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# FUNGAL ISOLATES OF GROUNDNUT (*ARACHIS HYPOGAEA* L) SEEDS IN OWERRI METROPOLIS, NIGERIA

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## ABSTRACT

Groundnut, an important crop grown in Nigeria, is highly susceptible to diseases caused by some plant pathogenic fungi, thereby leading to loss of yield. Isolations were made from fungi-infested groundnut seed samples. Four seeds from each sample were aseptically plated on Potato Dextrose Agar (PDA). Pure isolates were identified using cultural and microscopic characters, then stored in agar slants until they were ready for use. Data were statistically analysed using analysis of variance (ANOVA). Results of isolation identified six fungi associated with groundnut seeds to include *Aspergillus flavus* (88.90±19.22%), *A. parasiticus* (44.43±19.28%), *A. niger* (100.00±0.00%), *Penicillium chrysogenum (*44.43±19.28%), *Trichoderma virens* (33.30±0.00%) and *Chrysonilia sitophila* (77.80±19.22%). The percentage occurrence of *A. niger* was found to be the highest followed by *A. flavus* and *C. sitophila*. These fungi were found to be associated with groundnut seeds in storage. Therefore, good sanitary measures and planting of resistant varieties of groundnut should be used to prevent spoilage of groundnut seeds.

**Keywords:** Groundnut; fungi; isolation; Owerri; resistant varieties; seed; sanitary
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## INTRODUCTION

Groundnut (*Arachis hypogaea* L.), an important food crop grown in Nigeria (Tapheel and Jong, 2014), is also known as peanut, earthnut, Pinders, Manila nut etc (Beghin *et al*., 2003). Groundnut is a species in the Fabaceae family (Shazia *et al.,* 2004). It is an annual herbaceous plant growing up to 30 to 50 cm tall. It is cultivated in more than 100 countries in all the six continents around the world in semi-arid tropical areas in subsistence and commercial farming systems (Okoi and Afuo, 2009). The crop is popularly grown in the northern part of Nigeria such as Kano and Borno States, and in the eastern part like Enugu and Ebonyi States (Ibiam and Egwu, 2011).

Groundnut seeds are susceptible to diseases caused by some plant pathogenic fungi thereby limiting the successful production of the crop (Omugo *et al*., 2018); the seeds serve as a source of nutrients for fungi such as *Rhizopus* spp., *Penicillium* spp., *Aspergillus niger* and *A. flavus*. Fungi growing on stored seeds can reduce the germination rate along with loss in the quantum of carbohydrate, protein and total oil content. This leads to increased moisture content, free fatty acid content and other biochemical changes (Begum *et al*., 2013).

*Aspergillus* is a common mould in tropical and sub-tropical countries which causes aflatoxin contamination (Achugbu *et al.,* 2016) as a result of moldy appearance of badly stored commodities, such as groundnut, cereal and cotton seeds (Koïta, *et al.*, 2017).

Okoi and Afuo (2009) observed that groundnut seeds that were not removed from the shells were not attacked by microorganisms and insects like those that were removed from the shells. Ibrahim *et al.* (1986) reported that if grains were harvested dry, they would not be subject to great damage by microorganisms because these organisms require certain amount of moisture to grow and multiply.

Control of groundnut seed infestation in Nigeria has been reportedly achieved through some cultural practices and application of fungicides (Omugo *et al*., 2018). Recently, farmers and agriculturists have complained of cost of fungicides, which are very expensive and difficult to purchase by peasant farmers, who produce approximately 98% of the food consumed (Okigbo, 2016). Also, repeated application of fungicides might cause slow underground erosion, leading to soil pollution as well as a gradual loss of sensitivity in the target pathogens.

Consideration for effective storage of groundnut has always been of prime concern to consumers, marketers and farmers in production of groundnut especially in the tropics where environmental conditions are important, in view of the fact that groundnut seeds are susceptible to fungal attack. This study, therefore, was aimed to investigate the fungal isolates associated with the deterioration of groundnut seeds in different markets in Owerri Metropolis.

## MATERIALS AND METHODS

**Experimental site**

The isolation, inoculation, culturing and microscopic examination were carried out in the laboratory of the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria.

