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Screening of Cowpea (*Vigna unguiculata* (L.)Walp) Varieties for Resistance to Leaf Spot in Southern Guinea Savannah Agro-Ecology of Nigeria.

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Abstract

Five cowpea varieties (*Vigna unguiculata* (L.) Walp) namely UAM 09 1055-6, UAM 09 1051-1, IT 99k-573-1-1, IT 90k-277-2 and IT 99k-573-2-1 were investigated for fungi associated with the seed, leaf spot disease incidence and severity in Makurdi (07º 45'- 7º 50'N and 08º 45' - 08º 50'E ; 98 m)and Otobi (7°07' - 7°11'N and 8° 05- 8°10'E) in Benue State, Nigeria under natural infection. The effect ofleaf spot incidence and severity on the growth and yield of cowpea varieties were also evaluated. Fungi associated with the seeds and leaf spot lesions were isolated and identified. Diseased leaf samples from the study locations were found to be infected with *Fusarium verticillioides, Curvularia lunata*, *Aspergillus tamarii* Kite, *Lasiodiplodia theobromae, Aspergillus flavus* Link and *Aspergillus niger* van Tiegh, *Pythium spp, Fusarium solani, Macrophomina phaseolina* and *Phoma sp*. Otobi field had significantly higher incidence and severity of leaf spot disease than the Makurdi field. Cowpea variety IT 99k-573-1-1, IT 99k-573-2-1 and IT 90k-277-2 were classified as moderately resistant in Makurdi with a mean incidence of 22.23%, 16.97% and 16.67% respectively while varieties UAM 09 1051-1 and UAM 09 1055-6 were classified as Moderately susceptible and Highly susceptible with mean leaf spot incidence of 41.67% and 99.17% respectively. In Otobi, all the cowpea varieties screened were classified as Susceptible to leaf spot incidence with the exception of variety IT 90k-277-2 which was classified as moderately susceptible to leaf spot disease.

Key words: Cowpea, leaf spot, screening, resistant, susceptible.
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Introduction

Cowpea (*Vigna unguiculata* (L) Walp) is a legume widely cultivated in the tropics. Its grain is consumed as a source of cheap dietary protein while the tender leaves and immature pods are also eaten as vegetables (Dugje *et al*., 2009).In addition, the plant serves to provide fodder for livestock. Nigeria is the largest cowpea producer in Africa where cowpea is a source of livelihood for many subsistent farmers (Neya *et al*., 2015). In Nigeria, Cowpea is cultivated in the drier areas including Benue State where the rainfall range is between 500 and 1200 mm/year (Agber *et al*., 2017). The extra-early and early maturing cowpea varieties also thrive in the Sahel where the rainfall is less than 500 mm (Dugje *et al*., 2009).Cowpea production is affected by the incidence of pests and diseases at different stages of development. Leaf spot incidence on cowpea limits the leaf available for photosynthesis resulting in reduced yield. Yield losses of between 20 % and 40 % have been reported on cowpea due to leaf spot disease (Boa, 2014). Although *Pseudocercospora cruenta* or *Mycosphaerella cruenta* which are air borne

(Boa, 2014) have been previously known as the causal agent of leaf spot on cowpea, the disease maybe caused by other fungal pathogens due to the emergence of new leaf spot fungi infecting cowpea. There is the need to identify these fungi and to screen the field response of some new cowpea varieties and existing varieties to leaf spot infection in Makurdi and Otobi in the Southern Guinea Savanna of Benue State, Nigeria. This study seeks to determine the seed health and viability of five cowpea varieties and to evaluate the response of these varieties of cowpea to infection by these fungi under natural infections.

Materials and Methods

Seed health testing.

Seed health of Cowpea varieties collected from the seed bank of the Molecular Biology Laboratory of Federal University of Agriculture, Makurdi, Nigeria was evaluated. Germination of seeds was done following the International Seed Testing Association guidelines.

