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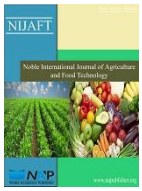

Original Article **Open Access**

MORPHOLOGICAL ANALYSIS OF FUNGI RESPONSIBLE FOR SPOILAGE AND POSTHARVEST LOSS OF COWPEA SEEDS IN IBADAN, NIGERIA

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ABSTRACT: Fungi and insects play significant roles in the reduction of the quality of stored cowpea seeds in major local markets in Nigeria, resulting in drastic decline in value due to discoloration, astringent taste, unpleasant flavor and foul smell. The dearth of information in Southwestern, Nigeria was the impetus behind this research. Three major markets within Ibadan, Oyo State, Nigeria were randomly selected for cowpea seeds assessment. Standard laboratory techniques were used in the determination and identification of the pathogens. The fungi isolated and identified were Aspergillus flavus, A. niger, A. fumigatus, A. parasiticus, Rhizopus stolonifer, and Fusarim oxysporum. Aspergillus strains had higher disease dominance and ability to grow on poor substrates. All the fungal strains isolated had negative effects on the sprouting of cowpea seeds except Aspergillus niger. Cowpea seeds obtained from Ibadan markets were confirmed to be contaminated by different strains of fungi. The result of this study was an evaluation of the unforeseen danger to human health caused by microbes; this is a serious course for concern.

Keywords: Quality of Stored Cowpea Seeds; Astringent Taste; Unpleasant Flavor; Foul Smell; Fungal Pathogens.

1. INTRODUCTION

Vigna unguiculata (L.) Walp. (Cowpea) is a legume that is significant in the diet of millions of people around the world. It provides profitable income for most underdeveloped nations [1] and the seeds contain bioactive compounds that serve as better nourishment for growing tissues in the body [2]. It is often called "meat for the poor" because it is a cheaper source of protein. However, grains and pulses especially cowpea seeds are susceptible to fungal contamination when stored under poor environmental conditions [3]. Fungal encroachment of stored seeds can result in yield loss, decrease in seed viability and quality [4], discoloration, poor growth, mycotoxin production and decay [5, 6]. Fungi and insects play significant roles in the reduction of the quality of stored cowpea seeds, and the situation is mostly aggravated by high relative humidity and temperature [7].

The physiological impact of fungal encroachment on stored cowpea seeds include: increased seed temperature and mustiness, increased fatty acid production, reduced sugar quality and respiration rate, production of mycotoxins (which if consumed may be harmful to man and animals) and loss in seed weight and viability [8]. The quality of cowpea seeds were further reduced by discoloration, unpleasant taste, flavor or smell, and also decrease in nutritive value [8]. In the early 1970s, Cowpea seeds from Western Nigeria were reported to harbor moulds such as *Aspergillus flavus*, *A. niger*, *Fusarium vertilliodes*, *F. solani*, *Penicillium digitatum* and *Rhizopus* sp. [9] but there was paucity of information in the later years. Emechebe and Mcdonald [10] reported that cowpea seeds from markets in Northern Nigeria contained *Ascochyta* spp., *Colletotrichum lindemuthianum, C. truncatum, Rhizoctonia solani, F. oxysporum, F. solani, Macrophomina phaseolina, Septoria vignae* and *Corticium rolfsii*, still there is a dearth of information to climax the trend of cowpea seed contamination by fungi in most Nigerian markets.

Similar reports were given in South Africa and Benin, where cowpea seeds collected from local markets were contaminated with *Fusarium equiseti, F. graminearum, F. semitectum, F. proliferatum, F. chlamydosporum, F. sambucium,* and *F. subglutinans* [5]. The loss of cowpea seeds during storage to microorganisms has been a serious threat to farmers in Nigeria [11]. According to Embaby and Abdel-Galil [12] fungi are serious parasites to stored cowpea seeds, therefore, the current study seeks to isolate and identify fungi associated with stored cowpea seeds from some selected markets in Ibadan, Oyo State, Nigeria in order to determine their individual contribution to the problem of food shortage and mycotoxin poisoning in Nigeria.

2. METHODS

2.1. Sample Site

Three major markets within Ibadan, Oyo State, Nigeria were randomly selected for cowpea seeds collection. The markets were Sasa market (Akinyele local government), Bodija market (Ibadan North local government), Oja-Oba (Ibadan South local government). The Institute of Agriculture Research and Training, Moore Plantation, Ibadan, Oyo State, Nigeria was the source of collection of disease free cowpea seeds.

