International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 12 Issue 1, January-2023, Impact Factor: 7.957

The Role of *Aspergillus flavus* on Deterioration of Oil, Sugar and Protein Content of Different Groundnut (*Arachis hypogea*) Varieties

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ABSTRACT

The present study was carried out to study the role of *Aspergillus flavus* on deterioration of oils, sugars and proteins content of six groundnut varieties *Viz* Chandra, Indori , Rajasthani, TMV-2, AK-12 and Amber. Mass multiplications of the pathogen were done and there after were cultured on PDA plates. The spores were collected after 5 days and a spore suspension was prepared. The culture filtrate was used to infest the healthy seeds of groundnut varieties. After infestation for 48 hours the studies were conducted to check the impact of *Aspergillus flavus* on the six groundnut varieties. Reduction in the levels of oils, sugars and proteins were noticed in groundnut varieties *Viz* Chandra, Indori, Rajasthani, TMV-2, AK-12 and Amber

Keywords: Groundnut*; Aspergillus flavus;* oil quanity; proteins; sugars; PDA

INTRODUCTION

Groundnut is an important oilseed and food legume in tropical and subtropical areas in different agro climatic regions between latitude 40^{o} S and 40^{o}N. In India it is mostly grown as a rainfed crop in well drained sandy soils in low 750mm a normal and medium rainfall. Groundnut is not only a major oilseed crop but also an important food crop in India. India groundnut occupies an area of 40.68 lakh ha with production of 66.15 lakh tonnes and productivity of 1626 kg ha-1 in Kharif season (2017-18). In rabi-summer, it is being grown in 8.39 lakh ha with production of 16 lakh tonnes with productivity of 1909 kg ha-1 (2017-18). In Andhra Pradesh, in Kharif season, it is cultivated in an area of 6.48 lakh ha with production of 8 lakh tonnes and productivity of 1238 kg ha-1 . In rabi season, it is cultivated in 0.90 lakh ha with production of 2.07 lakh tonnes and Arachis hypogaea (L.), commonly known as groundnut or peanut, is an important oil, food and feed legume crop originally from South America .Groundnut is now widely grown throughout the tropical, sub-tropical and warm temperate areas in Asia, Africa, Oceania, North and South America, and Europe. Groundnut was introduced to West Africa by Portuguese explorers in the 16th century (Bakoye *et al*., 2019).

The *Arachis hypogea* is an annual legume, herbaceous plant that grows to a height of 20-60cm and is the major food legume crop in the world. The major groundnut producing countries are China, India, USA, Indonesia, and Senegal. The cultivation of groundnut is about 23.95 million ha worldwide with the total production of 36.45 million tons and an average yield of 1520 kg per ha in the year 2009 (FAO 2013). It is very good source of edible oil and is also consumed as dried fruit. 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). Groundnut is the 13th most important food crop source of edible oil and the3th most important source of vegetable protein. Groundnut seeds contain high quality edible oil of approximately 50% easily digestible protein of 25% and 20% carbohydrate Groundnut haulms are more palatable and rich in protein compared to stovers of cereals which have low N, high fibre content, and poor digestibility and therefore have low nutritive value and are used as supplementary feed (Singh and Singh, 1991., Richard Oteng-Frimpong *et al.,* 2017**).** Seeds are generally associated with certain saprophytic or parasitic micro-organisms which perpetuate in the seed lots on the advent of favourable conditions. Groundnut seeds are highly susceptible to fungi such as, Rhizopus, Aspergillus flavus, Penicillium, Fusarium. Infection of groundnut by species of Aspergillus occurs both at pre-harvest and post-harvest stages (G. Adithya *et al.,* 2017). Janardhan *et a.l,* 2011 found that *Aspergillus* is a common mould in tropical

and sub tropical countries and causes aflatoxin contamination as a result of moulding of badly stored commodities, such as groundnut, cereal and cotton seeds. Chavan and Kakde, 2008 reported that groundnut seeds are highly susceptible to diseases, as they serve as a source of stored nutrients for fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Alternaria dianthicola*, *Curvularia lunata*, *Curvularia pellescens*, *Fusarium oxysporum*, *Fusarium equiseti*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum* causes discoloration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oil seeds. 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 and Adithya, 2016). Such seeds are unfit for human consumption and are also rejected at the industrial.

