IDENTIFICATION OF FUNGI ASSOCIATED WITH COWPEA (*Vigna unguiculata*) GRAINS IN HUMID AGRO-ECOLOGY

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ABSTRACT

Cowpea (Vigna unguiculata (L). Walp) is an important pulse crop majorly consumed widely in Africa as an affordable source of nutrients including protein. Cowpea belongs to the family Fabaceae and it is a self-pollinating crop. Cowpea provides shelter as a cover crop and improves soil fertility. Grains and pulses especially cowpea grains are susceptible to fungal contamination when stored under poor environmental conditions and the infection of fungal pathogens on cowpea grains could result in contamination, reduction in the quality of the grains and postharvest loss of cowpea grains. Four major towns in Ondo State Nigeria were randomly selected for cowpea grains assessment. The fungi isolated and identified were; Aspergillus niger, Trichophyton spp., Aspergillus fumigatus, and Myrmecridim spp. The result of this study is to give accounts of fungi species associated with the spoilage and postharvest loss of cowpea

Keywords: Cowpea, Soil fertility, Post-harvest loss, *Aspergillius spp.*, Agroecology

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INTRODUCTION

The cowpea (*Vigna unguiculata*) is an annual herbaceous legume from the genus *Vigna* and family Fabaceae/Papilionaceae. All cultivated cowpeas are grouped under the species *Vigna unguiculata,* which is subdivided into four cultivar groups: Unguiculata, Biflora, Sesquipedalis and Textilis. They can be distinguished from one another by different physiological factors, such as seed size and color; taste, yield and maturity time (Hedayati, *et al.,* 2007). The plant is an herbaceous legume showing considerable adaptation to warm climates with adequate rainfall and is cultivated across Southeast Asia, Africa, Southern United States and Latin America. Cowpea provides food for millions of people, mainly in developing countries, with an annual worldwide production of about 4.5 million metric tons (Animasaun *et al.,* 2015). Although beans are the primary focus of the cowpea plant, both flowers and leaves are also considered as edibles in some parts of the world. Most cowpeas are grown on the African continent, particularly in Nigeria and Niger, which account for 66% of world production.

Cultivated cowpeas are grown as warm-season-adapted annuals in tropical and subtropical zones in all countries in sub-Saharan Africa. The vast majority of the world's cowpea production (over 95%) takes place in sub-Saharan Africa, with about 12.5 million hectares under cultivation worldwide in 2014 (FAOSTAT, 2014). In Africa cowpea can be cultivated up to 1 800 m altitude but is mainly grown in the lowlands. Cowpea grains provide a rich source of proteins and calories, as well as minerals and vitamins. This complements the mainly cereal diet in countries that grow cowpeas as a major food crop. Cowpea starch is digested more slowly than the starch from cereals, which is more beneficial to human health.

However, it does contain some anti-nutritional elements, notable phytic acid and protease inhibitors, which reduce the nutritional value of the crop. Methods such as fermentation, soaking, germination, deranging, and autoclaving are used to combat the anti-nutritional properties of the cowpea by increasing the bioavailability of nutrients within the crop. Although little research had been conducted on the nutritional value of the leaves and immature pods, what is available suggests that the leaves have a similar nutritional value to black nightshade and sweet potato leaves, while the green pods have less anti-nutritional factors than the dried grains. The loss of cowpea grains during storage due to micro-organisms has long been a serious production constrainto growers in humidagro-ecology. Many fungi are serious parasites of maturing and stored grains and their invasion can result in various damages including, reduced yields of seed both quantitatively and qualitatively, discoloration, decreased germinability, mycotoxin production and total decay (Shahnaz *et al*., 2015).

Various seed borne fungal species reported to be associated with cowpea grains are; *Aspergillus niger*, which causes black spots on cowpea grains, *Aspergillus flavus*, which is a mycotoxigenic fungus that has the ability to produce B aflatoxin in cowpea grains thereby affecting the crop's growth, yield and also result in loss of market value, *Pencillium spp*, which inhibits the seed germination of cowpea grains, *Alternaria alternata*, which causes black spots or blight on cowpea grains, *Fusarium oxysporum*, which is a serious wilt fungi pathogen that reduces the biochemical content of cowpea and also caused stunted growth, *Rhizopus stolonifera*, which causes decaying of plant materials (***Zanjare et al., 2020***). The control of seed-borne fungal disease using synthetic fungicides is widely practiced in Ghana, although the use of these synthetic fungicides is effective, the approach has the tendency to result in the unintended accumulation of toxic fungicide residue in the ecosystem which may also induce resistance in pathogens in store (***Etaware, 2019a) and in the open market*** (***Etaware, 2019b).***