**Sources of Plant Materials**

Samples of stored, shelled groundnut seeds with mold were procured from the following markets in Owerri, Imo State, Nigeria: Eke Onunwa, Nkwo Orji and Relief markets and stored separately in sterile paper bags and labeled accordingly. Owerri is the capital and the largest city in Imo State. Owerri Metropolis is located in the humid tropical ecological zone of South East, Nigeria between latitudes $5^0$ $25^1$N and $50^0$ $23^1$N and longitude $7^0$ $2^1$E and $149^0$ $33^1$E of the Greenwich Meridian (Metrological Unit, Imo State Ministry of Land and Survey, 2020 and Microsoft Corporation, 2009).

**Sterilisation of Materials**

All glass wares, paper towels and inoculating needles were wrapped with aluminum foil and sterilised in hot-air oven at $160^0$ C for 1 hour (Cheesbrough, 2002).

**Media Preparation**

Potato Dextrose Agar (PDA) was used for the isolation of the fungi associated with groundnut seeds and for the sub-culture, growth and maintenance of the fungal isolates. The PDA was prepared routinely according to the manufacturer`s specification and autoclaved at 121ºC for 15 minutes (Cheesbrough, 2002).

**Isolation of Fungi**

Direct isolation method was employed. The groundnut seed samples were surface-sterilised to remove surface contaminants by dipping completely in 10% sodium hypochlorite (NaOCl) solution for 1 minute and rinsed once in sterile distilled water (SDW) (Ritchie, 1991). The seeds were then placed on sterile paper towels for air drying, after which five seeds were plated per plate on PDA in four Petri dishes in three replicates for each sample and incubated at 27± 2ºC for 5 days. The Petri dishes were sealed with paraffin to prevent contamination. The plates were examined daily for the development of fungal growth (Okigbo *et al*., 2009).

**Identification of Isolates and Frequency of Groundnut Seed Fungi**

Isolates were identified based on colony characterisation, strain morphology and microscopically for spore types and hyphal structure. Thereafter, they were compared with published fungal identification atlas by Campbell *et al*. (2013). The incidence of groundnut seed fungi was recorded and their frequencies determined by calculating the percentage occurrence of each fungus following the method described by Rathod *et al.* (2010).

**Pure Culture**

Test fungi were sub-cultured to get pure isolates by transferring mycelia from mixed young culture with the help of sterile inoculating needle. The pure cultures were stored in agar slants for subsequent studies (Cappuccino and Sherman, 2002).

**Statistical Analysis**

Data were subjected to Analysis of Variance (ANOVA) test, using SPSS version 10.0. The means were separated using Duncan`s Multiple Range Test (DMRT) at $p \leq 0.05$.

## RESULTS

Table I shows the morphological and cultural characteristics of fungal isolates from groundnut seeds on potato dextrose agar. The table shows six (6) fungal isolates to include *Aspergillus flavus* (Plate I. A-C), *Aspergillus parasiticus* (Plate II. A-C), *Aspergillus niger* (Plate III. A-C), *Penicillium chrysogenum* (Plate IV.A-B), *Trichoderma virens* (Plate V. A-C) and *Chrysonilia sitophila* (Plate VI. A-C).

Table 1: Morphology and cultural characteristics of fungal isolates from groundnut seed on Potato Dextrose Agar

| Isolate | Macroscopic morphology | Microscopic morphology | Probable Organism |
|---|---|---|---|
| A | Lemon green powdery growth of mycelia at day three | Green conidiosphores with septate hyphae | *Aspergillus flavus* Link |
| B | Cottonry Lemon green powdery growth of mycelia | Conidiosphores with septate hyphae, Globrous spores | *Aspergillus parasiticus* Speare |
| C | Powdery, dark brown, flatty spread on the surface of the solid medium | Septate and branched hyphae with conidia in chains | *Aspergillus niger* Van Tieghen |
| D | Blue-green, white edge colony, velvety surface with rapid growth and maturing in 5 days | Septate, hyaline hyphae, branched (brush-like) bearing biverticulate conidiophores | *Penicillium chrysogenum* Charlse Thom |
| E | Compact mycelia, coconut smell, white and greenish colony with deposit of yellow pigment on media | Septate highly branched hypha, pyramidal or ampule shaped phialide | *Trichoderma virens*(Miller, Giddens and Foster) Von Arx |
| F | White to light orange, contory ('lid lifter') mycelia, colony exhibits rapid growth, maturing within 72 hours | Smooth-walled hyaline, Septate hyphae with rectangular arthroconidia | *Chrysonilia sitophila* UKSI.Arx |

