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Research Article

Isolation of fungi associated with necrotic lesions on groundnut (*Arachis hypogea L.walp)* pods and seeds in Samaru Zaria, Nigeria

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Abstract

Fungi associated with necrotic lesions on groundnut pods and seeds in Samaru, Zaria, Nigeria were studied. The percentage and mean percentage occurrence of the fungi isolated were determined. Potato Dextrose Agar (PDA) was prepared using potato extract mixed with 20g each of agar and glucose and autoclaved at 121˚c for 15 minute. Pieces of both infected pods and seeds were surface sterilized in 0.1% mercury chloride for 1 minute after which they were rinsed three (3) times in sterile distilled water and plated asceptically in petri dishes containing PDA. They were incubated at room temperature and observed for the growth of pathogens after every 24 hours for 7 days. The isolated pathogens were then sub cultured on fresh PDA and used for identification. The following fungi were isolated from both the pods and seed parts. *Fusarium Sp, Macrophomina Sp, Phoma Sp, Aspergillus niger* and *Chloridium Sp,* with *Fusarium Sp* having the highest mean percentage occurrence of 6.7%. The presence of *Fusarium Sp* and *Macrophomina Sp* was considered as the possible cause of groundnut pod rot disease. The identification of the pathogens was confirmed at the Department of Crop Protection, diagnostic service unit, Institute for Agricultural Research (IAR) ABU Zaria.

Key words: Groundnut, Necrotic, Lesion, Pathogen, Potato Dextrose Agar (PDA)

INTRODUCTION

Groundnut (*Arachis hypogeal* L.) is an annual legume belonging to the family FABACEAE. It is cultivated in both tropics and suptropics. Groundnut is an important annual oil seed crop (Brown, 1999). It is a popular source of vegetable oil, about 50% and protein 25% (Chater, 2002).

In the 1970s, about 94% of the total world crop was produced *in* Africa, the situation remains much the same today but the optimization of production which is still grossly at the subsistence level in the region continues to be hampered by pest and diseases. Some of the diseases are known to be caused by seed-borne pathogens most of which are fungi, bacteria and viruses (Maude, 1996). The majority is caused by fungi and several of them are yield reducers in certain regions and seasons (Mayee, 1987; Mayee and Datar, 1988; Ganesan and Sekar, 2004a).

The plant can be attacked at all stages of development as seed or seedlings and also after harvest (Chater, 2002). These diseases can be identified through physical symptoms and laboratory tests. Various diseases affect pods of these legumes, some of which include, pod rot and black hull of groundnut pod (Chater, 2002).

Diseases lead to high economic loss of these legumes. (Alabi, 1994). Occurrence of necrotic lesions damage the fruit thereby leading to reduction in market value. It also reduces viability as well as yield of the crops. Therefore this study was carried out to determine whether the necrotic lesions observed on groundnut pod are induced by the same pathogen from literature or entirely by "new" pathogen.

MATERIALS AND METHODS

Infected groundnut pod with seeds were collected from the farmers field in Samaru, Zaria. Potato Dextrose Agar (PDA) was prepared by weighing 250g of cleaned pealed Irish potato tubers. The tubers were sliced and boiled in 500ml of distilled water on hot plate. The extract was filtered using cheese cloth into a one liter conical flask then 20g agar was mixed with 250ml of distilled water. 20g of glucose was dissolved in another 250ml of distilled water. The three (3) components were mixed together in a one liter conical flask and melted in a water bath. The mixture was adjusted to one (1) liter. The homogeneous mixture was autoclave at 121˚c for 15 minute.

Small portion of the infected groundnut pods including portion of the healthy side was cut using a sharp sterile razor blade. The pieces were then soaked in sterile distilled water for 1 minute and then surface sterilize in 0.1% mercury chloride for 1 minute after which they were rinsed 3 times in sterile distilled water and plated asceptically four (4) per petri dish containing PDA. They were incubated at room temperature and observed for the growth of pathogens after every 24 hours for 7 days. The isolated pathogens were then sub cultured on fresh PDA and used for identification.

The seeds inside the affected pods were soaked in sterile distilled water for 1 minutes and surface sterilized as above; they were also plated and incubated as above.

