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FUNGAL SPECIES ASSOCIATED WITH *Vignaunguiculata* (L.) Walp (COWPEA) SEED FROM PARTS OF ENUGU STATE, NIGERIA.

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ABSTRACT

A study was conducted on fungal organisms associated with Cowpea (Vigna unguiculata (L.) Walp) seeds obtained from three Local Government Areas in Enugu state, namely Nsukka, Igbo-eze and Udenu L. G. A. In each L. G. A., the seeds were collected from two different towns: Lejja and Orba for Nsukka L. G. A;Aku and Ukehe for Igbo-eze L. G. A and lastly Ezimo and Ubollo for Udenu L. G. A. Standard Blotter Method and Agar method were used for the isolation of Fungi. Cowpea seeds from different locations had varying levels of infestation with fungi. Aspergillus niger Van Tieghem, Botryodiplodia theobromae (Pat) Novel, Fusarium oxysporum Schlecht and Rhizopus stolonifer Ehrenb ex Link were isolated from both blotter and agar methods. Botryodiplodia theobromae had the highest mean occurrence (42.37%) followed by Fusarium oxysporum(30.02%), Aspergillus niger (21.99%) and lastly, Rhizopus stolonifer (10.15%). The occurrences of fungi isolate according to the location were observed with Fusarium oxysporum occurring most in Lejja and Aku by 24 isolates and 18 isolates respectively. In Orba, Aspergillus niger occurred most by 19 isolates. In Ukehe, Ezimo and Obimo, Botryodiplodia theobromae occurred most by 21 isolates, 19 isolates and 11 isolates respectively. Seed pathogens cause reduction in cooking quality, nutritive values, and decrease germinability of seeds and total decay. Cowpea serve as an important food in Africa. There is need for regular seed testing and adequate seed treatment so as to ensure that growers produce healthy plant produce

Keywords:*Vignaunguiculata* (L.) Walp seed, fungi, Isolation, Pathogenecity.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) belongs to the legume family Leguminoseae and sub-family Papillinoadeae (Enwere, 1998). It is an annual herb with a strong principal root and many spreading lateral roots in surface soil.

In the Igbo speaking part of Nigeria, it is commonly known as “Akidioji”. It is a climbing plant, with each pod containing

several edible seeds. It is grown mostly in the eastern part of Nigeria e.g. Ebonyi state, Enugu state, Abia state etc (Udensi*et al*., 2007).The presence of nodular bacteria specific to cowpea (Bradyrhizobium spp.), make it suitable for cultivation in the hot, marginal cropping areas of Southern Africa, as well as in the cooler, higher rainfall areas. However, cowpeas are much less tolerant to cold soils (Martins *et al*., 2003).

Cowpea is a higher drought-tolerant crop than many other crops. It grows in areas with average annualrainfall down to 500 mm; best grown in areas with annualrainfall between 750-1,100 mm. One of the most remarkable things about cowpea is that it thrives in dry environments; available cultivars produce a crop with as little as 300 mm of rainfall. Cowpea also has a great tolerance to water logging. Well-distributed rainfall is important for normal growth and development of cowpeas (Udensi *et al*., 2007)..

Cowpea is one of common names in English: cowpea, bachapin bean, black-eyed pea, southern, crowder pea, china pea and cowgram; in Afrikaans: akkerboon, swartbekboon, koertjie; in Zulu: isihlumaya; in Venda: munawa (plant), nawa (fruits) Mimbumba, indumba; in Shangaan: dinaba, munaoa, tinyawa. It is also known internationally as lubia, niebe coupe or frijol (FAO, 2005).

Cowpea contains about 22-25% protein, 3.4-3.9% ash, 1.3-1.5% fat and 5.9-7.3% fibre (Duke, 1981).Cowpea is one of the most important sources of proteins, carbohydrate and vitamins in the diet of many populations especially in developing countries (Philips and Mc Watters, 1991).They are nutritious and provide complementary proteins to cereals. Some people eat both the fresh pods and leaves, and the dried seeds are popular ingredients in a variety of dishes in the southern USA and Nigeria. Mixing of different cowpea varieties for food is common in northern and eastern Nigeria (Ntoukam, 2000).Cowpea grown to maturity can be used as a feed (grazed or harvested for fodder). They are used as fresh cut-and-carry forage, and for hay and silage.