2.2. Sample Collection

A total of 500g of mixed cowpea seed samples were collected from the market locations and healthy samples from the control location. They were aseptically packaged in sterile polyethylene bags, labeled appropriately and transferred to the mycology/pathology laboratory of the Department of Botany, University of Ibadan, Ibadan, Nigeria for further analysis.

2.3. Isolation of Fungi

Twenty five (25) diseased and healthy cowpea seeds from each location were surface sterilized using 70% ethanol for 30 seconds after which they were rinsed in 3 successive changes of sterile distilled water, blotted and air-dried in a laminar air flow chamber for 30mins. The seeds were aseptically placed in Petri dishes containing freshly prepared full strength PDA. The inoculated plates were incubated at 25 ± 2^{0}C and examined for 7days. Pure culture of each isolates was obtained by careful, consistent and exclusive sub-culturing of individual mycelia growth from the original culture.

2.4. Identification of the Isolates

Secondary characterization of fungal isolates was through color formation, mycelia texture and orientation on PDA, while primary identification was based on somatic structure morphology, type of reproductive body produced, spore formation and type, and their distinctive appearance under a high power digitized trinocular microscope. The production of primary or secondary metabolites and other extracellular substances were investigated too.

2.5. Pathogenicity and Sprouting Inhibition Test

Pathogenicity test was conducted using the method described by Agrios [13]. Healthy cowpea seeds from the control Centre [Institute of Agriculture Research and Training (IAR&T)] were immersed in the spore suspension of each isolates [calibrated at 3.6×10^{3}cfu/mL] for one hour. The inoculated cowpea seeds were aseptically transferred into a sterile inoculating vessel containing double layers of sterile blotter papers using a germ-free scalpel. Each inoculating vessel contained 10 cowpea seeds, and the inoculated seeds were kept in an incubated calibrated at 25 ± 2^{o}C for 7days. Close observations were recorded daily for possible signs and symptoms of the initial disease(s), while the percentage number of sprouted seeds for each isolates was determined.

2.6. Data Analysis

Tables and graph(s) were prepared using Microsoft Excel Worksheets 2007 service pack and data collected from the experiment was represented as means.

3. RESULT

Cowpea seeds contain a very high amount of protein (24.80%) and carbohydrate (63.60%) and other essential nutrient in small quantities as stated in Table 1 [14].

Table 1. Percentage nutrient content of mature cowpea seeds

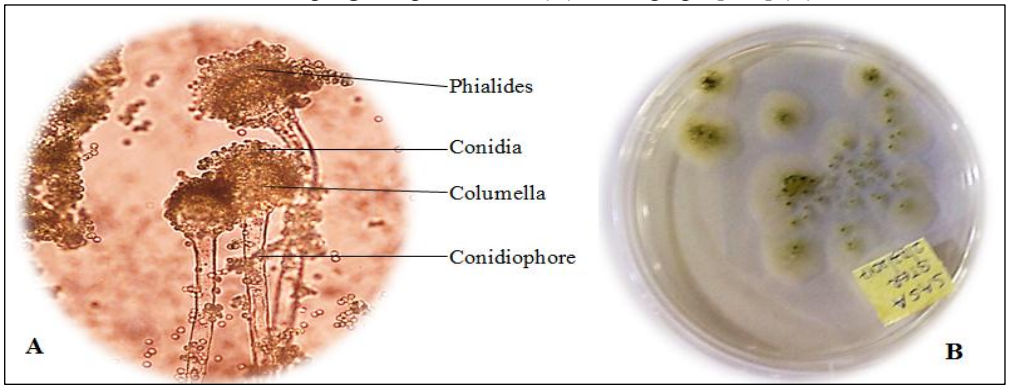
Nutrient Content	Percentage
Protein	24.8
Fat	1.90
Fibre	6.30
Carbohydrate	63.6
Thiamine	0.00
Riboflavin	0.00
Niacin	0.00

Source: Singh and Rachie [14].