Isolation and identification of seed borne fungi

The blotter method was used in isolating seed borne fungi. Cowpea seeds were sterilized in 10% Sodium hypochlorite for 1 minute and rinsed in three changes of sterile distilled water (SDW). Ten seeds were placed on moist blotter in sterilized 9cm diameter Petri dishes moistened with 10ml SDW and incubated for seven days at ambient conditions of light and temperature. A total of ten replicates were maintained for each of the five varieties giving a total of 500 seeds arranged in a completely randomized design. The incubated seeds were observed for germination and fungal infection. Fungal isolates were further sub-cultured on PDA to obtain pure cultures. Isolated fungi were identified using manual and confirmed with the Germplasm Health unit of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Screening of Cowpea varieties for leaf spot infection under natural infection

Two field trials were set up simultaneously at the Teaching and Research Farm of the Department of Crop and Environmental Protection (07° 45'- 7° 50'N and 08° 45' - 08°50'E; 98 m) and National Root Crops Research Institute Otobi Sub-station Benue State, located between 7°07' - 7°11'N and 8° 05- 8°10'Ein the Southern Guinea Savannah of Nigeria.Two newly released varieties (UAM 09 1055-6 and UAM 09 1051-1) and three cowpea varieties (IT 99k-573-1-1, IT 90k-277-2, IT 99k-573-2-1) obtained from the Molecular Biology laboratory of Federal University of Agriculture, Makurdi were evaluated in the locations.

The sites fall within the Southern Guinea Savannah agro-ecological zone of Nigeria, where the rainfall is bi-modally commencing between March/April and terminating in October/November with the highest peak in July/August. The total annual rainfall is about between 900 mm to 1200mm and maximum and minimum temperatures of 37ºC and 21ºC, respectively, while the relative humidity is between 70-80% (Agber *et al*., 2017).

Experimental Treatment and Design

The experiment evaluated five cowpea varieties which were laid out in a Randomized Complete Block Design with three replications on a field of 17m x 13m (221m²) with plot sizes of 3m × 4m(12m^2). The site was manually cultivated before planting. Two newly releasedcowpea varieties UAM 09 1055-6 (early maturity) and UAM 09 1051-1 and three other cowpea varieties IT 99k-573-1-1(Medium maturity), IT 90k-277-2 (Medium maturity), IT 99k-573-2-1 obtained fromthe Molecular Biology laboratory of the Federal University of Agriculture, Makurdiwere used.

Three seeds were planted per hill at an intra row spacing of 30cm and inter row spacing of 75cm. The Cowpea seedlings were later thinned to two seedlings per hill. Single Super Phosphate fertilizer (SSP) was applied at the recommended rate of 30kg/ha at planting and NPK 15: 15:15 fertilizer at the rate of 15kg/ha at 4 weeks after planting (WAP). Weeding was done at 3 and 5 weeks after planting. Cowpea field was sprayed with Cypermethrin and dimethoate insecticide at the rate of 50 g a.i/ha at 3, 6, 7 and 8 weeks after planting.

Isolation and Identification of fungi

Cowpea leaf samples with necrotic spots were collected from the two locations for isolation and identification of fungi. Small sections (3-5mm) were cut from the edges of infected leaves to contain both diseased and healthy tissues. The

tissues were sterilized for 1minute in 10% Sodium hypochlorite solution after which they were rinsed in three changes of SDW and blotted dry on sterile filter papers. Potato Dextrose Agar (PDA) was prepared by adding 39g in 1litre of Sterile Distilled Water (SDW) in a conical flask. The flask was autoclaved at 121^{o}C for 15 minutes; the media was allowed to cool. Streptomycin Sulphate was added at the rate of 0.2g/L and the media allowed to solidify. The plates were then incubated on the laboratory bench at ambient conditions of light and temperature (30± 2^{o}C) for 3 days. Pure culture was obtained by sub culturing unto fresh PDA plates.

Microscopic examination was done by examining the colony characteristics. A sterile needle was used in taking a little portion of the hyphae containing spores on the sterile glass slide stained with lactophenol cotton blue and examined under the microscope for fungal structures. Pure cultures were identified using compound microscope and compared with reference manual (Wanatabe, 2010). The cultures were further confirmed at the Germplasm Health Unit of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Data Collection

Incubated seeds were examined for the presence of fungi to determine the seed health. Infected seeds were counted and recorded as percentages.

For the field screening, data collection was from the two inner rows. Data collection wason emergence percentage, disease incidence and severity, plant height, number of branches, number of leaves, number of pods per plant, days to maturity, number of days to 50% flowering, pod weight, pod, number of seeds pod, pod length ,100 seed weight, yield plot were recorded per plot.

