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Mycological evaluation of the phylloplane of *Vigna unguiculata* (L.) Walp

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ABSTRACT: Cowpea a leguminous crop consumed by millions of people in Asia and sub-Sahara Africa often faces pre- and postharvest attack like fungal infection. Twelve cultivars of *Vigna unguiculata* (L.) Walp obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria were investigated for fungal infection using pour plate technique and eleven fungal isolates were obtained from nine of the cultivars. Pathogenicity of *Fusarium oxysporium* (cultivars IT8ID-994, IT98K-452-1); *Alternaria alternata* (cultivars IT98k-205-8, TVX3236, IT845-2246-4); *Colletotrichum truncatum* (cultivars IT86D-719, Ife brown, (IA4B45)244, IT90k-277-2) and *Sclerotium rolfsii* (cultivars Ife brown, (IA4B45)244, IT845-2246-4) was confirmed on these nine cultivars of cowpea showing varying percentage of disease occurrence. The incessant attack on cowpea plant both on the field and in storage calls for more research in the area of disease resistant varieties for the ever growing population.

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Cowpea (*Vigna unguiculata* L. Walp.) is one of the world's dicotyledonous leguminous crops and a major food crop of millions of people in the developing countries (Summerfield *et al*., 1974). This legume which is commonly known as black-eyed peas, southern pea, field pea, china pea, crowder pea, lubia, niebe, coupe or frijole is one of the most important food legume in the semi-arid tropics covering Asia, Africa, Southern Europe, Central and South America. The legume is widely produced in Africa under marginal production systems. The legume belongs to the family Fabaceae, and the Sub-family Faboideae which belongs to the genus Vigna section catiang, species unguiculata.

Cowpea as a pulse crop is of major importance to the livelihoods of millions of relatively poor people in less developed countries (Inaizumi *et al.,* 1999; Abizari *et al.,* 2013). It is valued because it is highly nutritious, has the ability to tolerate drought and also has the ability to fix atmospheric nitrogen allowing it grow and improve on soil with poor fertility (Senff *et al.,* 1992). This makes cowpea an important component of traditional intercropping systems, especially in the complex and elegant subsistence farming systems of the dry savannah in sub-Saharan Africa. Cowpea is mostly cultivated for its seeds, pods and leaves which is a good staple food in many parts of Africa where every parts of the plants is eaten (Fatokun, 2002). All the parts of the cowpea that are used for food are nutritious providing protein, vitamins and minerals. Fatokun, (2002) and Lambot, (2002) stated that cowpea fruit contain 51% carbohydrate, 22 to 32% protein, 13% fats and 3.5% minerals on a dry weight basis. Thus, most of its nutritional value is provided by protein and carbohydrate. The high protein content represents a major advantage in the use of cowpea in nutritional products, for infants and children's foods and they compensate for the large proportion of carbohydrate often ingested in African diets. Cowpea being a major source of protein in the diet of many people in the Sub-Saharan. The protein present in cowpea consists of 90% water soluble globulin and 10% water soluble albumins. Cowpea is also a good source of calcium, iron, zinc, vitamins and carotene (Adedire and Akinneye, 2003; Abizari *et al.,* 2013). According to FAO, about 3.32 million metric tonnes of cowpea dry grains were produced worldwide annually on about 9.8 million hectares, about 9.3 million hectares of these in West Africa. Africa accounts for about 75% of total world population (FAOSTAT, 2000). Nigeria produces 2.1 million metric tonnes making it the largest producer accounting for about 22% of total production, followed by Niger (650,000 tonnes) and Mali (110,000

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tonnes). World average yield is 337 kg/ha and average yield in Nigeria is 417 kg/ha.

The phylloplane, the surface of plant leaves, is a complex habitat that is characterized by a variety of microorganisms including bacteria, filamentous fungi and yeast. Pathogens, saprobes and epiphytes occur in this habitat and numerous studies have described the phylloplane populations from various plant species (Dorsey and Levetin, 2006). The non-pathogenic fungi that inhabit the phyllosphere depend on nutrients exuded from the leaf or those deposited from the atmosphere. In addition to nutrient levels, growth and abundance of phylloplane fungi are also influenced by environmental conditions such as water availability, UV radiation, and temperature (Dorsey and Levetin, 2006). Many of these fungi grow on the surface of living leaf, which is the resident inhabitant, they may be pathogenic or non-pathogenic while other fungi, which are unable to grow under such circumstances for a longer time are casual inhabitants. The phylloplane inhabitants have to be distinguished from the primary saprotrophs (not being able to grow on full extent until the onset of senescence). Cowpea is a kind of crop that faces different kinds of pest and diseases. One of the major problems of cowpea production is its susceptibility to various bacterial, viral and fungal infections (Ajibade and Amusa, 2001). Therefore, this study aim is to identify the kind of fungi that infect cowpea plants.