3.1. Aspergillus parasiticus

- **Secondary Identification (Laboratory)**: The mycelia appeared white with cotton-like texture from day two on PDA. As the culture grew older, the cotton-like mycelia developed pale green patches surrounded by a fluffy mass of white mycelia colony. At day seven (7), the pale green coloration deepened with the production of fruiting bodies i.e. conidiophores and conidia (Plate 1b). Metabolites was produced on PDA and Aflatoxin B_1, B_2, G_1, G_2, was detected.
- **Primary Identification (Microscopy)**: The conidiophore was hyaline, double walled and non-septate. At the tip of the conidiophore is the columella (Plate 1a). The columella was also hyaline and globosely formed with the attachment of metullae and phialides on which the conidia (spores) are attached. The conidia produced were ovoid, ellipsoidal or round in shape and they were attached singly each to the tip of the phialides (the spore production cells of the fungus).

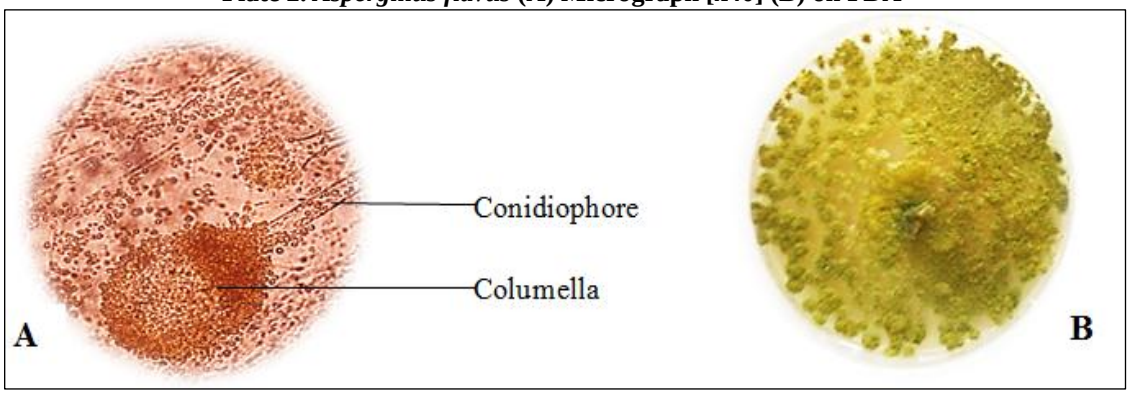
Plate 1. *Aspergillus parasiticus* (A) Micrograph [x40] (B) on PDA



3.2. Aspergillus flavus

- **Secondary Identification (Laboratory)**: The young mycelia colony appeared cotton white from day two with a coarse mycelia texture on potato dextrose agar (PDA). As the culture grew older, the mycelia orientation appeared grass-like with the formation of a yellowish-green colony that is surrounded by a white barricade of fluffy mass of mycelia. At day seven (7), the yellowish-green mycelia colony coloration deepened with the production of spore mother cells, conidiophores and conidia (Plate 2b). Aflatoxin B_1 and B_2 was identified in the culture medium of *A. flavus*. It was detected and quantified using PDA as the basic medium for the extraction.
- **Primary Identification (Microscopy)**: The conidiophore was hyaline, double walled and non-septate. The columella appeared hyaline also with a club-shaped appearance (ellipsoidal at the tip like an egg). The metullae and phialides were attached to it in a brush-like orientation. The conidia produced were hyaline, double walled, ovoid, ellipsoidal or round in shape (Plate 2a).

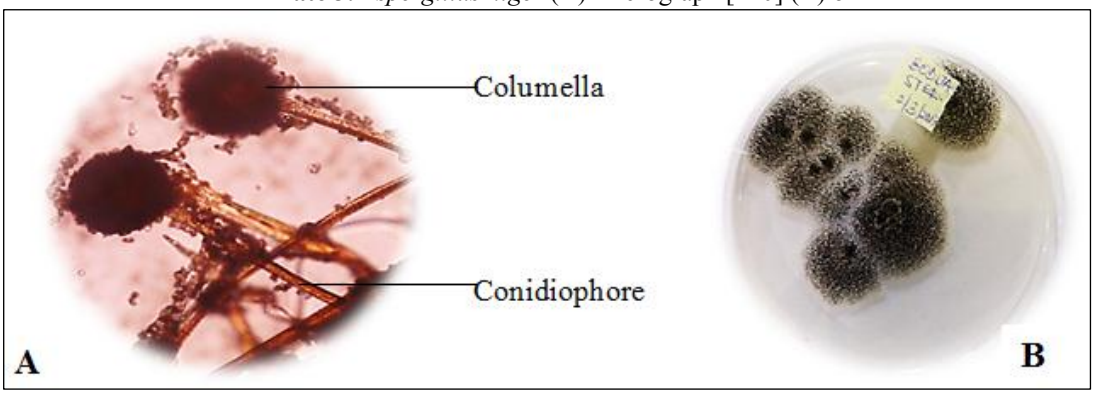
Plate 2. ***Aspergillus flavus*** **(A) Micrograph [x40] (B) on PDA**