Cowpea is susceptible to various pathogens such as *Colletotrichum lindemuthianum* (Tu, 1982), *Cercospora vignicaulis* (Mulder and Holliday, 1975) and *Fusarium oxysporum* (Swanson and Van Gundy, 1985). *Fusarium oxysporum* is responsible for vascular wilt in cowpea.

Seeds have been recognized as efficient vehicles in the dissemination of plant diseases between countries and continents (Jones, 1987). Seeds under storage are immensely exposed to attack by fungal pathogens. Seed borne diseases reduce yield of plants and quality of seeds. Therefore there is need to study the mycoflora of cowpea seeds in order to be conversant with the pathogenecity of these fungal pathogens. This study was carried out to isolate and identify the fungal organisms associated with cowpea seeds in three Local Government Areas in Enugu State.

MATERIALS AND METHODS

Sources of cowpea seeds

The cowpea seeds, *Vigna unguiculata* (L.) Walp used for this study were collected from three Local Government Areas in Enugu state, namely Nsukka, Igbo-eze and Udenu L. G. A. In each L. G. A., the seeds were collected from two different towns: Lejja and Orba for Nsukka L. G. A; Aku and Ukehe for Igbo-eze L. G. A and lastly

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Ezimo and Ubollo for Udenu L. G. A. The seeds were stored in an airtight black polythene bags and kept in a refrigerator for adequate preservation until when needed.

Isolation and identification of fungi associated with cowpea seeds.

The isolation of fungi associated with cowpea seed was carried out using the Standard Blotter method recommended by the International Rules for Seed Testing (ISTA, 1976) and Agar method (Klement and Voros, 1974).The apparently healthy seeds of *Vigna unguiculata* (L.) Walp were surface sterilized by soaking in 70% Ethanol for 5 minutes, rinsed with sterile distilled water for three consecutive times. The sterile seeds were picked with sterile forceps one by one and plated out in tens on filter papers (Whatman 9cm) moistened with sterile distilled water placed in sterilized glass Petri-dishes. Each set was incubated at room temperature (28 ± 2^oC) for 7days.

Inoculation/ Preparation of Pure Culture

The work area was first surface sterilized using 70% ethanol and cotton wool. Using an inoculating needle flamed to red hot and dipped in alcohol to cool, a small portion of the fungi colony was picked and transferred into a sterile plate containing solidified Potato Dextrose Agar. The culture was allowed to grow in a protected place.

After the period of incubation, the seeds were examined for fungal growth under Stereobinocular microscope. A light microscope was used to view the spores and the fruiting bodies of the organisms at a magnification of $X_{400.}$

All identified fungi were sub-cultured on Potato Dextrose Agar (PDA) medium incorporated with lactic acid in dishes to get single spore pure cultures. The inoculated Petri dishes were incubated at room temperature for 7days in a dark room.

Pathogenicity tests

One hundred grams of healthy-looking cowpea seeds were weighed out into 250ml conical flasks, plugged with non-absorbent cotton wool and covered with foil and then autoclaved at 121^oC for 15 minutes to eliminate any seed-borne microorganisms. After autoclaving, the flasks were allowed to cool and 100mls of sterile distilled water was added to each flask and shaken for 15 minutes to wet all the seeds. Using asterile cork borer (1.5cm in diameter) a disc of 7-day-old mycelial spores of each fungus obtained from the pure culture of isolated fungi was inoculated into each flask containing seeds. The flasks were shaken for about 15minutes to obtain homogeneity or to allow the fungus to be well distributed. The control flask, received the same treatment, but there was no fungus added to it. The conical flasks were placed in separate air tight plastic containers (which were previously surface sterilized) and incubated for 14 days after which the seeds were plated out. Pathogenecity tests were carried out on the healthy seeds to ensure that the organisms were actually associated with the seeds.

The identification of the isolated fungi was carried out with reference to Ataga *et al*. (2010). Frequency of occurrence of fungi was determined based on the Score Method recommended by Ataga and Akueshi (1986). The data generated were analysed by one way analysis of variance.