The Changes in the protein composition, reducing and non-reducing sugars were observed in grains of cowpea (*Vigna sinensis*) infested with either *Aspergillus nidulans* or *A. tereus* under different temperatures. Infection by *A. nidulans* was more deleterious than by *A. terreus* (Ouili et al., 2022**)** and Mahesh (2018). The same authors reported that the biochemical content of cowpea seed was changed by Fusarium oxysporum f.sp. tracheiphilum, *Aspergillus flavus, A. niger* and *Macrophomina phaseolina* infection. Cowpea seed samples collected from South Africa and Benin were contaminated with Fusarium species including *F. equiseti*, *F. graminearum, F. semitectum, F. proliferatum, F. chlamydosporum, F. sambucium,* and *F. subglutinans* (Kritzinger *et al.,* 2016). *Alternaria alternata, Lasiodiplodia theobrome, Drechslera tetramera* (*Cochliobolus spicifer*) and *Fusarium verticillioides* (*Gibberella fujikuroi*) were isolated from cowpea grains using the

standard blotter and agar plate methods. Emechebe and McDonald (2011) reported pathogens fungi detected on cowpea seed in Northern Nigeria from markets included Ascochyta spp., *Colletotrichum lindemuthianum, C. truncatum*, *Rhizoctonia solani, F. oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Septoria vignae and Corticium rolfsii*. Isolates of Apergillus and Fusarium produced mycotoxins in dry beans, cowpea and lupine (Lupinus sp. L.). Houssou *et al*. (2013) found two cowpea samples to be naturally contaminated with fungal species including Aspergillus that produced aflatoxins in Sudan. Houssou *et al*. (2013) and Kritzinger et al (2013) also confirmed the presence of aflatoxins in cowpea seed from West Africa. Grains and pulses especially cowpea grains are susceptible to fungal contamination when stored under poor environmental conditions by marketers and the infection of fungal pathogens on cowpea grains could result in contamination, reduction in the quality of the grains and postharvest loss of cowpea grains (Etaware, 2019).

The objectives of the study were;

(1) To isolate and identify fungi species that are associated with the spoilage and post-harvest loss in cowpea grains stored by farmers, and sold to consumers in Ondo and Ekiti states, Nigeria.
(2) Quantify the fungi mycotoxins in cowpea grains
(3) Determine phylogenetic relatedness among these fungi in the seed samples.

MATERIALS AND METHODS

Two types of grains (good and bad grains) were sourced from four locations each in Ondo State (Akure, Ore, Akoko, and Ita-ogbolu) and four locations in Ekiti state (Ilupeju-Ekiti, Otun-Ekiti, Aiye-Ekiti and Iyin-Ekiti).

Equipment/apparatus

Petri dishes (Pyrex), cork borer, wire loop. water bath, autoclave, incubator, drying oven, pipettes, filter paper, cotton wool, weighing balance, ruler, test tubes, McCartney bottle, refrigerator, ruler, cowpea grains, paper tape, NaoCl (sodium hypochlorite), and Film.

Method and Processes

Sterilization of working area using 70% ethanol& cotton wool
Disinfectant of grains by dipping in 1% of sodium hypochlorite (NaOcl) for 10 mins

Seed germination test

Filter paper would be moist with water and placed in the petri dishes
30 grains of good and infected once from each location would be placed in the petri dish containing filter paper in 3 replications. The petri dish would be covered and paper tape film would be use to cover its side plating on PDA for 7 days. 10g of cowpea seed was put into 90ml of distilled water, 15ml of PDA into each plate plating on PDA (culturing process)

Preparation of stock

The stock solution was prepared by weighing 10 g of sample of AKRG1 was dissolved into 90 ml of distilled water, and shaken thoroughly in a conical flask. For serial distillation 1 ml of my stock into a test tube that contains 9 ml of distilled water and was labelled it 10^{-1} and another labelled as 10^{-2}

Pour plate method of culturing

1ml of 10^{-2} and 10^{-4} into petri dishes and add 15-20 g/ml of prepared PDA into plate and a mixture of antibacterial was added at room temperature at 25°c for 5-7 days

Sub culturing

10g/ml of PDA was add into petri dishes and the fungi isolate, and incubated it at room temperature at 25°c for 3-5days. This is to identify the isolated fungi for morphologically and each plate was been labelled well after identification. During incubation of the plated sample, developing fungal colonies were subculture on freshly prepare PDA plated to obtain pure culture of the fungal isolate. The fungal isolated were identified based on the growth pattern and the color of the mycelia and they are also identified morphologically using compound microscope. Each fungi isolated would be viewed under the microscope for their microscope for their microscopic characteristics.

Sub culturing into slant bottle

15g/ml of PDA was poured into the slant bottle and antibacterial (chloramphenicol) was added, and allowed to gel, inoculated the isolated. Incubation was done at 25°c and stored in a cool and dry place.