Disease incidence

Disease incidencewas assessed by counting the number of infected plants in relation to the total number of plants per plot expressed as a percentage at 5, 8 and 11WAP corresponding to vegetative, flowering and pod stages.

Disease severity per plot

Disease severity was taken at 5, 8 and 11 weeks after planting. The average severity score was taken on each plot using the key from Onuegbu and Emiri (2011):

0 = leaves without spot.
1 = leaves with less than 5 spots.
2 = leaves with 5-10 spots.
3 = leaves with more than 10 spots.
4 = dead leaves.

Leaf spot Disease Rating

The reaction of the Cowpea varieties to leaf spot disease across the two locations was evaluated using a modified rating scale adopted from Oladiran (1983):

0= No leaf spot (Highly Resistant)
1-25% leaf spot (Moderately Resistant)
26-50% leaf spot (Moderately Susceptible)
51-75% leaf spot (Susceptible)
>76% leaf spot (Highly Susceptible)

Data analysis

Data were subjected to analysis of variance using Genstat 9th edition. Significant means were separated using Fishers least significant difference (F - LSD) at 5% level of probability. The emergence and growth data for both locations were averaged and analyzed.

Results

The data presented in Table 1 shows the fungi isolated from cowpea seeds before planting.*Aspergillusflavus* was isolated from all the cowpea varieties with highest isolation frequency of 6.40 % followed by *Fusarium verticillioides* which was isolated from all cowpea varieties with a frequency of 2.00 % and absent on IT 99k-573-2-1. *Fusarium solani* and *Curvularia lunata* were each isolated only from cowpea variety IT99k-573-1-1 and IT90k-277-2 respectively.

Table 1: Fungi isolated from seeds of five cowpea varieties and their frequency of isolation in Makurdi

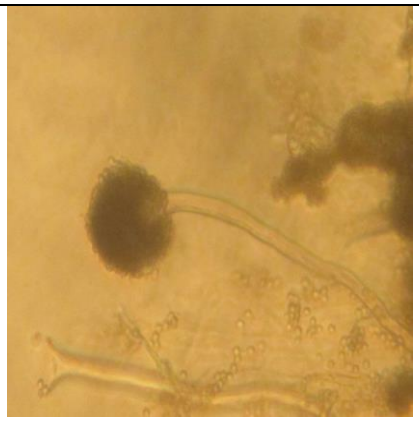
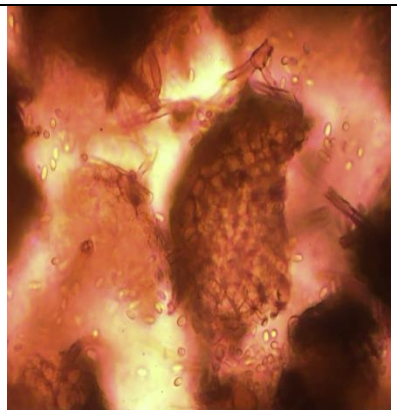
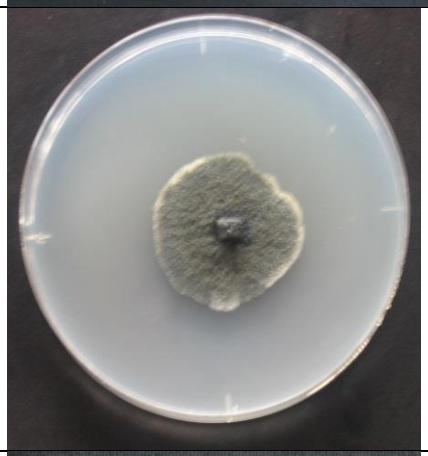
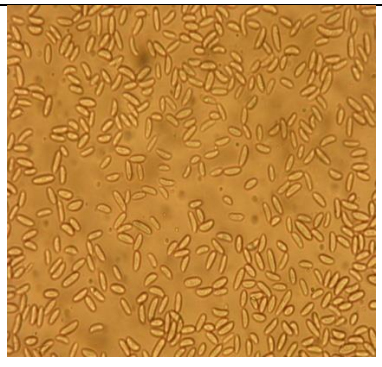
Fungi Isolated	Cowpea variety					Total no. of times isolated	Isolation frequency (%)
	IT99k-573-1-1	IT90k-277-2	UAM 09 1051-1	UAM 09 1055-6	IT99k-573-2-1		
F. solani	+	-	-	-	-	1	0.20
A. flavus	+	+	+	+	+	32	6.40
A. niger	+	+	+	-	+	8	1.60
F. verticillioides	+	+	+	+	-	10	2.00
A. flavus/*F. Verticillioides*	-	-	+	-	+	2	0.40
Curvularia lunata	-	+	-	-	-	1	0.20