Plate I (a) *Aspergillus flavus* pure culture on PDA (back)

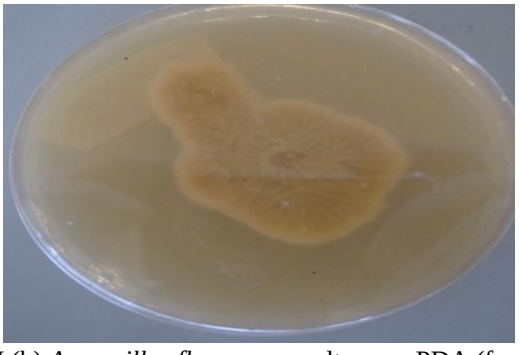

Plate I (b) *Aspergillus flavus* pure culture on PDA (front)

Plate I (c) Micrograph of *Aspergillus flavus* showing biserate vesicle (metulae and phialides) (LPCB 1000)

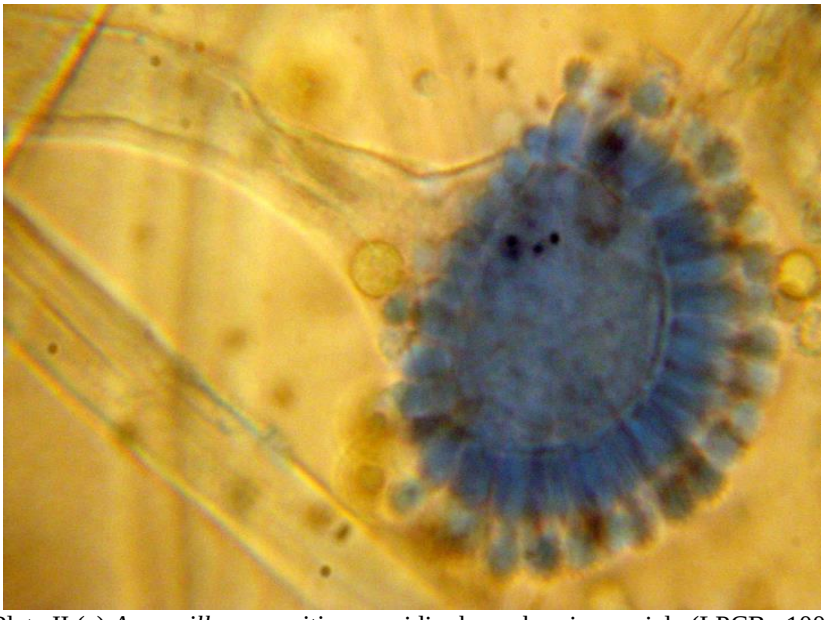

Plate II (a) *Aspergillus parasiticus* conidiophores bearing vesicle (LPCB x1000)

