

**EVALUATION OF FUNGAL PATHOGENS IN STORED COWPEA
(*Vignaunguiculata* (L)Walp) SEEDS FROM
THREE DIFFERENT LOCATIONS IN SOUTHEAST NIGERIA**

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ABSTRACT

The study examined the fungal pathogens in stored cowpea (*Vignaunguiculata* (L)Walp) seeds in three different locations in Southeast Nigeria (Okigwe, Onicha and Awgu) in 2014 and 2015. Cowpea seeds obtained from these three different locations were assessed for seed-borne fungi. The fungi were isolated and identified by standard methods of culturing and sub-culturing. The isolated fungi species were, *Fusarium*spp, *Aspergillusniger* and *Asergillusflavus*. There were on the average 38.33%, 88.00% and 43.33% of disease incidence of fungi found associated with the cowpea seeds from Okigwe, Onicha and Awgu respectively in 2014 and 2015. Storage patterns have significant roles in protecting crops from the menace of pathogens.

Keywords: Stored seeds; Fungi; pathogens; different locations; Southeast Nigeria.

INTRODUCTION

Cowpea (*Vignaunguiculata* (l) walp) is a Legume in Africa. It serves as a major source of indigenous plant protein in Nigeria (Ndemet al;2004). World statistics revealed that 4.5million metrictons of cowpea grains were produced annually on 12-14 million ha farmland, and 64-70% of the production obtained in different location across Nigeria. Singh et al (2002) reported that Nigeria is the world's largest producer of the crop with 2.0 million metric tones of grain from 5 million ha of farmland per annual. Cowpea contributed significantly to farmer's income and diets, it grain very highly nutritious and free metabolites, it provide a good supplement for scarce animal protein for 110 million consumers of the crops in the third world economics of the tropics and sub tropics (Timkoet al., 2007). Besides, cowpea also plays a major role in farming systems in the fixation of nitrogen through symbiosis with nodular

bradyrhizobium species of bacteria, fixing on the average 240kg N/ha per annum (Awurum, 2000,Aveling 2007).

Many phytopathogenic fungi and bacteria have long been known to produce enzymes capable to produce of hydrolyzing the polymeric carbohydrate constitute of higher plants cell wall. This factors might be responsible for the penetration of the fungi into the cowpea seeds. Pathogenic seed-borne.

Fungicauses' loss in yield, quality, nutritional values and viability of fod in the food- stuffs. Fungi and their mycotoxins constitute health hazards to animals and man following the consumption of contaminated grain. Many disease in animals are known to be of fungi origin (Makunet al; 2009, 2010).

Cowpea seed is vulnerable to attack by fungal pathogens in storage and at all stages of growth of the crops. Seed-borne fungi associated with cowpea include: a, *colletotrichum*, *fusarium*, *aspergillus* and *curvularia* species. These organisms have been implicated in low seed viability and germination. Seedling mortality and yield reduction in cowpea (Abdelmonenet al 2000).

Fungi attack on stored cowpea is among the major problems facing in the production of cowpea. It results in scarcity of food and propagating materials for increased production and profitability.

Identification of fungi pathogens in stored cowpea will open window for proper storage to avoid fungi attacks in the store.

The objectives of this study were to identify the incidence of the various pathogens in the stored cowpea seeds and the pathogens associated with the seeds in different areas of production.

MATERIALS AND METHODS

This experiment was conducted at the Pathology Laboratory of Federal College of Agriculture Ishiagu, Ebonyi State, Nigeria in 2014 and 2015. Seeds were obtained from farmers in Onicha in

Ebonyi State, Okigwe in Imo State, Awgu in Enugu State.

STERILIZATION OF MATERIALS

Petri dishes (12 cm) used were washed thoroughly with detergent and rinsed with sterilized water. They were sterilized in oven at 160°C for 2 hours. Analytical weighing balance was treated with absolute alcohol to make it germ free while Whatman papers used as blotter papers were stem sterilized in an oven at 100°C for one hour. Ten surface sterilized seeds of cowpea (*Vigna Ungiculata (L) walp*) from each location were placed in the Petri dishes equidistantly apart and incubated at 23 ± 2° C for seven days in an incubator.

PURE CULTURE PREPARATION

The standard medium used was potato dextrose Agar (PDA) and its composition is as follows:

Potato Dextrose Agar	
Potato extract	4.0g
Dextrose	20.0g
Agar	15.0g
Distilled water	1000ml

Monocultures of the isolates were prepared by sterilization of petri dishes in oven at 150°C for 2 hours. The potato dextrose agar (PDA) was sterilized in an autoclave at 151b pressure and at

121°C for 15 minutes.