Seeds were selected from the sample of whole seeds that shows fungal growth and were surface sterilized and wash as above. They were then soaked in sterile distilled water for 20 minutes. The cotyledon, embryo and testa were carefully separated using sterile forceps, under aseptic condition and plated separately on PDA and observe as above.

Identifications of pathogens were carried out based on studies of both macroscopically and microscopically. The gross morphology of the pathogens on plates was studied. A small portion of the culture was teased and mounted in lactophenol cotton blue on a glass slide and covered with a clean cover slip and then observe under the microscope. The vegetative and reproductive structures were studied and the pathogens identified. The identification was confirmed at the department of crop protection, diagnostic service unit, Institute for Agricultural Research (IAR) ABU Zaria.

RESULTS

Fungal growth was observed on groundnut pods(Plate I) and seeds (Plate III) . Pods with irregular brown lesions (Plate I) have the highest % occurrence (66.7%) of fungi (Table I). This indicated that it has the highest fungal infection. It was followed by pods with light brown lesion and pods with numerous brown lesions respectively (33.3% each). *Fusarium spp, A. niger* (Plate V) and *Macrophomina spp* (Plate 4) were isolated from the pods, seed as well as the testa. However, *Phoma spp and Chloridium spp* were found to be localized to the pods only.

Fusarium spp was observed to have the highest mean % occurrence(16.7%) followed by *Phoma spp, Chloridium spp, A. niger* and *Macrophomina spp* (8.3%) respectively (Plate V and Table 2).

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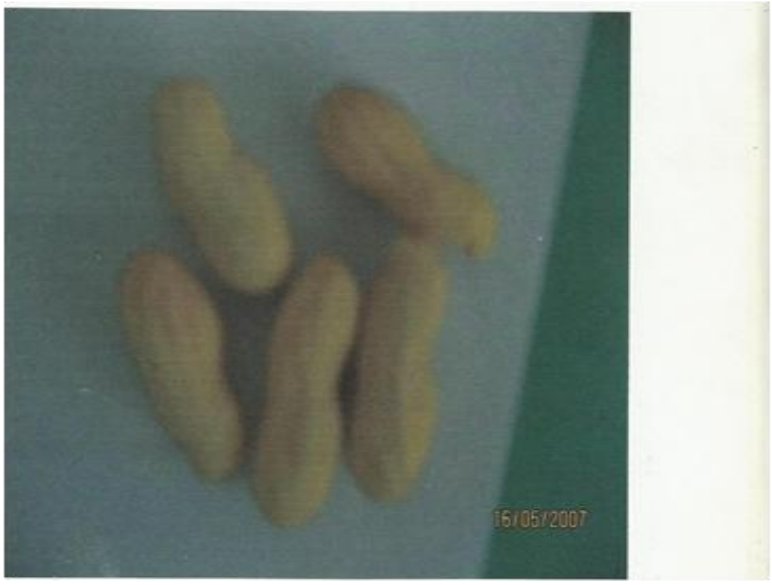