Broth process for molecular analysis

60g/ml of PDA into the conical flask and allow it to settle for about 1-2 hour, sieve it into another conical flask and added antibacterial(chloramphenicol) and add 15 g/ml of the mixture into all the four slant bottle each. Sterilization was done for 30 mins and cooled, and inoculated the isolated fungi into the four slant bottle.

Molecular analysis

Nucleic extraction protocol for (fungi)

- Making of buffer to grind: for a sample, pick 1ml of Extraction buffer and add 3ul of Proteinase K, and inverse mix.
- Get a mortar and pestle, scoop the growth part of the isolate and grind with 1ml Extraction buffer and pour it inside 1.5ml Eppendorf tube.
- Incubate in water bath at temp 60°C for 10mins.
- After 10mins in water bath, bring it out in room temperature for 5mins.
- Add 600ul of PCI mix, and shake vigorously.
- Centrifuge @ 12,000rcf for 10mins.
- Collect 500ul of the clear supernatant into a fresh new 1.5ml Eppendorf tube.
- Add 300ul of isopropanol inverse mix 2-3times.
- Incubate in -20°C for 1hour.
- Centrifuge @ 12,000rcf for 10mins to sediment the pellet.
- Discard the supernatant and avoid the pellet been disturbed.
- Add 500ul of 70% cool ethanol to wash the pellet and spin 2mins for the respective speed.
- Discard the ethanol and tap dry to make sure there is no trace of much ethanol.
- Dry the pellet in 37°C incubator for 20mins.
- Suspend with 50ul of sterile distilled water, and store in -20 or -80°C for further experiment.

Polymerase Chain Reaction:

- Dilute the stock DNA extracted to ratio 1:50 dilutions if the concentration of your Stock DNA is 100ul/ng higher.
- Dilute by pipetting 2ul of the stock DNA, and add 98ul of sterile distilled water and vortex to reduce the concentration DNA to be used for PCR setup.
- The PCR was setup inside work station, which has been disinfected before using for setup of PCR.

Reagents	**1Reaction**
SDH20	12.88ul
5x colorless Buffer	5ul
Mgcl2	1.5ul
DNTPs	0.5ul
ITS1	0.5ul
ITS4	0.5ul
Taq polymerase	0.12ul

- Mix all the reagents in 1.5ml Eppendorf tube, vortex the mix and aliquot 21ul in pcr tube and add 4ul of your diluted sample.
- After adding diluted samples 4 ul of your samples called templates, the total reaction will be 25 ul for 1reaction, cover and short spin down with micro centrifuge.
- And load it in PCR machine, and run with the conditions below:
- PCR were amplified under the following conditions: Initial Temp 5mins at 94°C and 35 cycles of 30 secs at 94°C, 30 mins at 55°C and 30 mins at 72°C. A final stage step at 72°C for 7 mins.

Purification of PCR product

- Pick all the 25ul of the PCR product in 1.5ml tube and add 2.5 vol of 95 % cold ethanol, and incubate in -20°C for 1 hr.
- Bring the product out and spin in the centrifuge for 10 mins at 13,000 rpm. Note that you may not see the pellet after spin.
- Decant the supernatant carefully and add 500 ul of 70% cold ethanol to wash the pellet, and spin for 2 mins at same speed. This step will be done twice.
- Decant the last wash of the ethanol and carefully tap dry.
- Leave the tube open while incubating the incubator at 36°C for 15 min, to evaporate the remaining ethanol.
- Add 25 ul of Sterile Distilled Water, to suspend the pellet, put in 4°C for it to dissolve.

Gel Electrophoresis

- 2% gel was casted with 1x TAE buffer to run a check on the samples and wait for 30mins for the gel to solidify.
- Tear a Para film nylon and 2ul of loading dye to make it dense and visible to see, and vortex the sample and add 3 ul.
- Place the gel with the tray in electrophoresis tank, load the sample mixed above, and with molecular ladder of 100 bp, and run for 55mins.
- View the gel under the UV-Gel Doc to get the amplified results of the purified PCR product.

