Journal of Agricultural Technology 2015 Vol. 11(6):1359-1370
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Seed-borne fungi of some peanut varieties from Hadhramout and Abyan Governorates in Yemen

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Mohamed Othman Al-Amod (2015) Seed-borne fungi of some peanut varieties from Hadhramout and Abyan Governorates in Yemen. Journal of Agricultural Technology 2015 Vol. 11(6): 1359-1370

Seed health testing to detect seed-borne fungi is an important step in the management of crop disease. The main objectives of the study were to isolate and identified the fungal pathogens of three varieties of groundnut seeds in Seiyun, Hadhramout Governorate and two varieties of groundnut seeds in Al-kod, Abyan Governorate.
Effect of those fungi in the groundnut seed germination was studied. .The study showed three main fungal pathogens found to be responsible for seed-borne of groundnut varieties seeds namely *Aspergillus niger*, *Aspergillus flavus* and *Macrophomina phaseolina*. The percentage frequencies were 37.63% for *Aspergillus niger*, followed by *Aspergillus flavus* representing (26.07%) and *Macrophomina phaseolina*. (21.02%) of the total isolates .The results also indicated that there was significant difference ($p \leq 0.05$) in mean isolation frequency of fungi from groundnut seeds at different varieties, Kod-1 variety was the highest, while for seed germination percentage, the results showed there was significant difference ($p \leq 0.05$) between different varieties, MH variety was the highest in seed germination percentage

Keywords: groundnut, seed health, seed germination, seed-borne fungi

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an annual crop and belongs to the plant family leguminosae. 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 (Singh and Singh, 1991)**.** Groundnut It is cultivated for its seeds as a source of oil, for direct human consumption as a protein and Vitamins A, B and some members of B_2 group supplement in humans and animal food (Purseglove, 1984). The bi-product, derived from the seeds after extraction of the oil could serve as an essential ingredient of poultry

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and animal feed. The shell could be used as fuel for manufacturing coarse boards, and cork substitute. The kernels could be eaten raw, roasted or sweetened. The oil could also be used for soap making, manufacturing of cosmetics and lubricants etc. (Ibiam and Egwu, 2011). Groundnut is an important leguminous agricultural plant in Yemen, is considered as available crop cultivated over an area of 2357 hectares in Yemen with a production of a bout 1678 kg per hectare The crop is popularly grown in the southern part of Yemen, (Abyan and Hadhramout Governorates) (Jehlan, 2013). It is grown on 24.4 million metric tons and an average productively of 1.4 metric ton per hectare (FAO, 2004).

Fungi can be rendered as the most harmful microorganism and so far, 46 fungal diseases were recorded on groundnut and 67 fungi were associated with various symptoms type (Wikipedia, 2012). Groundnut is attacked by a number of pathogenic fungi of economic importance. Several authors isolated the following fungi from peanut pods, shells and seeds like *Aspergillus niger, Aspergillus flavus, Alternaria dianthicola, Curvularia lunata, Curvulariapellescens, 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 oilseeds (Elwakil *et al.*, 2001; Chavan and Kakde, 2008). Janardhan *et al.* (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. Umechuruba (1986)**,** , Isolated *Macrophomina phaseolina, Penicillium spp., Fusarium equiseti, F. solani, F. moniliforme var. subglutinans, F. sambicum, F. semitectum, F. moniliforme, Colletorichum dematium, Aspergillus niger* and *A flavus* from thirteen unshelled groundnut samples from Nigeria. *Aspergillus niger, Fusarium, Penicilium* and *Cladosporium* are the predominant fungal genera associated with grains in storage, and aflatoxins of all mycotoxins is of utmost concern (CAST, 2003). This is due to their carcinogens and immunosuppressive effects in both humans and domestic animals (Turner *et al.,*

2003).The seeds are found to be responsible for disease transmission because they carry a number of pathogens, which get associated either in the field or in the post harvest storage condition (Manimurugan, 2003).

