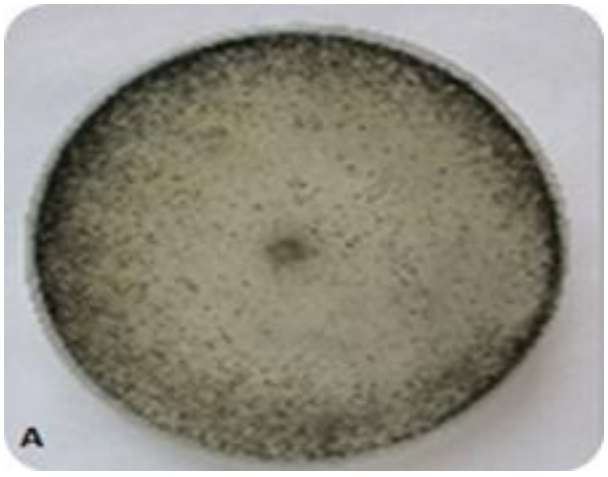


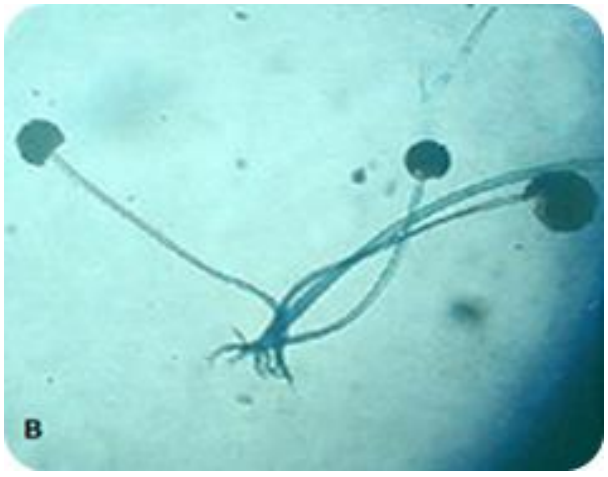




Macroscopic (A) and Microscopic (B) view of *Penicillium* spp.

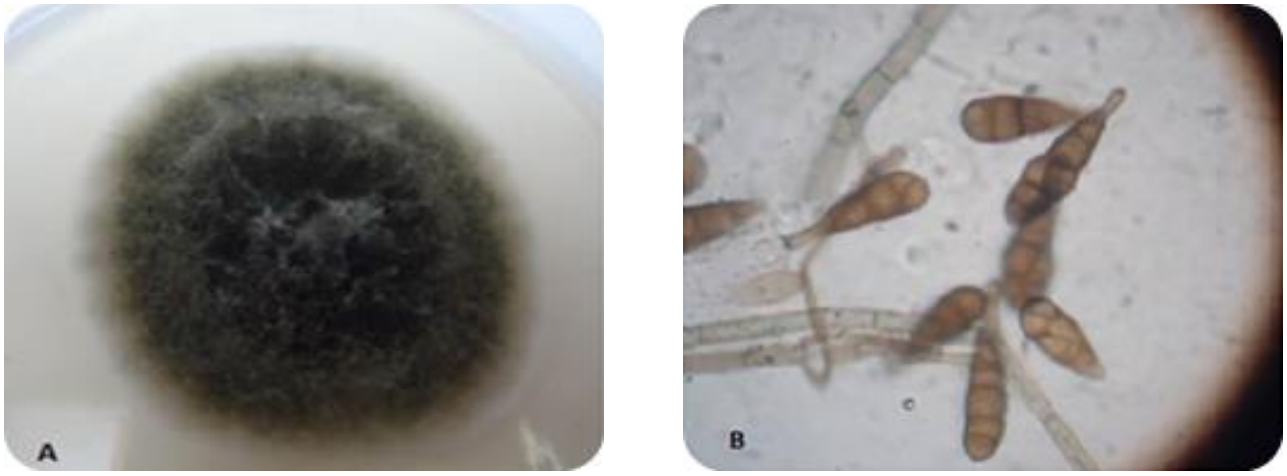

Microscopic (A) and Macroscopic (B) view of *Mucor* spp.




Macroscopic (A) and Microscopic (B) view of *Rhizopus* spp.

***Alternaria*spp.:** Rapid growth was shown by the colonies of the pathogen like flat and downy to cottony. The surface was grey having brown to black reverse. These characteristics were also described by Schell (2003). Hyphae is septate whereas conidiospores were also septatic and dark, large having 8-16 x 23-50 µm, singled or in acropetal chain with both, transverse and longitudinal septations. Similar characters were described by Larone (2002) and Schell (2003).




Macroscopic (A) and Microscopic (B) view of *Alternaria* spp.

Total 40 seeds of each seed sample were taken randomly from the 400 seed samples and observed. Frequency of infection in each variety was recorded as discussed in the Table 1. The maximum percent incidence was found associated with the seeds of cultivar (cv.) Zaki (41%) followed by variety Golden (37.75%), whereas the minimum overall fungal incidence was found associated with BARI 2011 (24.6%). Aspergillus was the most abundant fungal genus with overall incidence of 54.5% followed by *Penicillium* spp. with overall incidence of 44.75%. The highest incidence of *Aspergillus* was recorded upon the seeds of ZAKI (71%) and Golden (70%).

