ISOLATION, IDENTIFICATION AND PATHOGENICITY OF FUNGI ASSOCIATED WITH COWPEA (Vigna unguiculata L. (Walp) IN ASABA, DELTA STATE.

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ABSTRACT
The Ife Brown was used as it is a locally improved variety of cowpea that is highly susceptible to attack by microorganisms mostly fungi. A total of fourteen (14) fungi were identified and they include Most frequently encountered fungi were Sclerotium rolfsii, Fusarium oxysporium and Collectrichum lindemuthianum. Pathogenicity of the fungal isolates were shown. The Fusarium oxysporum and Sclerotium rolfsii had disease severity of 20%, Colletotrichum lindemuthianum had 15% while Cercospora cruenta, Phythium aphanidematum, Aspergillus niger and Rhizoctonia solani had 10%, respectively.
Keywords: Pathogenicity, Vigna unguiculata L. (Walp)

INTRODUCTION
Cowpea (Vigna unguiculata) (L.) Walp is an important crop for many subsistence farmers in tropical areas especially in Africa. The green plant parts of cowpea can be used as a vegetable or as fodder for cattle whereas the cowpea contain a high level of proteins and are used as human food (Philip and McWatters, 1991). The crop is well adapted to stress and has excellent nutritional qualities (El-Ameen, 2008). It is a very important food source in developing countries where animal protein is limited (Tenebe et al, 1995) thereby, supplementing the low protein menus due to high cost of animal source of protein (Fawole et al., 2006, Miko and Mohammed, 2007).

Almost all the parts of the cowpea are used as food. They contain proteins, vitamins and minerals (Fatokun, 2002). Its high protein level makes it extremely valuable in communities where many people cannot afford protein food derived from meat, fish and egg (IITA, 2007).

The amino acid of cowpea compliments those of cereals (Fashakin and Ojo Nelson, 1988). Cowpea also contains minerals such as potassium, iron, calcium, phosphorus and magnesium. Cowpea has received a particular attention from the onset of research on grain legumes at the International Institute of Tropical Agriculture (IITA), Ibadan. Many African countries depend on its seeds and leaves for food. Adams (1984) reported that Nigeria is known to be the greatest consumer of cowpea in the World.

Cowpea can be eaten alone or can be eaten with other food such as rice and yams. 45% of the low income households are cowpea three times a week. The value of cowpea lies in its high protein content and ability to tolerate drought. As a legume cowpea fixes atmospheric nitrogen, allowing it to grow on and improve poor soils (IITA, 1985). Cowpea grain contains about 25% protein making it extremely valuable when many people cannot afford protein foods such as meat and fish. Despite the many uses and its relevance to the tropics particularly the Nigerian agriculture; cowpea crop losses due to insect pests were estimated at 55-62%. Several fungi attack the legume plants during growth, harvest and storage. While more than 25 different species of fungi are known to invade stored grains and legumes (Duan et al., 2007), 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 odors and color and change appearance of stored food grade grains and decrease germinibility and total decay (Quenton et al., 2003 and Castillo et al., 2004).

In Nigeria, there is a need for more information on the identification and pathogenicity of fungi associated with cowpea. This present study aimed at isolating the fungi and determining the pathogenicity of the isolates.

MATERIALS AND METHODS
Study was carried out at the Teaching and Research farm of the Faculty of Agriculture, Delta State University, Asaba Campus. Ife-Brown, a locally developed variety of cowpea were planted. Each plot consisted of six (6) rows of 36 cowpeas per row. Plants were observed for infection at all stages of infection.

Diseased parts were incubated on Potato Dextrose Agar. Percentage frequencies of isolation (PFI) of all the fungi were calculated by the formular:

$$PFI = \frac{\text{No of times a fungus is encountered}}{\text{Total no of times all fungi were encountered}} \times 100$$

Identification of isolates was done using light microscope and standard mycological manuals by Barnett and Hunter (1999).