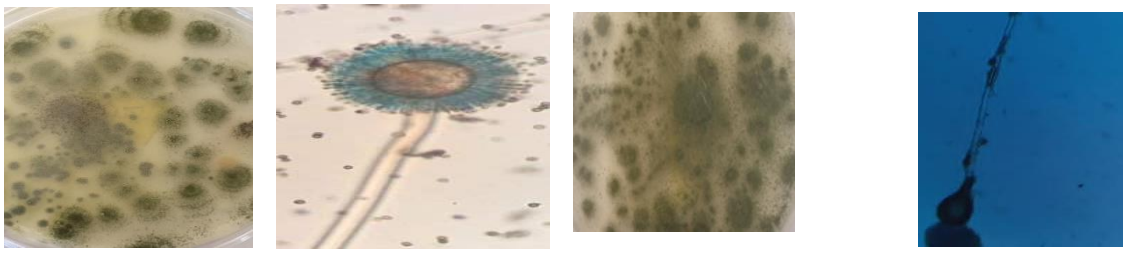
RESULTS

Morphological identification

Identified four fungal species on the cowpea grains were sampled from Ondo and Ekiti States:

Aspergillus fumigatus

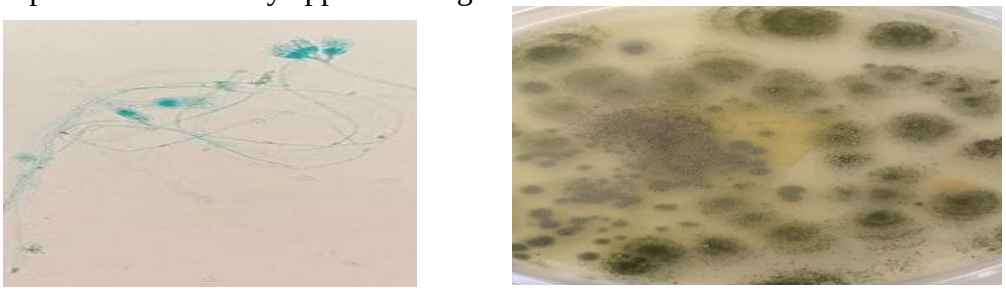
- The colonies are typically green with suede-like surface consisting of a dense felt of conidiosphores
- Conidial heads are typically columnar but often much shorter and smaller and uniseriate stripes are short, smooth walled and have conical-shaped terminal vesicles .which support a single row of phialides on the upper two thirds of the vesicle.



a b c d

Plate 1: Colony form and morphology of *Aspergillus fumigatus (a and b) from Ondo state and (c and d) from Ekiti state, Nigeria*

Aspergillus niger

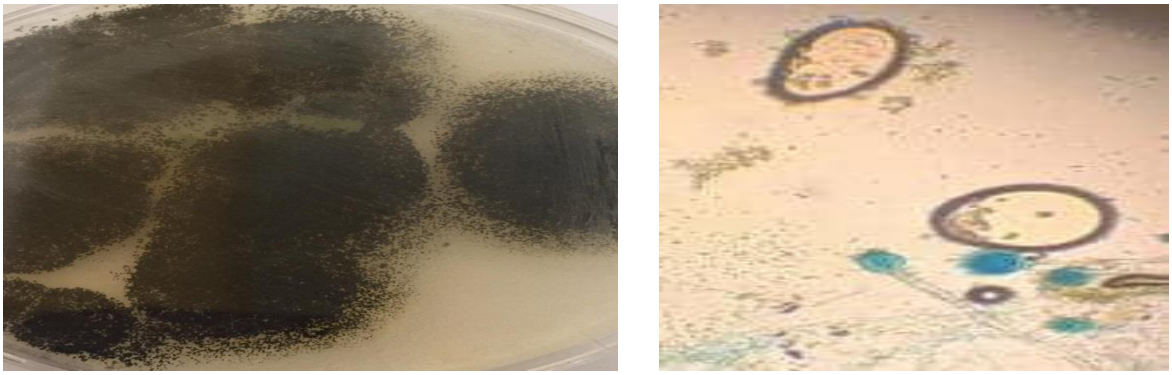
- Colonies are typically plain –black to dark brown as observed with the conidial heads
- Conidial heads are large, globose dark-brown, becoming-radiate and tending to split into several loose column with oge; conidial heads are biseriate with the phialides borne on brown, often septate metulae. They appeared rough-walled
- 

a b

Plate 2: Colony form and morphology of *Aspergillus niger*

Aspergillus nidulans

- The colonies are typically green in colour with a fluffy form surface consisting of a dense felt of conidiosphores.
- Conidial heads are typically columnar but often much shorter and smaller (uniseriate)smooth walled and have conical –shaped terminal vesicles which support a single row of phialides