Table 1. Percent Incidence of Mycoflora associated on different varieties of Peanut.

| Sr. No. | Name of Fungi | % Incidence of Mycroflora | | | | |
|---|---|---|---|---|---|---|
| | | BARI-2000 | BARI-2011 | ZAKI | Golden | |
| 1 | *Aspergillus* spp. | 45 | 32 | 71 | 70 | 54.5 |
| 2 | *Fusarium* spp. | 30 | 8 | 45 | 40 | 30.75 |
| 3 | *Penicillium* spp. | 50 | 56 | 38 | 35 | 44.75 |
| 4 | *Mucor* spp. | 30 | 28 | 21 | 20 | 24.75 |
| 5 | *Rhizopus* spp. | 15 | 8 | 46 | 45 | 28.5 |
| 6 | *Alternaria* spp. | 20 | 16 | 25 | 15 | 19 |
| | | 31.6 | 24.6 | 41 | 37.5 | Mean % |

Various disease producing organism, *Fussarium solani, Fussarium oxysporum* are the cause of damping off in peanut seedlings. Infection of roots in peanut, caused by *Fussarium solani* and *Rhizoctonia solani* with *Glomus mosseae* (Reddy and Rao, 1980). *Macrophomina phaseolina* cause infection and reduce germination and vigor of seeds of sunflower beside producing pre and post-emergence diseases, seedling blight and charcoal rot. *Aspergillus flavus* attack germinating peanut seeds (Clinton, 1960). *A. niger* caused crown rot disease of peanut. According to Sullivan (1984), groundnut seeds are highly susceptible to diseases because they are rich in nutrients useful for numerous fungi such as *Rhizopus* spp., *Penicillium* spp., *Aspergillus niger* and *A. flavus*. Groundnuts stored in different storage facilities are susceptible to attack by fungi, insects and other microorganisms under favorable conditions (Aliyu and Kutama, 2007).

**Effect of Mycoflora on Seeds Germination:** Germination test was carried out by using standard rolled paper method (ISTA, 1985). After germination, the comparison among healthy and infected seeds was recorded that is shown in Table 2. The maximum percentage of low germinated seeds was observed in Golden which was 34% while the minimum percentage was observed in BARI-2011 i.e 26%. The maximum percentage of moderately germinated seeds was observed in ZAKI i.e. 58 % and minimum percentage was observed in BARI-2000 i.e. 50%. Whereas maximum healthy seeds germination was found 22% in BARI-2011 followed by BARI-2000, ZAKI and Golden with 16%, 12% and 8%

respectively. The minimum germination was found in Golden and maximum growth in BARI-2011.

Table 2. % Effect of Mycoflora on Seeds Germination.

| Sr. no | Variety | Low germinated seeds (%) | Moderately germinated seeds (%) | Healthy seeds (%) |
|---|---|---|---|---|
| 1 | BARI-2000 | 34 | 50 | 16 |
| 2 | BARI-2011 | 26 | 52 | 22 |
| 3 | ZAKI | 30 | 58 | 12 |
| 4 | Golden | 38 | 54 | 8 |

**Fatty Acid Profile Determination:** According to GC analysis five prominent free fatty acids were identified and quantified which are palmitic acid (C16), Stearic acid (C18), Oleic acid (C18:1), Linoleic acid (C18:2) and Linolenic acid (C18:3). High level of Linoleic acid percentage and oleic acid present in peanut seeds.

Analysis of free fatty acids showed that w/w percent of Palmitic acid was found to be 9.81 in BARI-2011 followed by BARI-2000, ZAKI and Golden viz 9.26, 8.10 and 5.63 respectively. Stearic acid with maximum percentage having 2.33% in BARI-2011 and minimum in ZAKI with 1.32%. Oleic acid was 49.38% in BARI-2011, 42.63 in BARI-2000, 37.05 in ZAKI and 36.49 in Golden which is the least and Linoleic acid was high in BARI-2000 having 47.84% followed by BARI-2011 44.42%, Golden 36.20% and ZAKI 34.74%. The Linolenic acid contents of peanut varieties BARI-2011, BARI-2000, ZAKI and Golden were 0.49, 0.58, 0.46 and 0.51%.

Table 3.Fatty Acid Percent in Peanut Seeds.

| Varieties | Fatty Acid Percentage (%) | | | | |
|---|---|---|---|---|---|
| | C16:0 | C18:0 | C18:1 | C18:2 | C18:3 |
| BARI-2011 | 9.81 | 2.33 | 49.38 | 44.42 | 0.49 |
| BARI-2000 | 9.26 | 1.97 | 42.63 | 47.84 | 0.58 |
| ZAKI | 8.10 | 1.32 | 37.05 | 34.74 | 0.46 |
| Golden | 5.63 | 1.61 | 36.49 | 36.20 | 0.51 |

The results are similar to the previous findings of Twiddy (1994) who evaluated that the deterioration of *Arachis hypogaea* L. seeds due to fungal activity is normally associated with the production of off-colours and flavors, rancidity, discoloration effects on yield and quality of oil, loss of seed viability and formation of mycotoxins.

Storage fungi can cause decrease of germination capability, loss in weight, discoloration of kernels, heating and mustiness, chemical and nutritional changes, and mycotoxin contamination. They can change fat quality of peanuts by hydrolytic enzymes producing free fatty acids and glycerol (Pomeranz, 1992). Moisture content is the most important factor affecting fungal growth in stored products (Ahmed and Young, 1982). Peanuts are stable at 70% relative humidity between 7 - 9% moisture content, at which conditions fungal growth is arrested (ICAR, 1987).

**CONCLUSION**

It is concluded from the present study that the seed borne mycoflora associated with peanut seeds changes the fatty acid composition by producing toxic metabolites in the seeds.

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