3.3. Aspergillus niger

- **Secondary Identification (Laboratory)**: Mycelia appeared black with a single colony formation around the point of inoculation from day one on PDA. In older cultures, the dark mycelia colony was surrounded by a white barricade of fluffy mass of mycelia. At day seven (7), the dark mycelia colony totally ramified the medium and the white barricade of fluffy mycelia mass fades away (Plate 3b).
- **Primary Identification (Microscopy)**: The conidiophore was transparent and double walled when observed under the microscope. There was no cross walls formed across the conidiophore. The columella was totally covered by the metullae, phialides and spores in a forest-like orientation. The conidia produced were hyaline, double walled, ovoid, ellipsoidal or round in shape (Plate 3a).

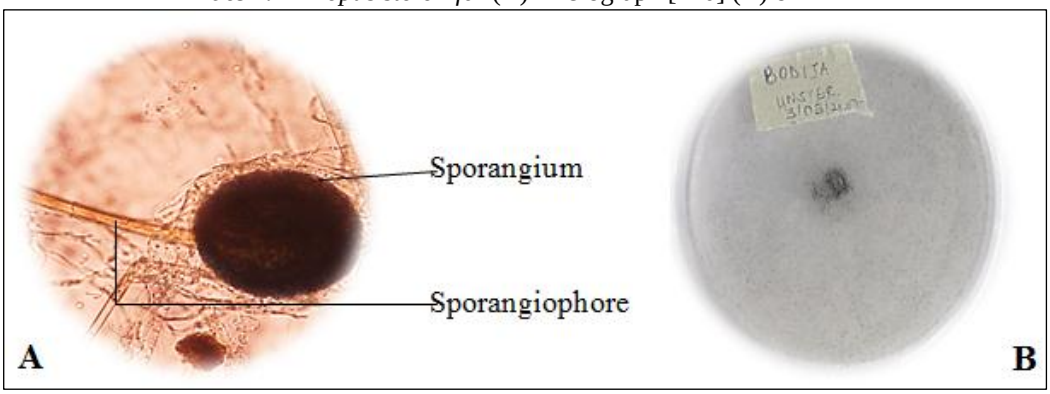
Plate 3. *Aspergillus niger* (A) Micrograph [x40] (B) on PDA



3.4. Rhizopus stolonifer

- **Secondary Identification (Laboratory)**: Mycelia growth was rapid on PDA. The mycelia texture was cotton-like or more precisely a loose cotton appearance. The culture appeared white from day one and later turned light grey as the culture ages. In older cultures, the grey mycelial colony produced dots of dark patches due to the production of sporangiospores. At day seven (7), the grey coloration become intense and the dark dots become more pronounced all over the culture medium (Plate 4b).
- **Primary Identification (Microscopy)**: sporangiophores are hyaline and double layered when observed under the microscope. There was no cross walls observed in the micro-image view of the sporangiophores (non-septate) and each sporangiophore bears a single sporangium at the tip. The sporangia produced are sub-globular in shape and they contain the sporangiospores. The sporangiospores were globule, round or oval in shape, and they were hyaline with double layered coating (Plate 4a).

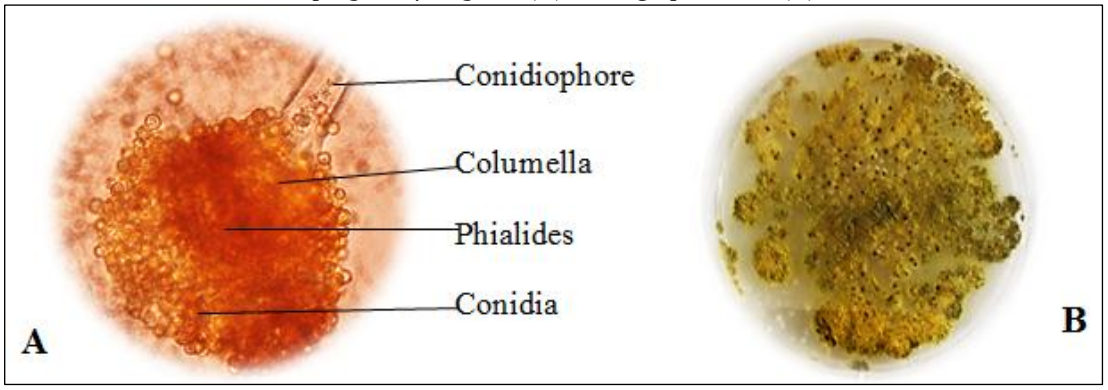
Plate 4. *Rhizopus stolonifer* (A) Micrograph [x40] (B) on PDA