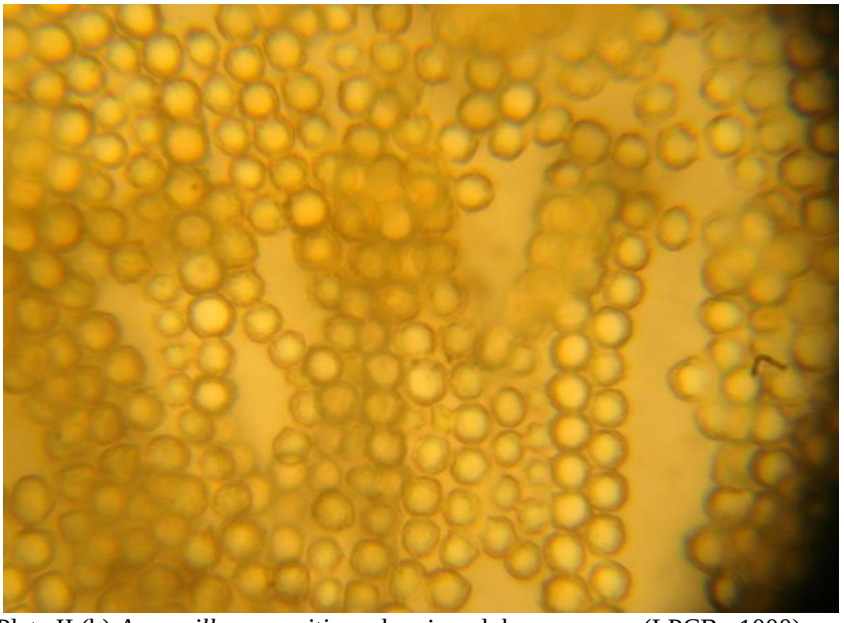

Plate II (b) *Aspergillus parasiticus* showing globrous spores (LPCB x1000)

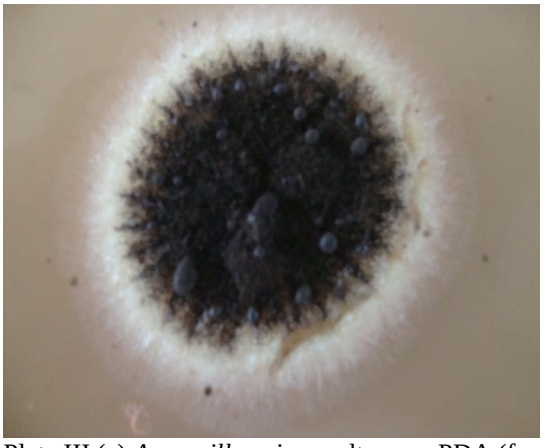

Plate III (a) *Aspergillus niger* culture on PDA (front)

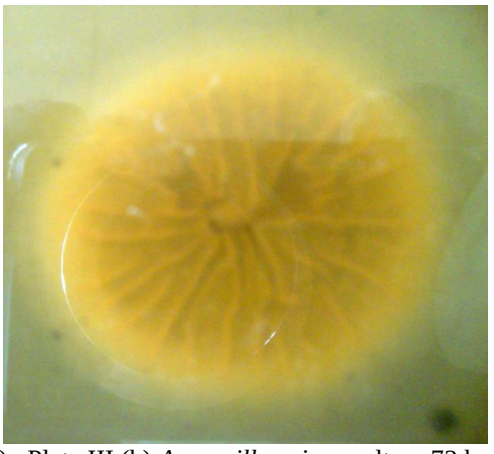

Plate III (b) *Aspergillus niger* culture 72 hours at 31°C on PDA (back)

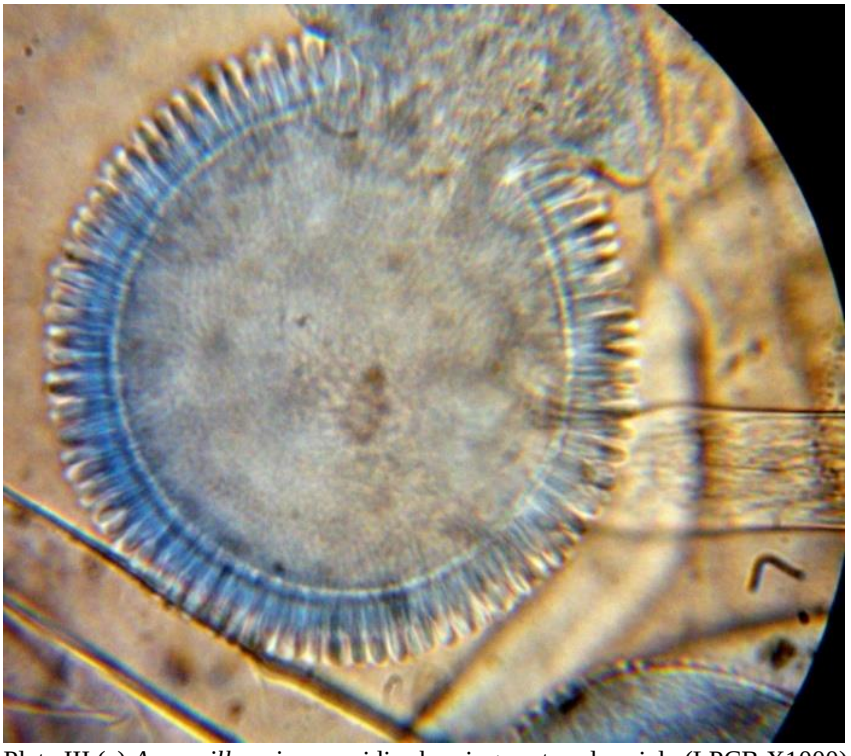

Plate III (c) *Aspergillus niger* conidia showing ruptured vesicle (LPCB X1000)