The sterilized medium was allowed to cool to 45°C and 20ml volume dispersed to the Petri dishes. They were allowed to solidify (gel) before inoculation proper began.

INNOCULATION

Inoculation needle was dipped into absolute alcohol, flamed to redness and cooled before used. The needle was used to cut out separately from the advancing edge of a 3-days old culture and transferred into the solidified medium in Petri dishes. They were incubated at room a temperature to grow for 7 days.

IDENTIFICATION:

Identification of the separated isolates was carried out with the help of electronic microscope and identification manual.

RESULTS AND DISCUSSION:

The table below shows the number of seeds plated in each petri dishes, No of seeds affected and disease incidence in each location. The seeds infected after inoculation of the seeds were observed, the disease incidence, number of seeds plated and the number of seeds infected were tabulated as follows. Base on the location of each seeds collected;

Table 1: Seeds from Okigwe (Imo state)

No of Seeds plated	Disease incidence (DI) (%)	Fungi isolated
100	40.00	<i>Aspergillusniger</i>
100	20.00	<i>Aspergillusniger</i>
100	30.00	<i>Aspergillusniger</i>
100	50.00	<i>Aspergillusflavus</i>
100	40.00	<i>Aspergillusflavus</i>
100	50.00	<i>Fusariumspp</i>

The table above shows the number of seeds plated in each Petri dishes, number of infected with fungal, disease incidence and isolated fungi in stored cowpea in Okigwe, Imo state. It was observed that there are three main species of fungal that attack stored cowpea in this location. The main species isolated were: *Aspergillusniger*, *Aspergillusflavus*, and *Fusarium* species. *Aspergillus* species were

the common isolates followed by *Fusarium* from the location.

Table 2: Seeds from Onicha (Ebonyi State)

No of seeds plated	Disease Incidence (DI) (%)	Fungi isolated
100	90.00	<i>Fusarium</i>
100	80.00	<i>Aspergillusflavus</i>
100	80.00	<i>A. flavus</i>
100	100.00	<i>A. flavus</i>
100	80.00	<i>A. niger</i>
100	100.00	<i>A. niger</i>

The figure above shows stored cowpea seeds in Onicha (Ebonyi State) with highest infection of fungal micro-organisms. The data obtained, also shows that Onicha is infected with average percentage of 88% of fungal micro-organisms, also observed from the microscopic the species

of fungal that attack stored cowpea seed in onicha. The record review that the main species that attack stored cowpea seeds are: *Fusarium*, *Aspergillusflavus*, and *Aspergillusniger*. It was also observed that *Aspergillusniger* is the most fungal isolated.

Table 3: Seeds from Awgu (Enugu State)

No of seeds plated	Disease Incidence (DI) (%)	Fungi isolated
100	40.00	<i>Aspergillusflavus</i>
100	40.00	<i>A. flavus</i>
100	30.00	<i>A. flavus</i>
100	60.00	<i>A. niger</i>
100	40.00	<i>A. niger</i>
100.	50.00	<i>Fusarium</i>

The table above shows that cowpea seeds stored in Awgu. The data obtained also shows that Awgu has average percentage of infection of 43.3% fungal. Microscopic analysis also shows the species of fungi that attack stored cowpea seeds in Awgu are: *Aspergillusflavus*, *Aspergillusniger*, and *Fusarium*. But *Aspergillusflavus* is the common isolate.