MATERIALS AND METHODS

Sources of Plant Seeds: All the cowpea seeds that were used for the purpose of this research as listed in Table 1 were collected from the Grain Legume Improvement Unit (GLIU), International Institute of Tropical Agriculture (IITA), located in Ibadan, Oyo State Nigeria.

Table 1: List of Cowpea genotypes used in the mycological work.

Code	Accession Number
1	IT98K-205-8
2	IT98KD-288
3	IT86D-719
4	IT81D-994
5	IFE BROWN
6	TVX 3236
7	IT845-2246-4
8	(IA4B45)244
9	IT97K-499-35
10	(IA4B55)257
11	IT90K-277-2
12	IT98K-452-1

Sources of fungi: The fungi used for this project were isolated from the leaves of some of the strains of the cowpeas that were infected during the cause of the experiment. The cowpea cultivars used were planted in rows on separate plots and they include: Drought tolerant (IT98K-205-8), Drought susceptible (IT86D-719), Bruchid susceptible (TUX 3236), Bruchid susceptible (IFE BROWN), Bruchid resistant (IT81D-994), Bruchid resistant (IT845-2246-4), and Vegetable [(IA4B45)244], Striga resistant (1T98K-452-1), and Striga susceptible (IT90K-277-2).

Isolation of the fungi: Isolation of fungi was done by cutting four millimetre of the diseased part of the leaf, this was then dipped in 40 % bleach and left for 60 seconds in the bleach. It was later removed and rinsed in three changes of distilled water before it was then inoculated on Potato Dextrose Agar plates which were prepared according to manufacturer's specification (Oxoid, Basingstoke, England). The developing fungal colonies were sub-cultured aseptically into fresh Potato Dextrose Agar plates until pure cultures of the isolates were obtained.

Identification of fungi: For identification, morphological studies of the fungi, i.e., the shape, size and spore formation after 72 hours were observed on plates containing the fungi. After 3 to 4 days growth of the fungi, the mycelium containing the spores were teased out on a slide with the aid of an inoculating needle cleaned with ethanol and subsequently stained with lactophenol blue and was observed under light microscope. The pictures of the organisms were taken using Motic Camera 2000. The fungi identified were confirmed by comparing their morphology with fungi descriptions of Talbot, (1971); Deacon, (1980) and Bryce (1992).

Pathogenicity test of the isolates: Pathogenicity test using Koch's postulate was carried out on two sets of twelve rows of planted cowpea in a greenhouse environment. Each set consisting of 36 cowpea per row were sprayed with spore suspension of each isolate. Spore suspension of all isolates from the cowpea leaves were prepared separately by adding 10 ml of sterilized distilled water to 14 day old PDA Petri dish cultures, dislodging the spores with separate sterile glass rods and filtering through three layers of sterile muslin cloth with separate sterile conical flask for each isolate. Each spore suspension was made up to 5 ml. With the aid of haemocytometer, the number of spores was estimated to be 3.2×10^3. Mature cowpea leaves growing in plastic bags under green house conditions were inoculated with spore suspension of the isolates (4.0 x 103spores/ml) using an atomizer until runoff from pure cultures of the isolates was reached. The inoculated plants with the respective controls (sprayed with sterile water) were covered with disinfected transparent polythene bags for 48 hours to maintain high relative humidity. After

the development of disease symptoms in the plants (three weeks after inoculation), the pathogens were re-isolated from observed artificially infected cowpea plants. The character of the symptoms was compared with that of the original fungal isolates to proof Koch's postulate. Percentages were used to quantify the level of disease severity and disease isolation frequency. Percentage frequencies of isolation (PFI) of all the fungi were calculated by the formula:

$$\text{PFI} = \frac{\text{NTF}}{\text{TNTF}} \text{ x } 100$$

Where $No\ of\ times\ a\ fungus\ is\ encountered$ and $Total\ number\ of\ times\ all\ fungi\ were\ encountered$

RESULTS AND DISCUSSION

Mycological evaluation of the infected plants: The surface of plants are very important habitat for microorganisms. Some microorganisms can only grow in association with plants as either parasites or symbionts. The fungi associated with the leave surface of cowpea was evaluated in this study. A list of twelve cultivars of cowpea were used for the study. The mycological study of the cowpea plots showed the presence of eleven (11) fungi species which were in association with the leaves. These identified isolates with the respective percentage frequency of occurrence are depicted in Table 2.