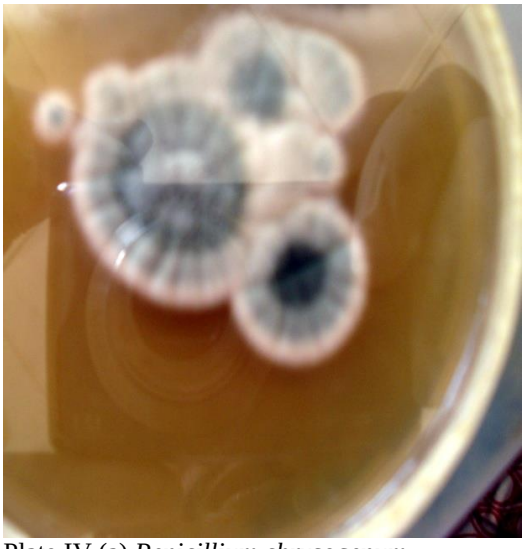

Plate IV (a) *Penicillium chrysogenum* 7days at 31ºC (front)

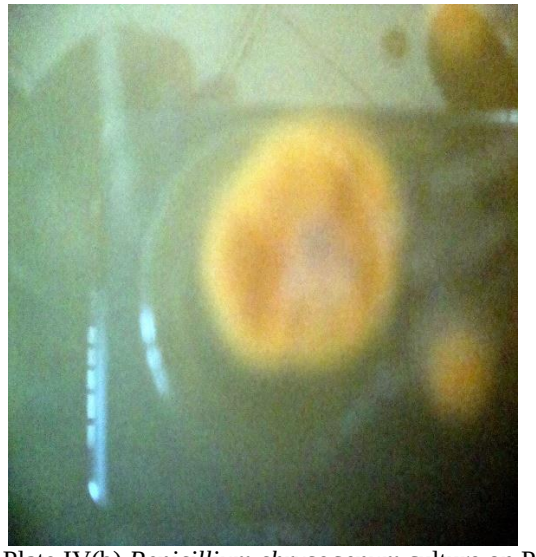

Plate IV(b) *Penicillium chrysogenum* culture on PDA culture (back)

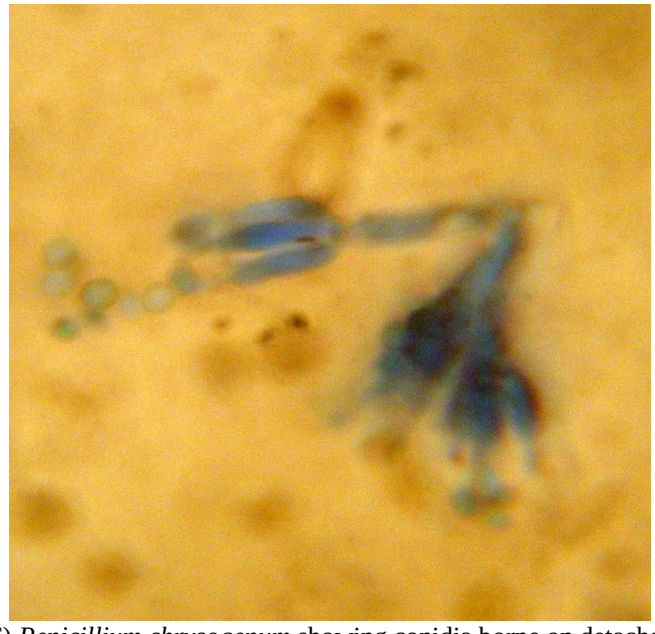

Plate IV (C) *Penicillium chrysogenum* showing conidia borne on detached verticilate phialides (LPCB x1000)