MATERIALS AND METHODS

The seeds of six varieties of groundnut,Chandra, Indori,Rajasthani,TMV-2,AK-12 and Amber were collected from germplasm collection of National Bureau of Plant Genetic Resource, New Delhi. The seeds were then placed in B.O.D for 24hrs at 25_0c. Seeds of each accession were examined under binocular microscope after incubating on moist blotter for presence of mycelium or fruiting body of fungi on seed surface. The identification of fungi was made according to Ainsworth and Bisbys (1973).A large number of seed borne mycoflora was obtained from seeds placed in Petri plates in incubator. Further study was carried out to study the role of dominant seed borne *Aspergillus flavus* on the deterioration of oil, sugar, fattyacids and protein content of groundnut varieties. The dominant seed borne mycoflora *Aspergillus flavus* was cultured on PDA for 7 days at room temperature to get large number of spores. The spores were washed off with sterile distilled water. Spore suspension was prepared by gently scraping the culture and transferring it to culture tube containing 100 ml distilled water.

The spore suspension obtained was adjusted to a concentration of $1\times10^{-}7$spores ml-1. The seeds of all the six genotypes of *Arachis hypogea* were taken in equal quantity (100 seed/each) these seed were surface sterilized by 1% NaOCl and then rinsed with distilled water and kept a side. Seeds were soaked in spore suspension of *Aspergillus flavus* and shake by hand for about 10 minutes and kept for 1 hour After words seeds were collected and redried for about 5 hours under laminar flow. The number of conidia were counted by a haemocytometer and approximated at 5×104 conidia seeds-1.Both inoculated and un-inoculated seeds were used for subsequent studies. 100 grams of each variety of ground nut both infested and control were grinded with the help of grinder and oil of each accession was obtained through Soxlet apparatus for 9 hours per sample using Hexane as solvent (Jham *et al.,* 1982).

The oil obtained was put in specific viols that were already sterilized. The oil obtained through Soxlet apparatus was then subjected to GLC analysis to study variation in free fattyacid (Uppstrom *et al.,* 1978). Protein estimation was done by Lowry's method (Lowery *et al* 1951) using spectrophotometer. Standard calibration graph Estimation of Sugar was done by method evolved by Dey (1990). Sugar was extracted from grinded seeds (1gm) both infested and control in ethanol (90%). It was kept in oven at 600C for 1 hour and final volume was made to 25ml. To 1 ml aliquot of 5% phenol was followed by addition of 5ml sulphuric acid. Optical density was read at 485 nm, the concentration were determined against curve by using glucose.

RESULTS AND DISCUSSIONS

Effect on Sugar Content

The present study investigated that there is reduction in sugar content in genotypes of *Arachis hypogeal* Chandra, Indori, Rajasthani,TMV-2,AK-12 and Amber by fungal infection. The sugar content in variety Chandra healthy seeds were found to be 14.19±1.25mg/gmfw as compared to 7.13 ±0.89 mg/gm.fw in infested seeds In case of healthy seeds of variety Indori the sugar was observed as 32.86 ±1.91 mg/gm.fwand it was reduced to 31.22 ±1.86 mg/gm.fw in indori infested. The sugar content of healthy seeds of genotype Rajasthani was observed as 30.69 ±1.85 mg/gm.fw and in the same variety infested the sugar content was reduced to 17.92 ±1.42 mg/gm.fw.the sugar content of healthy seeds of variety TMV-2 was 18.29±1.92 mg/gm.fw and that of 10.11±1.22 mg/gm.fw in infested seeds.The sugar content was reduced from 32.22±1.91 mg/gm.fw in healthy seeds of variety AK-12 to 25.22±1.28 mg/gm.fw in infested seeds.the healthy seeds of variety Amber contain25.23±1.83 mg/gm.fw as that of 24.73±1.91 mg/gm.fw in infested seeds.(Catherine et al.,1987) demonstrated decrease in sugar content with the infection of *Aspergillus flavus*, *Fusarium oxysoporium* in seeds of some autoclaved oil palm kernels. Mathur and Sinha1978 reported that fungi association with seeds brings certain biochemical changes in seeds by decreasing sugar content. Vegetable seeds were affected adversely due to association of seed-borne fungi during storage (Rao *et al.,* 2014). Decreased in sugar may be due to fungi utilized sugar as a substrate for its growth

Table 1: **Effect of *Aspergillus Flavus* on Sugar Content of *six varieties of Arachis hypogea***

VARIETY	Sugar Content mg/gm f.w	
	Control (Mean±S.E)	Infested Mean±S.E)
Chandra	14.19±1.25	7.13±0.89
Indori	32.86±1.91	31.22±1.86
Rajasthani	30.69±1.85	17.92±1.42
TMV-2	18.29±1.92	10.11±1.22
AK-12	32.29±1.91	25.22±1.28
Amber	25.23±1.83	24.73±1.38