Pathogenicity of the isolates was done in the screen house using sterilized soil in polyethylene bags. Five (5) kilogram of the soil sample was placed in each of the polyethylene bags. Three (3) seeds of the cowpea (Ife-Brown) were planted in each bag. The upper and lower surfaces of the cowpea were sprayed to run off point with the spore suspension of each fungal isolate. The leaves were inspected visually on daily basis for symptom development up to 21 days after inoculation.

The disease severity was rated on a subjective scale from 0-5,
Where 0 = No disease, 1 = trace, 2 = mild, 3 = moderate, 4 = severe and 5 = very severe.

Diseases symptoms expressed from artificial inoculation were compared with those observed on the naturally infected leaves of cowpea plants. Percentages were used to quantify the level of disease severity and disease isolation frequency.

RESULTS AND DISCUSSIONS

Most frequently encountered fungi pathogen from infected cowpea plants in Asaba were Sclerotium rolfsii, Fusarium oxysporum and Collectotrichum lindemuthianum as shown in Table 1.

Emechebe and Shoyinka (1985) isolated sixteen major pathogenic organisms including fungi from diseased cowpea plants in Nigeria. They also observed the occurrence of Collectotrichum lindemuthianum species from infected cowpea pods. Pathogenicity of the isolates are shown in table 2. Fungi such as C. lunata, C. tenius, Penicillum spp., Botridipodia theobromae, Microphomina phaseoline and Cladosporium vignae were not re-isolated after three weeks.

Amadi and Oso (1996) reported the presence and isolation of Cercospora cruenta from cowpea seeds. They suggested that these fungi cause field and storage diseases in cowpea. In conclusion, cowpea is attacked by many fungi in the field. Baggett and Frazier (1973) reported rapid depletion of glucose, sucrose and fructose in 10 varieties of pigeon pea infected with Fusarium oxysporum. Ameda and Oso (1976) implicated C. lindemuthianum as a major pathogen of cowpea that infects all stages of its growth.

Embaby et al, (2013) found that Aspergillus flavous and Fusarium moniforme were associated with most legumes and they also reported that these fungi produce mycotoxins such as aflatoxins.

Table 1: Frequency of Fungal Pathogens Isolated from infected Cowpea (Vigna unguiculata) plants in Asaba

Fungi	Total No of times isolated	Isolation frequency
Sclerotium rolfsii	98	22.02
Fusarium oxysporum	87	19.55
Curvularia lunata	6	1.35
Alternaira tenius	8	1.87
Penicillum notatum	22	4.94
Cercospora cruenta	25	5.61
Collectrichum lindemuthianum	80	17.98
Pythium aphanidermatum	10	2.25
Aspergillus niger	30	6.74
Botrodiplodia theobromae	15	3.37
Phytophthora vigne	13	2.92
Microphomina phaseoline	17	3.82
Cladosporium vignae	15	3.37
Rhizoctonia solani	19	4.27

Table 2: Pathogenicity of the fungal isolates from cowpea in Asaba

Fungi	Isolation from tissue 3 weeks after incubation	Disease Severity	Percentage Severity %
Sclerotium rolfsii	+	4	20
Fusarium oxysporum	+	4	20
Collectrichum lindemuthianum	+	3	15
Cercospora cruenta	+	2	10
Pythium aphanidermatum	+	2	10
Aspergillus niger	+	2	10
Rhizoctonia solani	+	2	10
Curvularia lunata	-	0	0

Alternaira tenius	-	0	0
Penicillum spp	-	0	0
Botrodiplodia theobromae	-	0	0
Phytophthora vigne	+	1	5
Microphomina phaseoline	-	0	0
Cladosporium vignae	-	0	0

Key + = Present
- = Absent

Pathogencity of the isolates are shown in Table 2 with F. oxysporum having 20% disease severity, S. rolfsii had disease of 20% while C. lindemuthianum had disease severity of 15%.