Plate V (a) *Trichoderma virens* culture on PDA 72 hours at $31^0$C

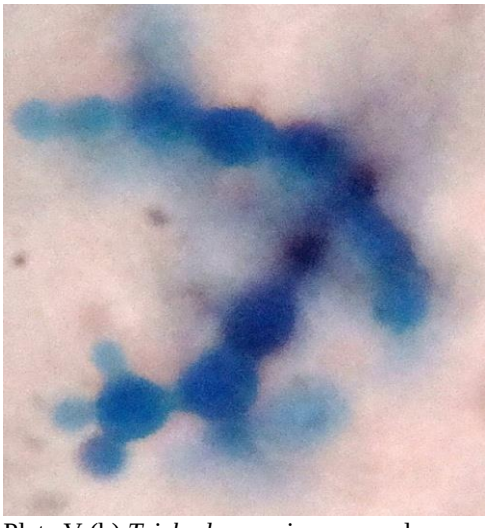

Plate V (b) *Trichoderma virens* ampule-shaped phialide (LPCB X1000)

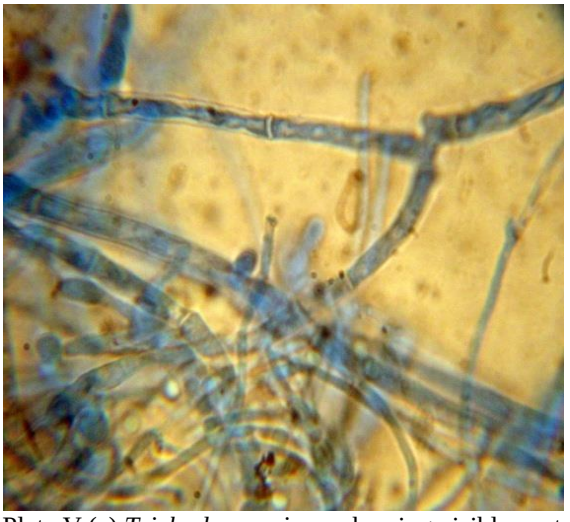

Plate V (c) *Trichoderma virens* showing visibly septate hyphae (LPCB X1000)

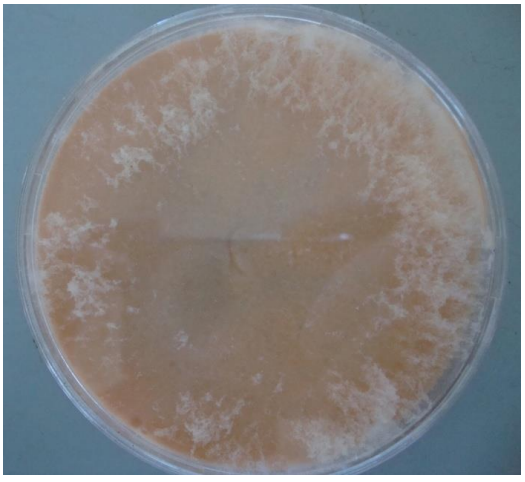

Plate VI (a) *Chrysonilia sitophila* culture on PDA 48 hours (front)

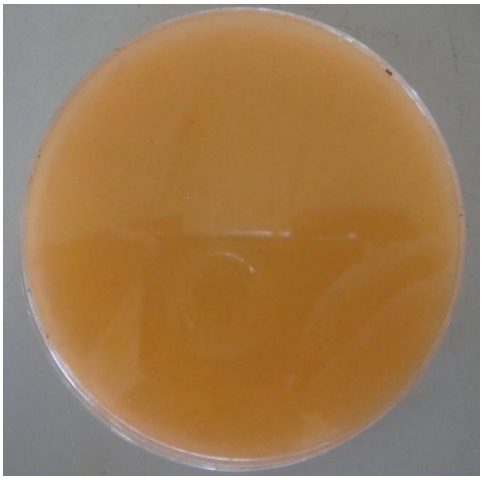

Plate VI (b) *Chrysonilia sitophila* culture on PDA 48 hours (back)

