BVG Trust

J. Sci. Trans. Environ. Technov. 2015, 8(3) : 142-145

Scientific Transactions in Environment and Technovation

The impact of seed borne fungi on the quantitative and qualitative changes in the oil of groundnut (*Arachis hypogaea*)

R. Bindu and D. Karkun

Department of Botany, Sree Narayana College, Alathur, Palakkad , Kerala

Abstract

Role of seed borne fungi on the quantitative and qualitative changes in the oil of groundnut (*Arachis hypogaea*) was analysed. The seeds were infested with different species of *Aspergillus* namely *A.flavus, A. terreus* and *A.fumigatus*. The seeds were kept in different containers such as earthen pot, polythene bags and metallic bin . Qualitative variation in mycoflora was observed in relative to the moisture contents of seeds, humidity and temperature of the atmosphere .The results revealed that the fungal activity was higher at higher relative humidity and the severity was found more in blotter method than the Agar plate method . There was a marked reduction in the percentage of oil level in the seeds infested with *A. flavus, A. terreus.* But the seeds infested with *A.fumigatus* did not exhibit marked difference. The test organisms caused increase in the molecular weight and thereby decreasing the Rf value.

Key words :- *Aspergillus, Arachis hypogaea , Benzene, Benze chloroform , Rf value*

INTRODUCTION

Seed is crucial and basic input to increase crop yields per unit area. The importance of seed in crop production is known to human being since Vedic period. There is clear mention in ancient literature yajarveda "May the seed viable,may the rains plentiful and may the grains ripe days and nights" . History of agriculture progress from early days is also the history of seed of new crops and varieties. The progress was very fast from last three decades. The green revolution was only possible with production of generally pure seeds possessing other qualities namely high generation, high vigour, high physical purity and sound health. Hence green revolution is in fact seed revolution. Only seeds of assured quality can be expected to respond to fertilizer and other inputs in expected manner, otherwise seed of hope may turn into seed of frustration. Among the inputs used by farmers seed is the cheapest input. It is the basic input and forms small part of the total cost of cultivation. The good seeds increase the efficiency of the factor of crop production.

Benefits of using high yielding cultivars, however, may get nullified by dangerous seed borne diseases as seed is just not a germplasm but a microhabitat as well. The pathogenic organisms can utilize and exploit nutrients according to their utilization efficiencies, thereby, lowering germinability and nutritional values of the seeds. Observations have been recorded for the presence of mycoflora on seed surfaces with qualitative and quantitative incidence . The objective of this study was to determine the presence of mycoflora on seed surface and its impact on the nutritional status of seeds of groundnut under stored condition.

Ground nut (*Arachis hypogaea*) is one of the highly nutritious legume crops. The seeds contained protein (25-28%) and oil (43-55%) (Bendre and Kumar, 2000). The high oil content of the seed has made ground nut an important oil yielding crop. Its economic importance increases tremendously as the demand for vegetable oil has increased. The crop enhances the soil fertility through biological nitrogen fixation through when used as alternate crop for crop rotation. The oil seeds of groundnut act as good substrate for the colonization of fungi, which in turn cause qualitative and quantitative changes in the seeds. The seeds are very often colonized by the species of *Aspergillus* and *Penicillium*, when the seeds are stored without completely dried or in the presence of relatively high atmospheric humidity. (Christensen and Kaufmann, 1968). They make the seeds non viable, non edible, toxic and alter the oil content both quantitatively and qualitatively (Prasad, 1980). The present article deals with the role of fungi on the quantitative and qualitative changes of the groundnut seeds.

MATERIAL AND METHODS

Groundnut seeds were collected from different fields and stored lots from farmers. The seed lots right from its harvest were stored in different types of containers such as earthen pots, polythene bags, GI sheets containers, etc., for 12 months . Monthly isolation of mycoflora was done for 12 months .Qualitative variation in mycoflora was observed inrelative to the moisture contents of seeds, humidity and temperature of the atmosphere. All three samples collected from seed lots of groundnut were tested on Czapek's Doxagar media and blotter technique simultaneously for the presence fungi. 100 seeds of groundnut – 50 seeds each for each Czapek's Doxagar media and blotter were plated in five replicates of ten each. After leaving them for 48 hours to ascertain sterility

*Corresponding Author :
email: rbindusona@gmail.com

Table 1 – Periodic screening for the qualitative variation in mycoflora of seed surface to Environmental Changes