Fungi growing on stored seeds such as groundnut, 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 and enhancing other biochemical changes. The tropical climate with high temperature and high relative humidity along with unscientific storage conditions adversely affect the preservation of cereal grains, oilseeds, etc., which lead to the total loss of seed quality. (Ameer Junaithal Begum, 2013.

Comparative seed health testing methods for detection of seed-borne pathogen were studied by several investigators. Mathur *et al.* (1975) found that the deep freezing method was more suitable for the detection of *Fusarium* spp in sorghum seeds. Khan *et al.* (1988) found that blotter and agar plate methods were suitable for the detection of *Fusarium* spp in rice seeds. Similar results have been observed by Dawar (1994) who reported that the blotter technique yielded significantly higher numbers of fungi than the agar plate and deep freezing methods with sunflower seeds

The objectives of this study was to investigate the incidence of seed-borne fungi on the groundnut varieties, isolate and identify the seed-borne fungi associated with some varieties of groundnut and obtain information on the pathological effect of these fungi on the peanut seed germination

2. Materials and Methods

2.1 Sources of seed samples

Groundnut seeds with their pericarp (shell) removed of two varieties, Ashford and Al-kod-1 from Al-kod Agricultural Research Station in Abyan Governorate and three varieties 1.e Ashford, Giza and MH from Seiyun Agricultural Research Station in Hadhramout Governorate, these varieties were screened to determine the seed-borne fungi associated with them in the laboratory of Department of Plant Protection, Irrigation and Agriculture Office in Wadi Hadhramout. These varieties seeds were collected in July, 2014, detailed information of the used groundnut varieties are given in table 1

Table 1: Source and varieties of groundnut seeds used in the experiment

Serial number	Name of varieties	Source of collection
1	Ashford	Agricultural Research Station, Seiyun
2	Giza	Agricultural Research Station, Seiyun
3	MH	Agricultural Research Station, Seiyun
4	Ashford	Agricultural Research Station, Al-kod
5	Kod-1	Agricultural Research Station, Al-kod

2.2 Seed health Testing

Seed health testing (SHT) for seed borne fungi was carried out following the Rules of International Seed Testing Association (ISTA, 1993), standard blotter method was selected for this study, Aliquots of 200 seeds were randomly taken from each sample variety for SHT.

2.3 Detection and Identification of seed-borne pathogens

Seed samples were analyzed for the detection of seed-borne fungi by blotter method following International Rules for Seed Health Testing

2.3.1 Blotter method

Standard method as described by the International Seed Testing Association (ISTA, 1996), was used for the isolation of the seed-borne fungi associated with the groundnut seed varieties samples. The seed samples were first sterilized in 1% sodium hypochlorite (chlorax) for five minutes and rinsed in several changes of sterile distilled water. The sterilized seed samples in their various forms according to their varieties were then inoculated on three moistened 9.0 cm filter papers in 9.0 cm Pyrex Petri-dishes. Five seeds were arranged at the periphery of the plate, four at the middle, and one at the centre. A total of two hundred seed samples per preparation, per variety, were used. The control for each sample per preparation, per variety was not treated with sodium

hypochlorite, but was only washed with distilled water. Both the control and the treatment were incubated in the dark at a temperature of 25±2 °C in the incubator for 7 days. Each seed was observed under stereomicroscope in order to record the presence of fungal colony 7 days after incubation based on growth habit.

In doubtful cases temporary slides were prepared from the fungal colony and observed under compound microscope. Appropriate keys (Malane and Muskette, 1964) were consulted for identification of the fungi. Each individual incubated seed was observed under stereomicroscope at 16x and 25x magnification in order to record the incidence of seed borne fungi. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlined by Mathur and Kongsdal, 2003. The results were presented as percent incidence for individual pathogen. Germination of the seeds was also recorded

2.3.3. Identification of fungi

For proper identification of fungi temporary slides were prepared from the fungal colony and observed under compound microscope at 100x and 400x and identified with the help of colony characteristics such as color and texture of mycelia and type of pigmentation. Microscopic characteristics of spores such as shape and color also used to identify the pathogens associated with the seed of Keys suggested by Malone and Muskette, 1964; Booth, 1971; Ellis, 1971; Chidambaram and Mathur, 1975 and Neergaard and Saad, 1962.