Table 2: Frequency of fungi isolates from the infected leaves of different cowpea strains

S/N	Fungal isolates	Number of times isolated	Percentage frequency of isolates
1	*Alternaria alternata*	54	10.63
2	*Aspergillus niger*	29	5.71
3	*Aspergillus wentii*	24	4.72
4	*Colletotrichum truncatum*	84	16.54
5	*Curvularia lunata*	8	1.57
6	*Fusarium oxysporium*	80	15.75
7	*Fusarium pallidoroseum*	73	14.37
8	*Microphomina phaseolina*	34	6.69
9	*Penicillium chrysogenum*	10	1.97
10	*Rhizoctonia solani*	16	3.14
11	*Sclerotium rolfsii*	96	18.90

Table 3: Occurrence of fungi in the nine genotypes of cowpea

Fungi	**Accession numbers**	**Genotypes**
Fusarium oxysporium	IT81D-994 IT98K-452-1	Bruchid resistant, Striga resistant.
Alternaria alternata	IT98k-205-8 TVX 3236 IT845-2246-4	Drought tolerant, Bruchid susceptible, Bruchid resistant.
Colletotrichum truncatum	IT86D-719 Ife brown (IA4B45)244 IT90k-277-2	Drought susceptible, Bruchid susceptible, Vegetable cowpea Striga susceptible.
Sclerotium rolfsii	Ife brown (IA4B45)244 IT845-2246-4	Bruchid susceptible, Vegetable cowpea Bruchid resistant.

Some of these fungi species are usually found living as parasites or symbionts on different parts of many plants. Fungi species identified in this study have been credited to diversely form an association one way or the other with cowpeas as reported by Ajibade and Amusa (2001) who noted different species of fungi associated with several cultivars of cowpea; Houssou *et al.,* (2008) and Bosah (2013) found these fungi on the various parts of cowpea. Kumar *et al.,* (2004) reportedly identified some in association with cowpea seeds while Adandonon *et al.,* (2004) found them causing stem rot in cowpea. Several studies have also confirmed these organisms as pathogens of crop plants which cause tremendous damage during the growing and fruiting seasons of the plants thereby leading to economical and nutritional losses. They are soil borne fungi of *V. unguiculata* (Shama *et al.,* 1988; Houssou *et al.,* 2008; Shahnaz *et al.,* 2015) isolated from varying cultivars of cowpea.

Pathogenicity of the isolates on cowpea plant: The study showed that four different types of pathogenic fungi can be isolated from nine genotypes of *Vigna unguiculata* which were affected during the course of the research. *Fusarium oxysporium, Alternaria alternata, Colletotrichum truncatum* and *Sclerotium rolfsii* were the four fungi re-isolated to confirm their pathogenicity. The identification of these pathogenic fungi in this present study, corresponds with the reports of Emechebe and McDonald (1979) who isolated species of *Fusarium* and *Colletotrichum* from *V. unguiculata.* Khare *et al.,* (2016) working with three cultivars of cowpea in Botswana also affirms the pathogenicity of *Fusarium* species on cowpea seed. Studies by Kumar *et al.* (2004) also showed that *Alternaria alternata* (a soil borne fungus) is a pathogen of cowpea. *Fusarium oxysporium* affected two of the genotypes (IT81D-994 and IT98K-452-1). *Alternaria alternata* affected three of the genotypes while *Colletotrichum truncatum* and *Sclerotium rolfsii* respectively affected four and three of the genotypes. This reports find credence in the submission of Bosah (2013) who identified some of these pathogen in Ife brown, one of the cultivars used in our study. These pathogenic isolates are seed borne and seed transmitted. The fungi affect yield, seed quality and marketability of the crop.

Conclusion: We hope that the information that we have obtained and provided in this study will add to the wealth of knowledge and provide a possible way of solving the numerous problems facing *V. unguiculata* one of which is fungal infection on the field. The observations pointed out in the study would

be of help to us as we intend to proceed in the research on cowpea breeding system.

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