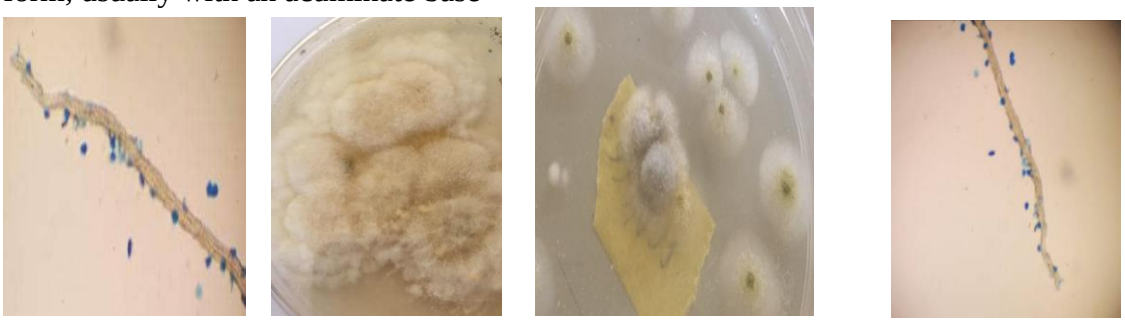


a b

Plate 3: Colony form and morphology of *Aspergillus nidulans*

Myrmecridium schulzeri

- Colonies are growing moderately rapidly, consisting of a rather compact, flat, submerged mycelium, whitish aerial mycelium, reverse side of the plate was orange in colour
- Conidial are sub hyaline, smooth walled or slightly rough-walled ,ellipsoidal, obovoidal or fusi form, usually with an acuminate base



a b c d

Plate 4: Colony form and morphology of *Myrmecridium schulzeri*

Table 1: Fungal isolates from Ondo state scores when blasted in NCBI (National Center for Biotechnology) in Ondo state

Lab ID	Sample ID	Scientific Name	Max Score	Total Score	Query Cover (%)	E Value	Percentage (%)	Accession
G1-CPS	Green	*Aspergillus fumigatus*	1031	1134	99	0	100	OR473251
B-CPS	Black	*Aspergillus niger*	1026	1026	100	0	100	OR473252
G2-CPS	Green	*Aspergillus nidulans*	966	966	100	0	100	OR473253
W-CPS	White	*Myrmecridium schulzeri*	952	952	100	0	100	OR473254

Table 2: Fungal isolates scores from Ekiti state when blasted in NCBI (National center for biotechnology information).

Lab ID	Sample ID	Scientific name	Max score	Total score	Query cover (%)	E value	Percentage identified (%)	Accession
B	Black	*Aspergillus niger*	1026	1026	100	0	100	OR473188
P	Pink	*Trichophyton spp.*	1105	1105	99	0	99.83	OR473189
G	Green	*Aspergillus fumigatus*	1105	1105	99	0	99.83	OR473190
W	White	*Myrmecridim spp.*	929	929	99	0	99.23	OR473191

The fungal isolated *vigna unguiculata* molecular assay include the following:
The green 1 isolated from cowpea seed with a sequence of
TCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCGA
CGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGACCCCAACATGAACGCTGTTCTGAAAGTATGCAGTC
TGAGTTGATTATCGTAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT
AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCT
GTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCCCGGGGGACGGGCCCGAAAGGCA
GCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCTGCTCTGTAGGCCCGGCCGGCGCCAGCCGACACCCA
ACTTTATTTTTCTAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAA
When queried on N.C.B.I (National Centre For Biotechnology Information) it recorded a 100% similarities with *Aspergilus fumagatus* (Accession number OR473251 in Table 1 above)

The Black from cowpea seed with a sequence of
TCATTACCGAGTGCGGGTCCTTTGGGCCCAACCTCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTC
GGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTC
TGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCG
ATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCC
TGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAAGGC
AGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTT
CCAACCATTCTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAA when queried on N.C.B.I (National Centre of Biotechnology Information) to have a 100% similarities with *Aspergilus niger* (Accession Number OR473252 in Table 1 above)

The Green two isolate from the cowpea seed with a sequence of
TCATTACCGAGTGCGGGCTGCCTCCGGGCGCCCAACCTCCCACCCGTGACTACCTAACACTGTTGCTTC
GGCGGGGAGCCCCCCAGGGGCGAGCCGCCGGGGACCACTGAACTTCATGCCTGAGAGTGATGCAGTC
TGAGCCTGAATACAAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAAC
GCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATT
GCGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTG
TTGGGTCGTCGTCCCCCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGTGTCCGGTCCTCGAGC
GTATGGGGCTTTGTCACCCGCTCGATTAGGGCCGGCCGGGCGCCAGCCGGCGTCTCCAACCTTATTTTT
CTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG when queried on N.C.B.I(National Centre of Biotechnology Information) to have a 100% similarities with *Aspergillus nidulans* (Accession Number OR473253in Table 1 above).