RESULTS

A total of four (4) fungal pathogens were isolated and identified from six locations in Enugu state. These organisms were;

Fusarium oxysporium, *Aspergillus niger*, *Botryodiplodia theobromae* and *Rhizopus stolonifer*. The most frequently encountered fungi pathogen from infected cowpea seeds in Enugu State was *Botryodiplodia theobromae*, followed by *Fusarium oxysporum*, *Aspergillus niger* and lastly *Rhizopus stolonifer* as shown in Table 1. The occurrence of fungi isolate according to the location was observed with *Fusarium oxysporum* occurring most in Lejja and Aku by 24 isolates and 18 isolates respectively. In Orba, *Aspergillus niger* occurred most by 19 isolates. In Ukehe, Ezimo and Obimo, *Botryodiplodia theobromae* occurred most by 21 isolates, 19 isolates and 11 isolates respectively.

Frequency of Occurrence of Fungi

The frequency of occurrence of the fungi isolated is shown in Table 2. It was observed that *Botryodiplodia theobromae* was the dominant fungus in all the locations with a mean occurrence of (42.37%), followed by *Fusarium oxysporum* (30.02%), *Aspergillus niger* (21.99%) and lastly *Rhizopus stolonifer* (10.15%).

Table 1: Fungi isolated from *Vigna unguiculata* Seed from six different locations in Enugu State.

Fungi	Nsukka		Igboeze		Udenu		
	Lejja	Orba	Aku	Ukehe	Ezimo	Ubollo	Mean
Fusarium oxysporum	1.7^{a}	1.3^{b}	1.7^{a}	1.3^{c}	1.4^{b}	1.4^{b}	1.5^{b}
Aspergillus niger	1.3^{b}	1.7^{a}	1.3^{c}	1.3^{c}	1.4^{b}	1.4^{b}	1.4^{c}
Botryodiplodia theobromae	1.6^{a}	1.7^{a}	1.5^{b}	1.7^{a}	1.9^{a}	1.6^{a}	1.7^{a}
Rhizopus stolonifer	1.3^{b}	1.4^{b}	1.0^{d}	1.5^{b}	1.3^{b}	1.2^{c}	1.3^{c}

Means followed by the same letter within columns are not significantly different (P= 0.05).
Score based on a scale in which 1= absence of fungus and 2= presence of fungus, therefore any mean score above 1 indicates presence of fungi.

Table 2: Frequency of Occurrences of Fungi according to Local Government Area (%)

Fungi	Location and Percentage Frequency			
	Nsukka	Igboeze	Udenu	Mean
Fusarium oxysporum	32.85	31.68	25.15	*30.02
Aspergillus niger	23.79	24.68	17.49	21.99
Botryodiplodia theobromae	31.19	49.8	46.11	42.37
Rhizopus stolonifer	12.21	7.36	10.89	10.15

***Mean value for three L.G.A.**

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DISCUSSION

Botryodiplodiatheobromae was the dominant fungus in all the locations followed by *Fusariumoxysporum*, *Aspergillusniger*and lastly *Rhizopusstolonifer*. Cowpea seeds from Igbo-eze L.G.A. were highly infestated with fungal pathogens as the number of isolates was more in Igbo-eze L.G.A. when compared to Nsukka and Udenu L.G.A.

Many seed borne fungi on cowpea have been reported to produce various symptoms and cause diseases ranging from fungal spots, rusts, wilting of plants and plant death (Saad*et al*., 1988).Emechebe and Shoyinka (1985) isolated sixteen major pathogenic organisms including fungi from diseased cowpea plants in Nigeria.

Bosah (2013) isolated and identified a total of fourteen (14) fungi including *Fusarium oxysporum* from cowpea in Asaba, Delta state.*Fusarium oxysporum*um is known to cause wilting in plants. Oluyemisi*et al* (2006) isolated nine fungal pathogens from cowpea seeds among which are *Fusariumoxysporum*and*Aspergillusniger*. Species of *Aspergillus*, *Penicillum*and *Fusarium* are responsible for most spoilage and germ damage before and during storage. They cause reduction in cooking or baking quality, and nutritive values, produce undesirable odours and colour and change appearance of stored food grade grains and decrease germinability and total decay (Castillo *et al*., 2004).

Cowpea serves as an important food for the teeming population of Africa. The results and observations derived from this study shows that there is need for adequate and regular seed testing with effective fungicidal treatment of seeds so as to ensure that growers produce healthy plants which will in turn provide healthy plant products.

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