GROUNDNUT SEEDS				CZAPEKDOX MEDIA						BLOTTER METHODS		
Sl.No.	Fungal Isolates	Months	Room Temp.	Earthen Pot		Metallic bin		Polythin bag		Earthen Pot	Metallic bin	Polythin bag
				% Moisture content of seeds	Incidence	% Moisture content of seeds	Incidence	% Moisture content of seeds	Incidence	Incidence	Incidence	Incidence
1	*A .flavus*	April	26.5°C±3 °C	31.4	++++	29.0	+++	28.3	++	+++++	++++	+++
		June	40°C±5 °C	22.0	+++	20.7	++	16.0	+	++++	++++	+
		August	25.5°C±1°C	35.0	+++++	30.5	++++	29.0	+++	++++++	+++++	++++
		Oct.	29.8°C±3 °C	28.4	++++	26.3	+++	26.8	++	+++++	++++	+++
		Dec.	28.1°C±2 °C	33.3	++++	29.1	+++	28.1	++	+++++	++++	+++
		Feb	26°C±2 °C	31.8	++++	29.5	+++	28.7	++	+++++	++++	+++
2	*A.fumigatus*	April	26.5°C±3 °C	31.4	+++	29.0	++	28.3	+	++++	+++	++
		June	40°C±5 °C	22.0	+	20.7	+	16.0	-	++	+	+
		August	25.5°C±1°C	35.0	++++	30.5	+++	29.0	++	+++++	++++	+++
		Oct.	29.8°C±3 °C	28.4	+++	26.3	++	26.8	+	++++	+++	++
		Dec.	28.1°C±2 °C	33.3	+++	29.1	++	28.1	+	++++	+++	++
		Feb	26°C±2 °C	31.8	+++	29.5	++	28.7	+	++++	+++	++
3	*A.terreus*	April	26.5°C±3 °C	31.4	++++	29.0	+++	28.3	++	+++++	++++	+++
		June	40°C±5 °C	22.0	+++	20.7	++	16.0	+	++++	++++	++
		August	25.5°C±1°C	35.0	+++++	30.5	++++	29.0	+++	++++++	+++++	++++
		Oct.	29.8°C±3 °C	28.4	++++	26.3	+++	26.8	++	+++++	++++	+++
		Dec.	28.1°C±2 °C	33.3	++++	29.1	+++	28.1	++	+++++	++++	+++
		Feb	26°C±2 °C	31.8	++++	29.5	+++	28.7	++	+++++	++++	+++

Table 2 : Microscopic characteristics used for identification of *Aspergillus* isolates

Sl.no.	Fungus	Microscopic features			
		Size	Stipe colour	Shape	Conidia surface
1	A.	flavus	400 - 800	Pale Brown	Globose Smooth finely roughened
2	A.	fumigatus	200 - 400	Gresh near apex	Globose small in columns Smooth
3	A.	terreus	100 -250	Uncoloured	Globose Smooth

Table 3 - Post inflectional variation in oil content of Groundnut

Sample	Benze chloroform solvent			
	5th day of Incubation		10th day of Incubation	
	Rf value	Comparative Quantity	Rf value	Comparative Quantity
Healthy Groundnut seeds	0.95	+ + +	0.90	+ + +
Seeds infested with				
A.fumigatus	0.92	+ + + +	0.58	+ + +
A. flavus	0.93	+	0.63	+ +
A. terreus	0.97	+ +	0.76	+ + +

+ + + + = High + + + = Moderate High + + = Moderate + = Low

Table. 4 - Post inflectional variation in oil content of Groundnut

Sample	Benzene solvent							
	5th day of Incubation				10th day of Incubation			
	Ist oil spot		IInd oil spot		Ist oil spot		IInd oil spot	
	Rf value	Comparative Quantity	Rf value	Comparative Quantity	Rf value	Comparative Quantity	Rf value	Comparative Quantity
Healthy seeds	0.75	+ +	0.92	+ + + +	0.83	+ +	0.95	+ + + +
Seeds infested with								
A.fumigatus	-	-	0.93	+ + + +	0.90	+	0.99	+ + +
A. flavus	-	-	0.90	+ +	0.87	+	0.96	+
A. terreus	-	-	0.96	+ +	0.86	+	0.93	+ +

+ + + + = High + + + = Moderate High + + = Moderate + = Low

they were used for plating of seeds and incubated at 28± 1°C for the growth of seed mycoflora. All the operations were done under aseptic conditions observations of the plates were started from fifth day for microscopic examination by stereoscopic binocular microscope to note conidial heads, stipes colour and, shape and roughness also colony features including diameter after 7 days .For the biochemical estimation, the surface sterilized seeds were inoculated with respective test organisms and incubated for seven days.