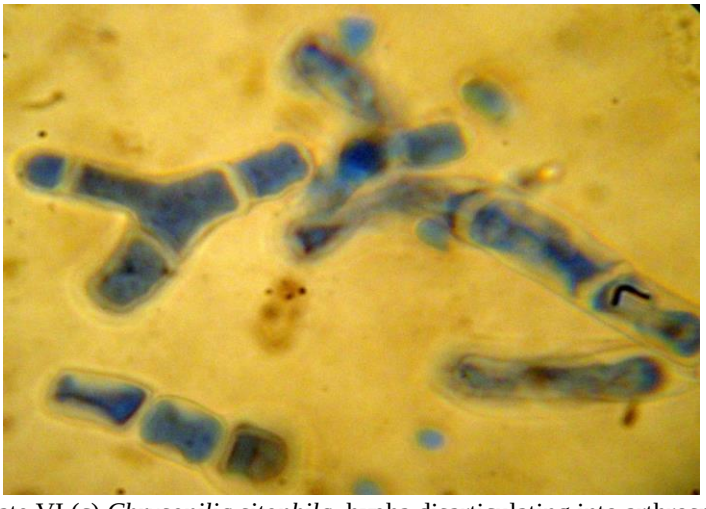

Plate VI (c) *Chrysonilia sitophila*, hypha disarticulating into arthrospores (LPCB X1000)

The result of the percentage occurrence of fungal isolates is shown in Table 2. The percentage occurrence of *Aspergillus niger* (100.00±0.00%), *A. flavus* (88.90±19.23%) and *C. sitophila* (77.80±19.23%) was the highest while that of *Trichoderma virens* was the lowest (33.30±0.00%). Analysis of variance showed a significant difference in the percentage occurrence of the fungal isolates ($p<0.05$).

Table 2: Percentage occurrence of fungal isolates from groundnut seeds

| S/N | Fungal Isolates | % Occurrence* |
|---|---|---|
| 1 | *Aspergillus flavus* | 88.90±19.23[b] |
| 2 | *Aspergillus parasiticus* | 44.43±19.28[a] |
| 3 | *Aspergillus niger* | 100.00±0.00[b] |
| 4 | *Penicillium chrysogenum* | 44.43±19.28[a] |
| 5 | *Trichoderma virens* | 33.30±0.00[a] |
| 6 | *Chrysonilia sitophila* | 77.80±19.23[b] |

Results are in Mean± Std

Means followed by the same letter within the same column are not significantly different at p =0.005

## DISCUSSION

Groundnut seeds are highly susceptible to diseases caused by some plant pathogenic microorganisms, thereby limiting the successful production of the crop (Begum *et al.*, 2013b). The result of this study identified six fungi associated with groundnut seeds. These include *Aspergillus flavus*, *A. parasiticus*, *A. niger*, *P. chrysogenum*, *T. virens* and *C. sitophila*. *P. chrysogenum*, *T. virens* and *C. sitophila* have been implicated in many studies as fungi inflicting rot and spoilage in many tropical crops (Ibiam and Egwu, 2011; Omugo *et al*., 2018).The genus *Aspergillus* is a common mould in tropical and sub-tropical countries and causes aflatoxin-contamination as a result of moldy of badly stored commodities, such as groundnut, cereal and cotton seeds. The *Aspergillus* genera have also been associated with grains in storage (CAST, 2003).

The percentage occurrence of *A. niger* was found to be the highest followed by *A. flavus* and then *C. sitophila*. This result agrees with the study of Raju and Krishnamurthy (2003) who observed a progressive increase in the *A. niger* in groundnut with prolonged storage period. The result is also consistent with the study of Ambang *et al.* (2011). Similarly, Yu *et al*. (2004) observed that *A. flavus* was the predominant species responsible for aflatoxin-contamination of groundnut seeds prior to harvest or during storage. *C. sitophila* has also been found to produce resistant spores and to grow on most cereal crops. Generally, the predominance of these fungi on groundnut seeds implies that they were able to utilise the stored nutrients in groundnut seeds as substrate for their growth.

## CONCLUSION

This study has identified *A. niger, A. flavus* and *C. sitophila* to be the three predominant fungi associated with stored groundnut seeds. Good sanitary measures and planting of resistant varieties should be used to prevent spoilage of groundnut seeds. Also, integrated pest management will be of immense benefit to groundnut growers in overcoming fungal and other pathogenic problems. There is also the need for improved storage method of groundnut seeds to reduce moisture and possible contamination by these fungi.

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