REFERENCES

Adams, M.W. (1984). Cowpea production constraint national programmes. Beans/Cowpea collaborative research support programmes. Michigan State University. 71-82

Amadi, J. E. and Oso B. A. (1996).Mycoflora of cowpea seed and their effects on seed nutrient contents and germination. Nigeria Journal of Science 30: 63-69.

Baggett, S. R. and Fraizer, N. A. (1973). Seasonal pattern of Fusarium root rot development in resistant Vs Susceptible cultivars of Phaseolus vulgaris L. Horticultural Science 8:214-216

Barnett, H. L. and Hunter, B. (1999): Illustrated Geneia of imperfect Fungi. Burgess Publishing Company.

Castillo, M. D. H. H. L. Gonzulez, E. J. Martinez; A. M. Pacin and S. L. Resnik (2004). Mycoflora and potential for mycotoxin production of freshly harvested black beans from Argentinean main production área. Mycopathologia. Kluwer Academic Publishers Dorderecht, Netherlands, pp. 107-112.

Duan, C. X., X. M. Wang, Z. D. Zhu and X. F. Wu (2007). Testin of seedborne fungi in wheat germ plasm conserved in the National Crop Genebank of China Agricultural Science 6:682-687

El Ameen, T. M. (2008) Genetic components of some economic traits in cowpea Vigna unguiculata. Journal of Agricultural Science, 33, 135-49

Embaby, E. M. Mohammed Reda, Mosaad A. Abdel-Wahhab, Hassan Omara and Asmaa M. Mokabel (2013) Occurrence of toxigenic fungi and mycotoxins in some legume seeds. Journal of Agricultural Technology 9(1):151-164

Emechebe, A. M. and Shoyinka, S. A. (1985). Fungi and Bacterial Diseases of cowpea in Africa. Pages 178-192 in Cowpea Research, Production and utilization. Edited by S. R. Singh and K. O. Rachie Chichester: John Wiley and Son.

Fashakin, J.B and Ojo (1988) Chemical composition and nutritive changes of some improved varieties of cowpea (Vigna unguicula (L) Walp) 2: New breeds of varieties from the International Institute for Tropical Agriculture, Ibadan, Nigeria. Tropical Sci., 28:191-199.

Fatokun, A.C. (2002) Breeding cowpea for resistance to insect pest; attempted crosses between cowpea and Vigna vexillate. In challenges and opportunities for Enhancing Sustainable Cowpea Production, IITA, Ibadan, Nigeria. Pp. 52-61.

Fawole, O. B., Ahmed, O. and Balogun, O. (2006), Pathogenicity and cell wall degrading enzyme activities of some fungi isolates from cowpea (Vigna unguiculata L. Walp) Biokemistri, 18, 45-51.

IITA (1987) Annual Report for 1983. Pp 218, Ibadan, Nigeria, IITA.

IITA (2007) Sustainable Food Production in sub-saharan African. IITA Contribution. Pg 208.

Miko, S. and Mohammed, I. B. (2007), Performance of improved cowpea genotypes in the Sudan Savanna: I. Growth and dry matter production. Biological and Environmental Sciences. Journal for the Tropics, 4, 12-18

Phillp, R. D. and McWatters, K. H. (1991) Contribution of cowpea to nutrition and health, food tech. Sept. 127-130.

Tenebe, V. A., Yusuf, Y., Kaigama, B. K. and Asenime, I. O. E. (1995). The effects of sources and levels of phosphorus on the growth and yield of cowpea (Vigna unguiculata (L.) Walp) varieties. Tropical Science, 35, 223-228.

Quenton, K., A. S. Theresa F. O. Walter, P. R. Johnon: V. D. W. Liana and S. S. Gardon (2003). Mycoflora and fumonisin Mycotoxin Associated with cowpea seed. Journal of Agricultural and Food Chemistry 51: 2188-2191