3.5. Aspergillus fumigatus

- **Secondary Identification (Laboratory)**: The mycelia were white with cotton-like texture from day two on PDA. As the culture grew older, the cotton-like mycelia turned greenish-yellow surrounded by a fluffy mass of white barricade of mycelia colony. At day seven (7), the greenish-yellow coloration deepened with the formation of dark dots of mycelia colony evenly distributed around the culture plate due to the production of fruiting bodies i.e. conidiophores and conidia (Plate 5b). Aflatoxin G_1 and G_2 were detected in the culture media where *A. fumigatus* was inoculated.
- **Primary Identification (Microscopy)**: The conidiophore was hyaline, double walled and non-septate. At the tip of the conidiophore was located the columella (Plate 5a). The columella was also hyaline and oval, with attached metullae and phialides. The conidia produced were ovoid, ellipsoidal or round in shape and they were attached singly each to the tip of the phialides (the spore production cells of the fungus).

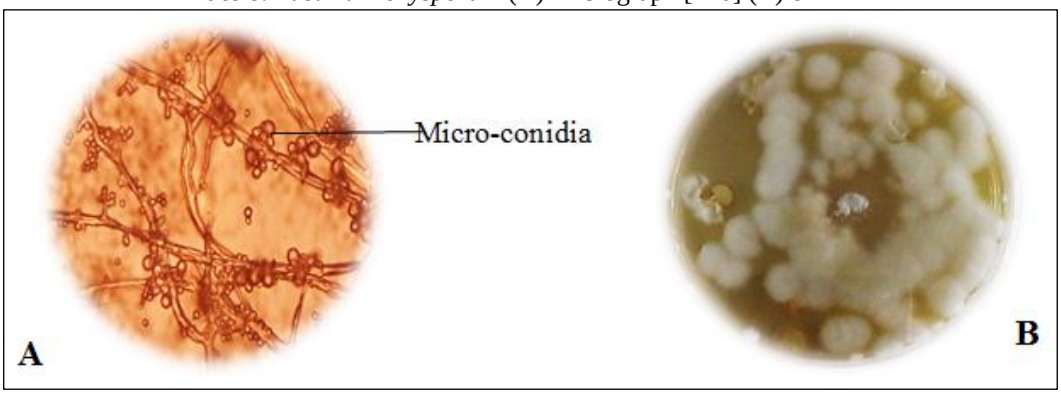
Plate 5. *Aspergillus fumigatus* (A) Micrograph [x100] (B) on PDA



3.6. Fusarium oxysporum

- **Secondary Identification (Laboratory)**: The mycelia appeared white with cotton-like or velvety coarse texture from day two of inoculation on potato dextrose agar (PDA). The mycelia orientation was rosette shaped in older cultures (Plate 6b).
- **Primary Identification (Microscopy)**: The somatic hyphae were dichotomously branched, non-septate, hyaline and double walled. The microspores produced were ovoid, ellipsoidal or round in shape (Plate 6a). The megaspores were larger and oval in shape.