2.4. Data Collected

Fungal species found growing on the surface of seeds, Type and frequency of occurrence of identified fungal species was recorded. Percentage frequency (PF) of occurrence of fungal was calculated by using the following formula:

PF= (No. of seeds on which fungus appear / Total number of seeds) X 100

Percent of germination (PG) of seed varieties are determined as proportion of germinated seed over the total number of seed and computed by using the following formula:

PG= No. of seeds germination/ Total number of seeds) X 100

2.5. Design of the Experiment and Statistical Analysis

The laboratory experiment was conducted following Completely Randomized Design (CRD) with twenty replications; the recorded data on percent of seed infection and seed germination were subjected to analysis of variance (ANOVA) using Genstat statistical package, 1995. Mean were compared using List Significance Difference (LSD) at 5% probability level.

3. Results

3.1. Prevalence of Seed Borne Fungi on peanut

The blotter method technique has proven more suitable for the detection of *Aspergillus niger*, *Aspergillus flavus* and *Macrophomina phaseolina* (Table-2), the highest incidence of these pathogens were recorded on Ashford and Kod-1 from Al-Kod Agricultural Research Station, Abyan Governorate. *A. niger*, *A. flavus* and *M. Phaseolina* were predominant on most of the studied varieties, among the different groundnut varieties from Seiyun Agricultural Station showed reduced of incidence in case of major identified seed-borne pathogens, although the finding about presence of seed-borne pathogens on groundnut seed is limited.

A total of three fungi species comprising two genera namely *Aspergillus flavus, Aspergillus niger*, *Macrophomina phaseolina.*, were isolated from peanut seed samples of Ashford, Kod-1, Giza and MH varieties collected from Seiyun Agricultural Research Station and Al-Kod Agricultural Research Station (Table 2). Three species were isolated by the blotter method. *Aspergillus niger* occurred on 37.63% of the samples tested on this blotter method; while *Aspergillus flavus* occurred in 26.07% of the samples tested on blotter method, another important storage pathogen *Macrophomina phaseolina* occurred in 21.02% of the samples tested on blotter method.

Table 2: Percentage frequency of seed-borne fungi of groundnut varieties from Al-Kod and Seiyun Agricultural Research Stations.

	Fungal species %			
Variety name and location	*Aspergillus niger*	*Aspergillus flavus*	*Macrophomina phaseolina*	Mean
Ashford (Al-kod)	42.67	30.42	15.20	29.43
Kod-1 (Al-kod)	52.86	36.05	32.93	40.61
Ashford (Seiyun)	34.91	25.10	22.47	27.49

Giza (Seiyun)	32.46	23.83	12.91	23.07
MH (Seiyun)	25.24	14.98	21.61	20.61
LSD 0.05	7.72			4.46
Mean	37.63	26.07	21.02	
LSD 0.05	3.46			

Table 2 also show that there was significant difference ($p \leq 0.05$) in mean isolation frequency of fungi from peanut seeds at different varieties, Kod-1 variety was the highest and significantly different with those varieties Ashford (Al-Kod), Ashford (Seiyun) Giza and MH, and also there was significant difference ($p \leq 0.05$) was observed between the mean isolation frequencies between related fungi. Mean isolation frequency of fungi from peanuts variety seeds was the highest and significantly different with *Aspergillus niger* and those fungi *Aspergillus flavus* and *Macrophomina phaseolina* and also significant different between *A. flavus* and *M. phaseolina* ($p \leq 0.05$. There was a highly significant interaction between peanuts varieties and isolated fungi in the effect in rate of isolation frequency of fungi, where that *A. niger* isolated from Al-Kod Agricultural Research Station showed a highly significant ($p \leq 0.05$).