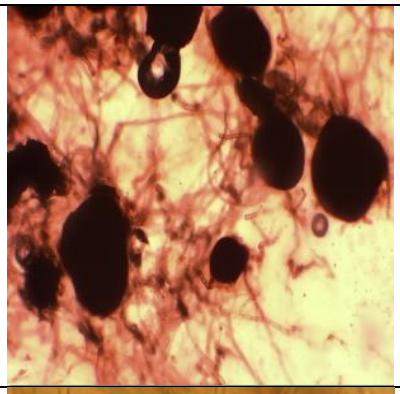
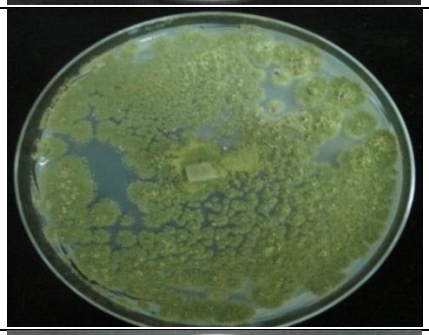
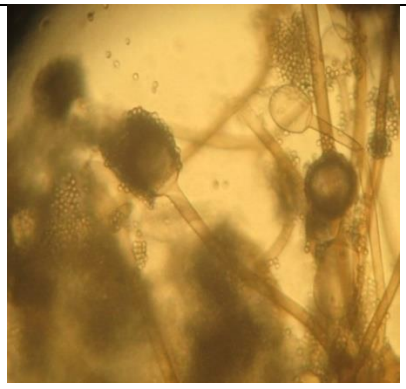
+ = Present; - = Absent