The white isolated from the cowpea with a sequence of
TCATTACGAGAGTGTCACCACTCCCAACCCATTGTTTACCTACCCGTCCACCGTGCTTCGGCAGGCAGT
CCTGTGGGACAGGGCCTCGCCCCCTCCGGGGGGTGCCTGCCGCTGGCCAACCAAAAATTCTAGCTGTT
TTTGTAACATCTGAGTCTTCCACAAATAAACAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGA
TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCACTAGTATTCTGGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCTGG
CTTGGTGTTGGGGCTCTGCGTTTGCAGTCCCTTAAATCCAGTGGCGGACACGCTAGGTCTCCGAGCGCA
GTATTTTTTTCTCGCTCAGGGCGTCCGGCGTGGGCTTGCCTCGCACCCATCTTATCAAGGTTGACCTCG
GATCAGGTAGGAATACCCGCTGAACTTAAGCATA poured when queried on N.C.B.I(National Centre of Biotechnology Information) to have a 100% similarities with *Myrmecridium schulzeri* (Accession Number OR473254 in Table 1 above)

The phylogenetic relationship between the isolates *Aspergillus fumigatus*, *Aspergilus niger*, *Aspergillus nidulans* , *Myrmecridium schulzeri* and other closely related fungi species are presented in Figure 1. The result shows the isolate G1-CPS very closely related to and result NR 121481.1 *Aspergillus fumigatus* also B-CPS are closely related with NR 111348.1 *Aspergillus niger*

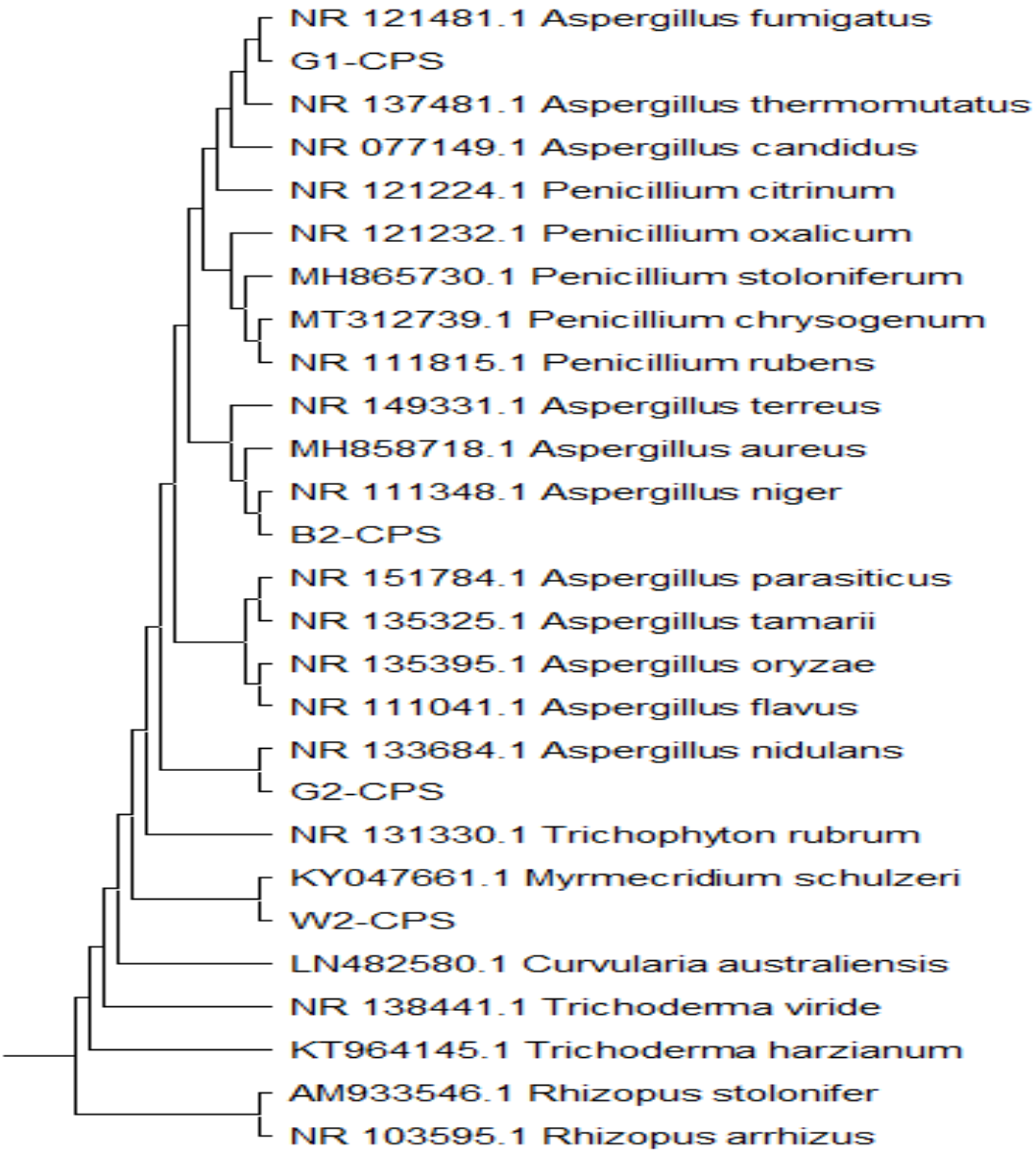


Figure 1: Phylogenetic tree of identified fungi species from Ondo state associated with the spoilage and postharvest loss of cowpea grains.