3.2. Seed germination

Results in (Table .3) show that the three varieties from Seiyun Agricultural Research Station i.e. Ashford-Seiyun, Giza and MH have average seed germination 50.9%, 53.9%, 55.2% respectively and the two varieties from Al-kod Agricultural Research Station i.e. Ashford-Al-Kod and Kod-1 have average seed germination 28.9%, 32.8% respectively, also (Table-3) show there was no significant difference ($p \leq 0.05$) in average percentage germination from groundnut seeds at different varieties from Seiyun Agricultural Research Station (Ashford-Seiyun, Giza, MH), Ashford-Seiyun variety was the highest (55.2%), and significantly different with those varieties from Al-Kod Agricultural Research Station (Ashford-Al-Kod and Kod-1), and there was no significant difference ($p \leq 0.05$) in average percentage germination between varieties in Al-Kod Agricultural Research Station (Ashford-Al-Kod and Kod-1 variety).

Table 3: Percentage seed germination of groundnut varieties collected from Al-Kod and Seiyun Agricultural Stations.

Variety / location	Germination percentage
Ashford (Al-Kod)	32.80
Kod-1 (Al-Kod)	28.90
Ashford (Seiyun)	55.20
Giza (Seiyun)	53.90
Giza (Seiyun)	50.90
LSD 0.05	9.64

4. Discussion

Results of the present investigation revealed that at least three important seed-borne fungal pathogens namely *A. niger*, *A. flavus* and *M. phaseolina* in Yemen, other investigators also found association of similar fungi with peanut seeds such as (Mukherjee *et al.,* 1992; Oladipupo, 2011), , the findings of the present investigation are in agreement with the findings of other investigators such as Mukherjee *et al.,* 1992; Chavan, 2011 and Oladipupo, 2011), which found *Aspergillus spp*, *Penicillium*, *Fusarium*, *Rhizoctonia* and *Alternaria* storage fungi on groundnut seed while Rasheed *et al.* (2004) found *M. phaseolina, Rhizoctonia solani, F. solani, F. oxysporum, A. niger* and *A. flavus* were predominant in groundnut and seed coat was greatly infected by fungi followed by cotyledon and axis.

Present study showed that saprophytic fungi viz., *A. niger* and *A. flavus* were predominant among the fungi isolated on blotter method. Such similar reports have been made by Rasheed *et al.,* (2004) on groundnut seed. These species have been reported to reduce the germination of seed and damage the seeds in storage. It was also reported that *A. flavus* was the important mycotoxins producer and produce aflatoxin B1, B2, G1 and G2 which are hepatocarcinogenic. Mycotoxins can cause severe damage to the liver, kidneys and nervous system of man even in low dosages (Rodricks, 1976). Blotter method was most practical method for routine analysis of seed health , this

method are in agreement with several investigators such as Khan *et al.,* 1988 in rice seeds and by Dawar and Ghaffer, 1991 on sunflower seed.; Oladipupo, 2011; Syed *et al.,* 2013.

Aspergillus species were predominant fungi on groundnut and those species were serious in reducing germination of seed and those species were reported to be responsible for a number of mycotoxins in groundnut. Mathur and Jorgensen, 1992; Oladipupo, 2011; Bahattcharya and Raha, 2002), these studies agreement with our study that showed those fungi were associated with the pathological effect on groundnut seed, such seed decay, low percentage germination was as a result of infection of seed-borne fungi on those seeds and the presence of *Aspergillus* spp which are the predominate fungi observed suggest that it inhibited the growth of other fungi due to competition for infection.

In general the germination of the groundnut seed of varieties in our study are low that agreement with the finding of the study of authors Patra *et al.,* 2000 which they reported that increase in storage period of groundnut seeds up to nine months, the viability decreased, while pathogen activity, moisture and sugar content in seed increased gradually.

There is therefore need for reducing the pathogenic fungi by treatment of seed for obtaining the good quality of seed and also reduce the mould fungi and mycotoxins production by improving the storage conditions

5. ACKNOWLEDGMENTS

The author is greatly acknowledged to the Agricultural engineer Mr. Ashoor Muftah Al-Zubery, Director of Plant Protection Administration- Agriculture and Irrigation Office, Wadi Hadhramout, for research facilities. The author also acknowledges to the Researcher Mr. Nabil Subaie, Seiyun Agricultural Research Station for providing seed materials to do this experiment.

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(received 18 July 2015; 1 September 2015)