Plate 1. Healthy groundnut pods

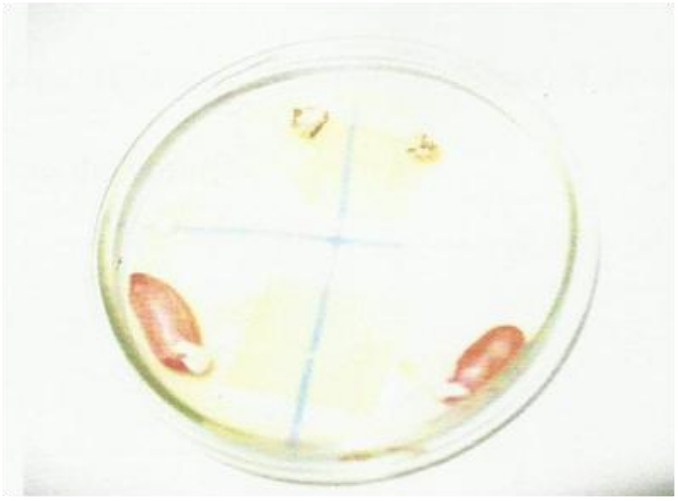

Plate 2. healthy groundnut pod and seed showing no fungal growth

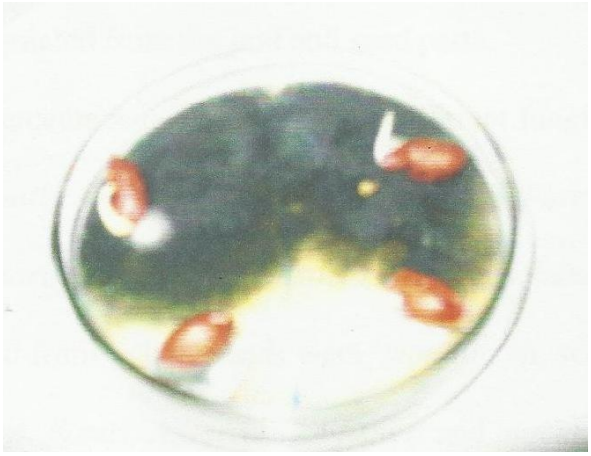

Plate 3. Fungal growth on groundnut seed

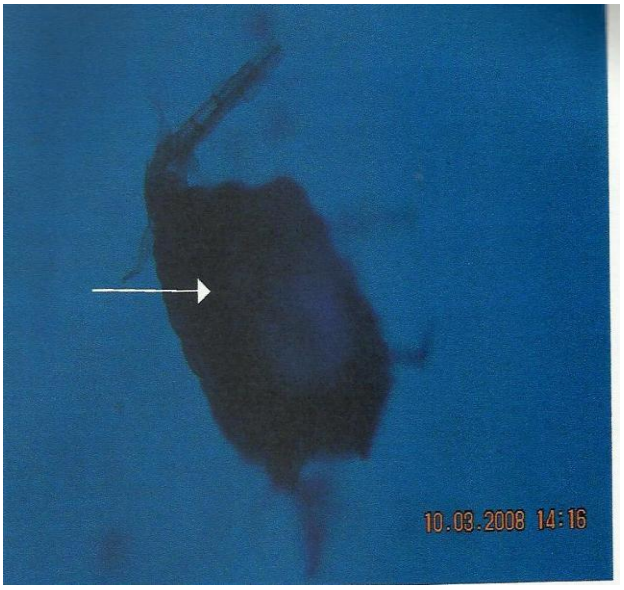

Plate 4. *Macrophomina spp*

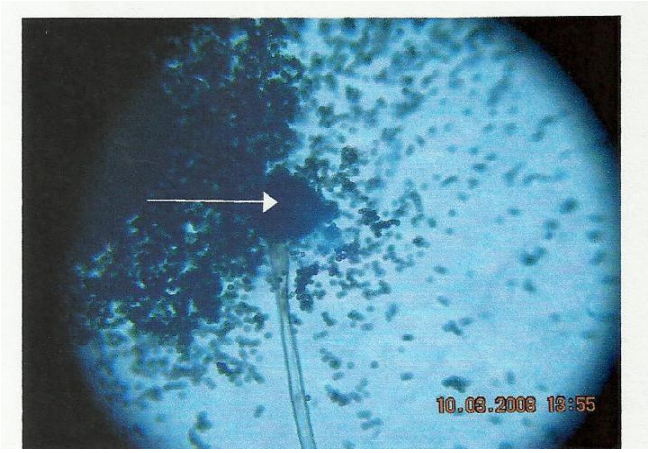

Plate 5. *Aspergillus niger* (arrow conidiophore)