The isolate B-CPS is very closely related to and result NR 111348.1 which is *Aspergillus niger*, G2-CPS is closely related to NR 133684.1 *Aspergillus nidulans* also W-CPS has a closely relatedness with KY 047661.1 *Myrmecridium schulzeri*

The phylogenetic relationship between the isolates, *Aspergillus niger*, *Trichophyton spp*., *Aspergillus fumigatus* and *Myrmecridim spp*., are closely related. Fungal species are presented in figure 1. The result showed that isolate G1 is very closely related to and resembles NR 121481.1, *Aspergillus fumigatus*. Also, B1 has a close relatedness with NR 111348.1, *Aspergillus niger*.

Isolate P1 is very close to NR 131330.1, *Trichophyton rubrum* and isolate W1 has a close relatedness with KY047661.1, *Myrmecridim schulzeri*.

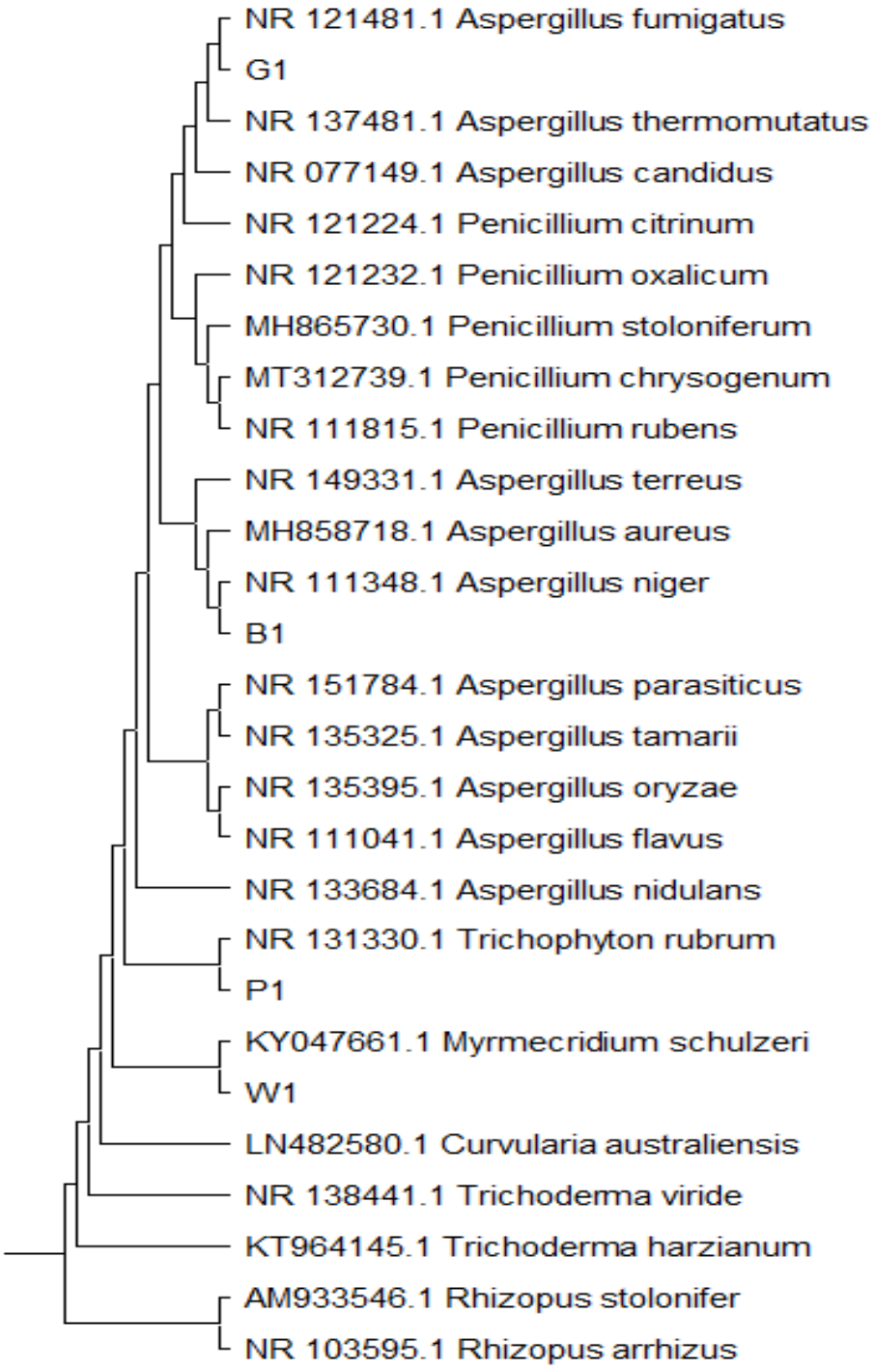


Figure 2: Phylogenetic tree of identified fungi species from Ekiti state associated with the spoilage and postharvest loss of cowpea grains.