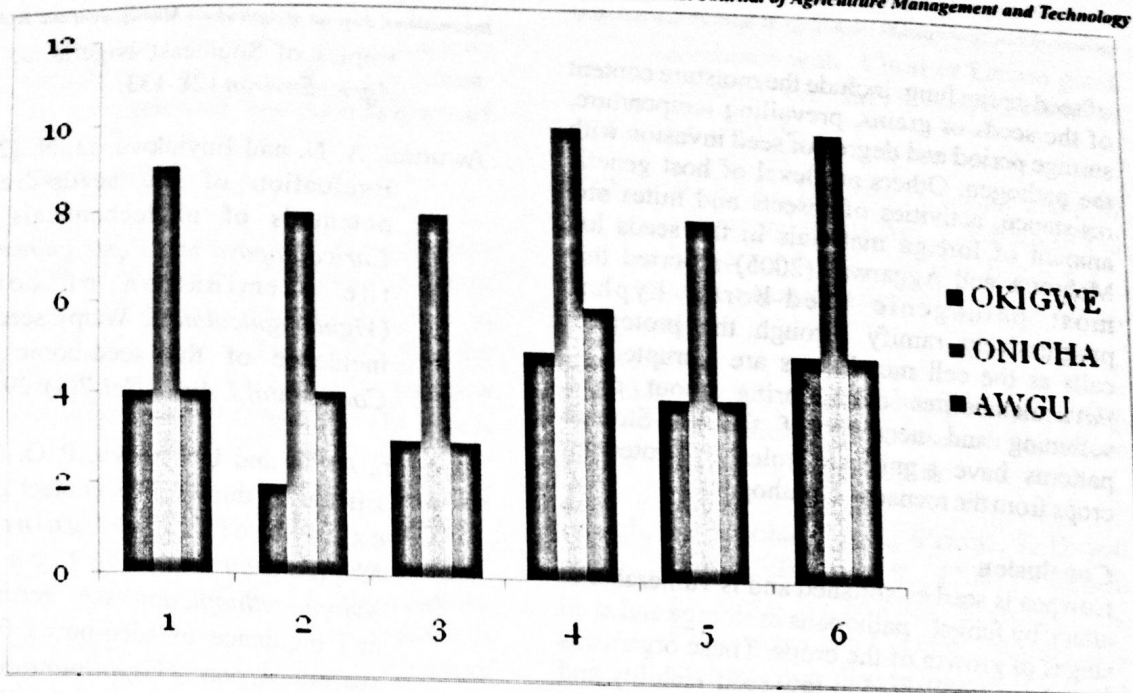


Fig: 1. The different levels of infections on the seeds from different locations

The chart above shows number of infected seeds in the three different locations. This shows that Onicha has highest number of infected seeds followed by Agwu, while Okigwe has the least number of seeds infected from the locations.

From the result got in the figure above shows that sample from Onicha has the highest percentage of fungal with 88% of disease incidence followed by Awgu with 43.33% while samples from Okigwe has the least disease incidence with 38.33%. It can be observed that stored cowpea seeds in Onicha (Ebonyi State) is highly infected with fungal micro-organisms followed by Awgu (Enugu State) while Okigwe (Imo State) has the least fungal micro-organisms. Cowpea is seed established and is vulnerable to attack by fungal pathogens in storage and at all stages of growth of the crops. Seed-borne fungi associated with cowpea include: *Aspergillus niger*, *fusarium*, *aspergillus flavus* and *curvularia* species. These organisms have been implicated in low seed viability and germination. Seedling mortality and yield reduction in cowpea (Abdelmonem et al 2000).

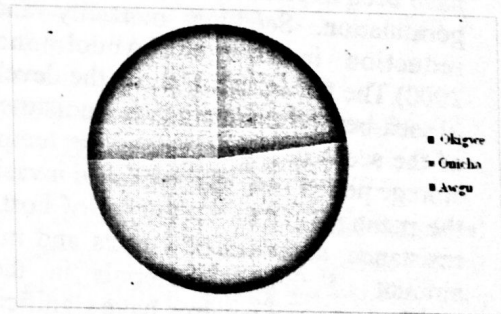


Fig. 2: Representing the disease incidence in the different locations.

The figure also shows that Onicha location has the highest percentage of infected seed with 186.73% while Agwu is the second infected location with 91.94% then Okigwe has the least percentage infection with 38.33%.

The survey also revealed that *A. niger* and *A. flavus* are the most frequent isolates. Microorganisms especially fungi are known to be the major cause of market and field losses of crops (Onifade, 2000). The incidence of fungi on other grains such as sorghum and millet have been reported by Brandyopadyay et al., (2005) to have included *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., and *Mucor* spp. Toxicogenic moulds and aflatoxin B1 has been detected from kernels, soybean oil, garri, yam flour, ginger, cowpea, maize, millet, rice, sorghum, cotton seed, and groundnut and melon seed crops from Nigeria (Bansi and Adebayo, 2003; Makunet et al., 2010). Factors influencing the development

of seed-borne fungi include the moisture content of the seeds or grains, prevailing temperature, storage period and degree of seed invasion with the pathogen. Others are level of host genetic resistance, activities of insects and mites and amount of foreign materials in the seeds lot. Mehrotra and Aggarwal (2005) reported that most pathogenic seed-borne hyphae progressively ramify through the protoplast cells as the cell membranes are disrupted. *A. flavus* metabolites could bring about rapid softening and necrosis of tissues. Storage patterns have significant roles in protecting crops from the menace of pathogens.

Conclusion

Cowpea is seed established and is vulnerable to attack by fungal pathogens in storage and at all stages of growth of the crops. These organisms have been implicated in low seed viability and germination. Seedling mortality and yield reduction in cowpea (Abdelmonem *et al* 2000). The factors influencing the development of seed-borne fungi include the moisture content of the seeds or grains, prevailing temperature, storage period and degree of seed invasion with the pathogen. Others are level of host genetic resistance, activities of insects and mites and amount of foreign materials in the seeds lot. Thus, storage patterns have significant roles in protecting crops from the menace of pathogens.

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