Plate 6. *Fusarium oxysporum* (A) Micrograph [x40] (B) on PDA

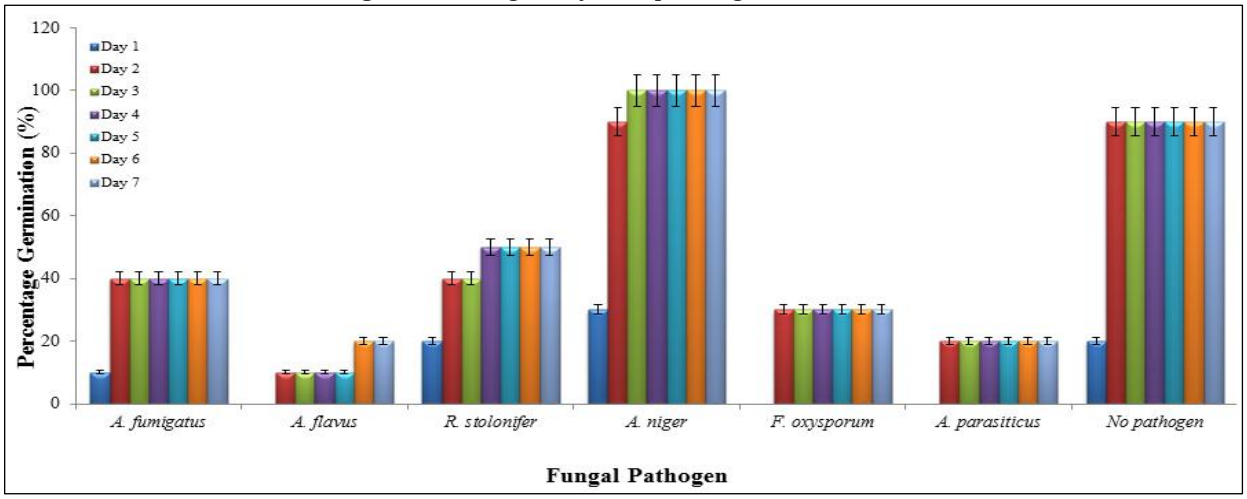


3.7. Pathogenicity and Sprouting Inhibition Tests

Aspergillus flavus was the most virulent of all the strains of fungal species isolated from the stored cowpea samples, the pathogen had severe effects on the sprouting of cowpea seeds as it inhibited the germination of 90% of the seeds from day 1 up till day 5. At day 6 and 7, it was only 20% of the seeds inoculated with *A. flavus* that sprouted [80% were lost to the activities of the pathogen]. A similar effect was observed for cowpea seeds inoculated with *A. parasiticus* but in this case the pathogen inhibited the germination of 80% of the seeds from day 1 to day 7 (Fig 1). 70% of Cowpea seeds inoculated with *Fusarium oxysporum* were lost to the activities of the pathogen as it only allowed the germination of 30% of the seeds subjected to the experiment from day 1 up till day 7.

It was also recorded that *A. fumigatus* inhibited the germination of 90% of the seeds on day 1 after which a total of 40% sprouted (day 2 to day 7), 60% of the seeds used for this experiment were lost to the pathological activities of *A. fumigatus* in the *in vivo* experiment conducted. *Rhizopus stolonifer* and *A. niger* had milder effects on the germinating cowpea seeds as they each inhibited 50% and 0% respectively of the inoculated cowpea seeds at day 7 (Fig 1). The effects of *A. niger* on the sprouted cowpea seeds was not significantly different from those cowpea seeds sprouted under normal conditions without the interference of other postharvest fungal pathogens (Fig 1)

Figure 1. Pathogenicity and sprouting inhibition tests



Source: Etaware and Ogungbemile (2020)

4. DISCUSSION

The fungi isolated from the samples analyzed were *Aspergillus flavus*, *A. n*iger, *A. fumigatus*, *A. parasiticus*, *Rhizopus stolonifer*, and *Fusarim oxysporum*. Similar results had earlier been reported by Khare, et al. [15] who affirmed the presence of these seedborne fungi in cowpea seeds from Botswana, these were further substantiated in the studies of other researchers who found similar mycoflora associated with cowpea seeds [16-18].

It was also observed that *Aspergillus* species had higher dominance (in terms of disease incitement) which may be associated with their wide range growth requirement and ability to grow on poor nutrient medium. Pitt and Hocking [19] also affirmed the predominance of *Aspergillus* genera in tropic

environments. The distribution of fungi isolates in cowpea seeds observed in this study can be linked to poor post-harvest handling practice and environmental factors amongst others as suggested by Salari, et al. [20].

All the fungal strains isolated had negative effect on the percentage germination of cowpea seeds except *Aspergillus niger*. This was in agreement with the findings of Montes-Belmont and Carvajal [21], who reported that storage fungi reduced the percentage germination and emergence of white cowpea seeds. In a different study, Fields and Kings [22] suggested that *Aspergillus* species significantly reduced the percentage germination of peas.

5. CONCLUSION

Cowpea seeds obtained from Sasa, Bodija and Oja-Oba markets in Ibadan, Oyo State, Nigeria were confirmed to be contaminated with different strains of fungi. The result of this study was a revelation of the unnoticed danger to human health which is a serious course for concern. Farmers and marketers should be advised on appropriate techniques for handling and storing cowpea seeds in other to avoid health hazards and food poisoning.

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