DISCUSSION

Vigna unguiculata is considered as an incredible source of many other health- promoting components, such as soluble and insoluble dietary fiber, phenolic compounds, minerals, and many other functional compounds, including B group vitamin, tocopherols, anthocyanins and carotenoids (Bai *et al*., 2020). The results of this studies have proved that the fungi isolates associated with the spoilage and postharvest loss of *Vigna unguiculata* include; *Aspergillus niger, Trichophyton spp., Aspergillus fumigatus*, and *Myrmecridim spp*. Aspergillus strains presented similar morphological characteristic to those isolated by Abdallah *et al*., (2019), Okayo *et al*., (2020) and Ono *et al*., (2021) with ability to produce aflatoxins and ochratoxin A (OTA), two toxins that could be the cause of liver cancer Mouhamed, (2012). The fungi species identified from the cowpea grains have also been reported to be associated with cowpea grains in different studies; *Aspergillus niger, Aspergillus flavus* and *Pencillium spp*. were found to be associated with *Vigna unguiculata* in India in a study by Zanjare *et al*., 2020. In a separate study, *Aspergillus fumigatus* is involved in the spoilage of *vigna unguiculata*. Also *Aspergillus nidulans* have been implicated and other fungi. This finding is consistent with previous research reports implicating *Aspergillus fumigatus* in the spoilage of V*igna unguiculata,* as well as other fungi such as *Aspergillus nidulans*.

Our study four fungi were isolated four locations each in Ondo and Ekiti states. They belong to the genera categorized as field and storage molds; among these, strains of *Aspergillus fumigatus* and *Aspergillus niger* exhibited notably high infection rates (ranging from 2 % to 100 %) compared to other strains. The phylogenetic tree constructed for the fungi in each state (Figures 1 and 2) suggest high similarities in their ordination and groupings at the genomic level. This affirms similar fungi cause high post-harvest losses in cowpea grains in storage and markets. The locations (Ondo and Ekiti states) where the fungi were isolated is characterized by weather pattern (rainfall, humidity, sunshine, and solar radiation) peculiar with the rainforest agro-ecology. The ability of these fungi to adapt to a wide temperature range in Ondo and Ekiti states may be the reason for their prevalence in the rain forest agro-ecological zones. Aspergillus showed a wide geographic spread but is more frequently found in areas with warm temperature. The majority of Aspergillus species prefer temperatures between 25˚C and 40˚C for optimum growth. For this reason, they grow very well in the so-called “dry” food products like cowpea. Thus, precautions must be taken during post-harvest activities and storage to avoid contamination of cowpea crops by these ubiquitous molds. The study demonstrate the prevalence and potential impact of these particular fungal strains on *Vigna unguiculata* spoilage.

CONCLUSIONS

The fungal species belonging to four genera were identified from 4 cowpea seed samples from Ondo and Ekiti states in this study. *Aspergillus flavus, Aspergillus niger, Aspergillus nidulans, and Myrmcridium schulzeri* are the species that were not only often observed but also had high seed infection rates reaching 100% in certain cases. These fungal species are mycotoxigenic, hence their abundance in cowpea seed samples could pose a health risk. Additionally, a range of harmful fungi are present in the cowpea grains grown in Nigeria. These include *R. solani, M. phaseolina, Rhizopus sp., Cladosporium sp.,* and *E. nidulans*. The nutritive, organoleptic, and germination value of grains may be diminished as a result of the frequency and abundance of these fungi in

samples. It is therefore important to develop methods to control the growth of these fungi in cowpea grains and crops in general to contribute to the preservation of consumer health and the reduction of food insecurities. *Aspergillus fumigatus* is a type of fungus that exists in association with cowpea grains and offers numerous benefits. These nitrogen-fixing bacteria form symbiotic relationships with cowpea plants, which enriches the soil with nitrogen and supports plant growth. Additionally, *Myrmecridium Schulzeri*, a type of mycorrhizal fungus, can enhance nutrient absorption in these plants. However, it is important to note that certain fungal species like Aspergillus and Penicillium can cause seed spoilage. Therefore, proper storage and handling protocols must be followed to prevent contamination. Overall, the presence of Aspergillus fumigatus and other beneficial fungi can provide significant advantages for cowpea production while also requiring careful attention to avoid potential issues.

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