The essential oil present in the matured dried and both healthy and infested seeds were detected by thin layer chromatography (TLC) technique. The concentrated ether extracts from healthy and infested seeds of *Aspergillus flavus, A. terreus* and *A.fumigatus* of 5th and 10th day were spotted on to silica gel plates in duplicate. One spotted plate was developed in Benzene and another one in Benzene : chloroform (1:1) solvent system. Healthy and infested seed extracts were developed simultaneously on two different plates in the same chamber to compare qualitative changes in the oil.

RESULTS AND DISCUSSION

Fungal activities were observed at different temperature and moisture content of the seeds at regular intervals of two months for one complete year, Fungal infestation were maximum in the seeds kept in earthen pots than those of metallic bins and polythene bags, The blotter method showed maximum frequency of fungi than the Czapek's Dox Agar media(Table no. I). The observation also confirm the superiority of ' Blotter method ' over ' Agar plate method' for determining the seed born fungi (Ram Nath et al., 1970 ; Agrawal et al., 1972)

Ground nut seeds were infected mainly by *Aspergillus flavus, A. terreus* and *A.fumigatus* and were found in the seeds throughout the year As the moisture content of the seeds increased in the months of July – August, the severity of *Aspergillus flavus, A. terreus* and *A.fumigatus* also increased . The maximum severity of test organisms was observed in the month of August possibly due to higher level of moisture content . According to Vidhyassekaran (1974) , at high humidity level , spores of the pathogen germinated well with in four hours in vitro as well as on the ragi leaves of both the varieties, which were moderately resistant . According to Singh (1977) , the fungi at lower relative humidity (33 and 55 %) unable to act upon the seed substrate to bring about any remarkable change in its chemical properties .

The identification of *Aspergillus* species on the basis of morphological study showed that *A. flavus* was larger in size compared to *A. fumigates* and *A. terreus.* The stipe colour of *A. flavus* was pale brown, *A. fumigatus* was grayish and uncoloured in A. terreus (Table 2).

Benzene chloroform solvent TLC of healthy ground nut seeds showed only one category of oil spot with relative frequency (Rf) value ranging between 0.95 to 0.90.As compared to healthy seeds, which contained high quantity (+ + + +) of oil, and the seeds infested with *A.fumigatus* showed relatively high quantity of oil after 5 days and same result was also obtained after 10 days of incubation. On the other hand, seed infested with *A. flavus* showed quite low quantity while *A. terreus* infested seeds showed moderate quantity of oil. It indicates that *A. flavus* and *A. terreus* were responsible for lowering down the oil content while *A. fumigatus* did not affect the quantity of oil in the seeds. Results also indicate that at 10 days of incubation. *A. fumigatus* and *A. flavus* brought about certain changes in the quality of oil content as the Rf value of the spot at this were low as compared to that of healthy seeds(Table 3) Benzene solvent TLC of groundnut resolved two categories of oil in the healthy seeds with Rf value of 0.75 and 0.92 respectively. The seeds infested with the *A. flavus, A. terreus* and *A.fumigatus*, on the 5th day of incubation , showed no trace of first category of oil (Rf 0.75). On the other hand , there was a substantial loss in oil quality

category (Rf 0.96) in seeds infested with *A. flavus* and *A. terreus* while *A.fumigatus* did not affect the quantity . Similar results were also observed in the seeds on the 10th day of incubation. Overall results indicate *A. flavus* and *A. terreus* caused substantial loss in oil content of groundnut (Table 4).

Suteri (1980)reported significant reduction of oil content in the seeds of various soybean seeds infested with chlorotic virus and yellow mosaic. Prasad (1980) observed that oil contents decreased significantly due to storage . According to Singh and Prasad (1977) loss in the oil content could be due to the increased lipolytic activity of these fungi at higher relative humidity. Estimation experiments for total oil content of the seeds revealed that ground nut suffered substantial loss of stored oil due to infection by *A. flavus* and *A. terreus* while *A.fumigatus* did not cause much damage in this respect. On the other hand, theses species bring about certain changes in the oil quality as Rf value of the oil at 10th day of incubation was quite low as compared to the oil of healthy seeds. It clearly indicates that infesting organisms caused increase in their molecular weight thereby decreasing the Rf value. Thus a long storage period with *Aspergillus* infection will definitely cause more saturation of the oil within the seeds, the consumption of which is quite harmful for the human health.

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