Data given are mean of three replicates ± standard error

Change in Oil Content

The present study observed that fungi *Aspergillus flavus* change the amount of oil in infested seeds compared to healthy seeds. The oil quantity of healthy seeds of genotype Chandra was observed to be 21.95 ±1.56 gm and it was reduced to 5.10 ±0.75 gm in infested seeds of the same genotype Chandra. There was also decrease in oil quantity of infected indori and Rajasthani genotype, the oil content of healthy seeds of genotype Indori was observed as 29.74 ±1.81 gm and the oil content was decreased to 25.32 ±1.67gm in infested seeds of the same genotype. The oil quantity of genotype Rajasthani healthy seeds was observed as 50.55 ±2.36 gm and it was reduced to 16.20 ±1.34 gm in infested seeds. The oil quantity of healthy seeds of genotype TMV-2 was observed to be 35.30 ±1.82 gm and it was reduced to 5.10 ±0.75 gm in infested seeds of the same genotype TMV-2.. There was also decrease in oil quantity of infected seeds of AK-12, the oil content of healthy seeds of genotype AK-12 was observed as 34.22. ±1.51 gm and the oil content were decreased to 24.22 ±1.63 gm in infested seeds of the same genotype. The oil quantity of genotype Amber healthy seeds was observed as 40.87 ±1.53 gm and it was reduced to 30.61 ±1.74 gm in infested seeds (Umechruba et al., 1992) also observed decreased in oil content of inoculated seeds of *Arachis hypogea*. *A.flavus* caused loss of 2.3% oil; *A.niger* caused oil loss of 3.6% and *M. phaseolina* 1.7% loss of oil. (Angelo and Ory, 1983) investigated decrease in oil content may be due to fatty acids hydrolyzed indiscriminately by the lipase enzyme of the fungus. The structural damage caused by fungal growth in the seed coat and the internal tissues of cotyledon will expose the lipid to oxidation, which is responsible for deteriorating oil quality (Dhingra et al.,1998).According to our consideration the reduction in oil content might be due to lipolytic activity of seed borne fungi. The colour of the oil of all infested seeds was changed as compared to healthy seeds, in all the three genotypes of *Arachis hypogea*. The oil from the infested seeds has unpleasant odour. (Ward and Diener, 1961) suggested that changes in colour may be due to pigments synthesized by invading fungi. The role of seed borne fungi in rancid odour development in oil has been reported in peanut (Wilson, 1947).

Table 2: Effect of *Aspergillus flavus* on oil content of groundnut varieties

VARIETY	oil Content in grams	
	Control (Mean±S.E)	Infested Mean±S.E)
Chandra	21.95±1.56	5.10±0.75
Indori	29.74±1.81	25.32±1.67
Rajasthani	50.55±2.36	16.20±1.34
TMV-2	35.30±1.82	15.11±1.11
AK-12	34.22±1.51	24.22±1.63
Amber	40.87±1.53	30.61±1.74

Data given are mean of three replicates ± standard error

Effect on protein content

The results in the Table 4 show that, protein content is decreased in infested seeds as compared to healthy seeds. All the six varieties showed reduction in protein content in infested seeds. The protein content in healthy seeds of Chandra was found to be 187 mg/gm and the protein content was decreased in infested seeds up to155mg/gm. The protein contents was decreased in variety Indori from 189mg/gm in healthy seeds to 150 mg/gm in infested seeds. In the healthy seeds of groundnut healthy varieties like Rajasthani,TMV-2,AK-12, Amber the protein content was found to be 161 mg/gm, 193mg/gm,178mg/gm,164 respectively and the protein content was decreased in infested seeds of the same varieties. The protein content was decreased to 143 mg/gm,122 mg/gm,142 mg/gm and 115 mg/gm in infested seeds of varieties Rajasthani,TMV-2,AK-12 and Amber varieties.. Srivastava and Sweta (2013) reported maximum protein content of

Jatropha crucas L. was reduced by the seed-borne fungi *Fusarium chlamydosporum*. Seed-borne fungi *Aspergillus flavus, A. niger, Macrophomina phaseolina, Fusarium oxysporum* caused reduction in proteins content (Ushamalini, 1998). Bilgrami et al., 1976 studied that, infesting seeds with A. flavus results insignificant decrease in the seed protein. Iyayi, (2004) stated that, decreased in reducing sugar and protein may be due to fungi utilized sugar as a substrate for their growth. This reduction in protein content of groundnut by *Aspergillus flavus* is by their proteolysis efficacy.The present investigation reveals that decrease in protein content may be due to proteolysis and formation of simple aminoacids that could be utilized by fungi.

Table- 4: Effect of *Aspergillus flavus* on protein content of groundnut varieties

VARIETY	Protein Content in mg/gram	
	Control	Infested
Chandra	187	155
Indori	189	150
Rajasthani	161	143
TMV-2	193	122
AK-12	178	142
Amber	164	115

ACKNOWLEDGEMENTS

The Authors are grateful to, Department of Botany, Government Motilal Vigyan Mahavidhyalaya, Bhopal, (M.P.) for providing necessary laboratory.

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