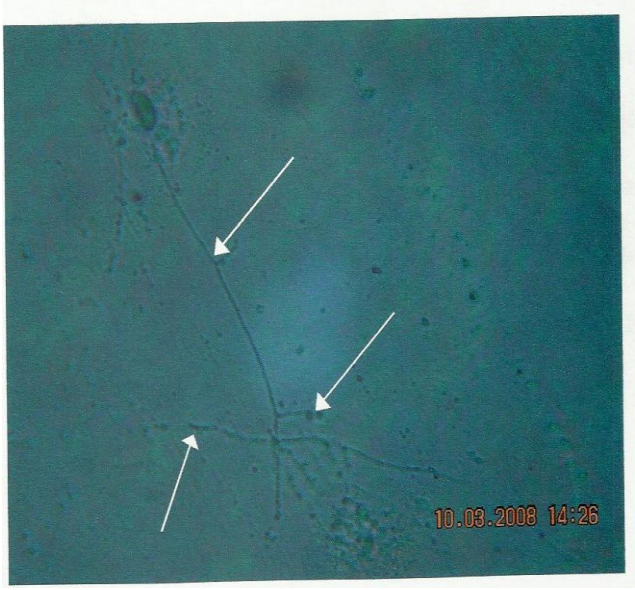

Plate 6. *Chloridium sp* (arrow hyphea, conidiophore and conidia)

Table I. Percent occurrence on pods, and seeds parts

Symptom	*Fungi isolated*	% Occurrence
Pods with irregular brown lesions.	*Phoma Sp, Chloridium Sp, A.niger,and Macrophomina Sp.*	66.7
Seed	*A. Niger*	16.7
Testa	-	-
Cotyledon	-	-
Embryo	-	33.3
Pod with light brown lesion		33.3
Seed	*Macrophomina Sp*	33.3
Testa	-	-
Cotyledon	-	-
Embryo	-	-
Pods with Numerous brown lesions	*Fusarium Sp*	33.3
Seed	-	33.3
Testa	-	33.3
Cotyledon	-	-
Embryo	-	-

Table 2. Mean % occurrence on pods, seeds and testa

Species	Pod (%)	Whole Seed (%)	Testa (%)	Cotyledon (%)	Embryo (%)	Mean (%)
Phoma Sp	8.3	-	-	-	-	1.7
Chloridium Sp	8.3	-	-	-	-	1.7
A. Niger	8.3	8.3	8.3	-	-	5.0
Fusarium Sp	16.7	8.3	8.3	-	-	6.7
Macrophomina Sp	8.3					
		8.3	8.3	-	-	5.0
Phoma Sp	8.3	-	-	-	-	1.7
Chloridium Sp	8.3	-	-	-	-	1.7
A. Niger	8.3	8.3	8.3	-	-	5.0
Fusarium Sp	16.7	8.3	8.3	-	-	6.7
Macrophomina Sp	8.3	8.3	8.3	-	-	5.0

DISCUSSION

Several workers have reported infections on both groundnut pod and seed (Cole, 1985; Bock, 1989 and Subrahmanyan *et al.*, 1992). The present study also isolation of various fungi from groundnut pods and seeds in addition to their identification and distribution. It was found that the distribution of some fungi on pods and seed parts varies. The very low percentage occurrence of fungal infection (16.7%) found on whole seed and testa indicated that a few fungi can cause infections on seeds. This in turn will have a serious implication on the viability of the seed. Also the high % occurrence of (66.7%) found on pod indicated that fungal infection is more on pod than seed parts. However, the isolation of some fungi from both pods and seed parts is an indication that they have uniform infection e.g .*Fusarium Sp, Macrophomina Sp* (plate 4) and *A.niger* (plate 5). The isolation of *Phoma spp* and *Chloridium spp* only from the pod, informed that the infection is found on the pod but may proceed to the seed later. Certainly, the isolation of *Fusarium sp* and *Microphomina Sp* revealed the obvious reason why the groundnut is affected by pod rot disease (Chater, 2002; Smith, 1992 and Singh and Oswalt, 1992). Similarly, the presence of these two fungal organisms showed that they are responsible for the irregular lesions. The isolation of *A.niger* indicated the possibility of crown rot disease (Singh and Oswalt, 1992). Although other workers reported its symptoms on leaves, the isolation of *Phoma spp* pointed at the possibility of having peanut web blotch disease (Robert, 1986).

CONCLUSION

It can be concluded that, the isolation of these fungal organisms and the different symptoms observed in this study indicated that symptoms can be used in field diagnosis of diseases. Similarly, the isolation of fungi from both pods and seeds parts revealed that fungi can cause serious economic loss as well as reduction in the viability of the seeds. In fact, the more terrible were those isolated from the embryo which inhibits its growth and subsequent germination.

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