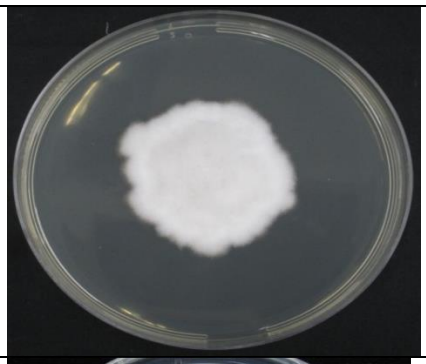
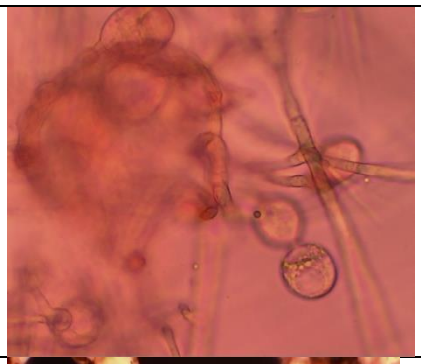
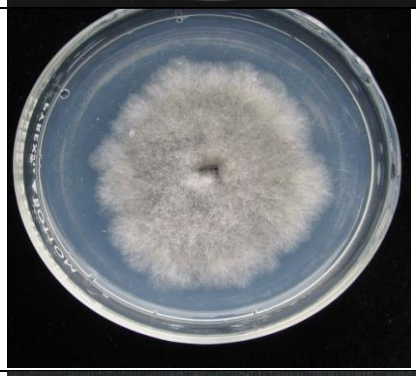
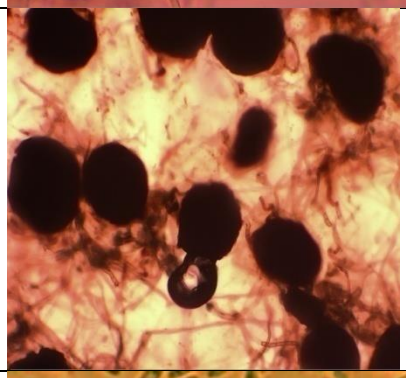
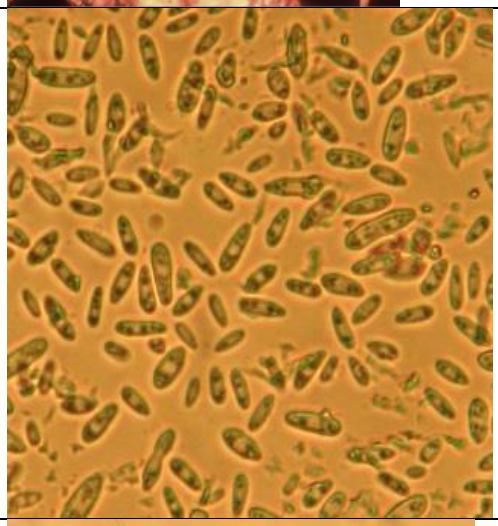
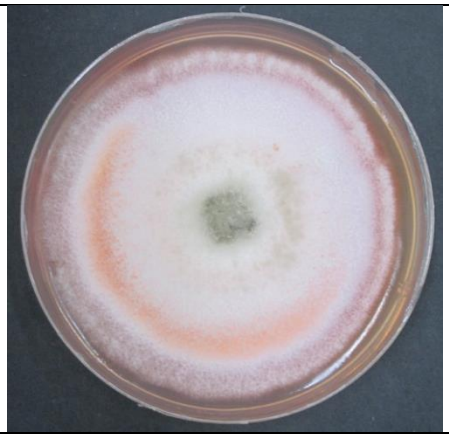
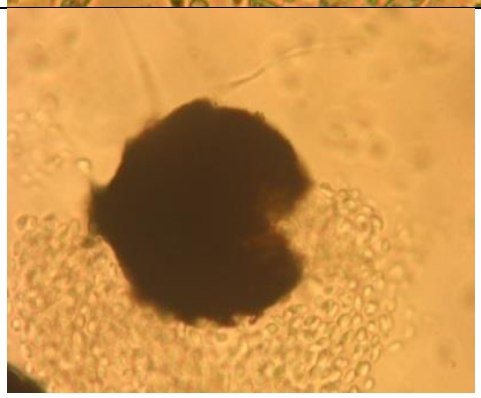
Isolation of fungi associated with Leaf spot Lesions of Cowpea Varieties

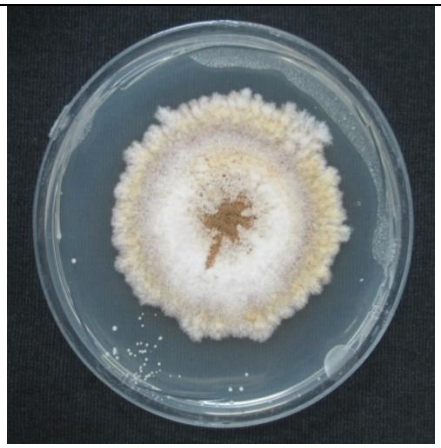
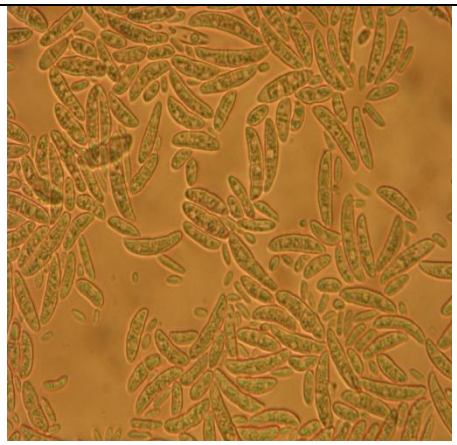
A total of eight genera were isolated from the infected cowpea leaves and seeds. Cultural characteristics of fungi associated with cowpea seed and leaf spot in Makurdi and Otobi are presented in plates 1- 14.

S/N	Fungi	Mycelium growth	Microscopic description
1.	*Pythium* sp from the leaves of IT 99k 573-1-1		

2.	*Aspergillus tamarii* from the leaves of IT 99k 573-1-1		
3.	*Phoma* sp from the leaves of IT 99k 573-1-1		
4.	*Curvularia lunata* from the leaves of IT 90K-277-2		
5.	*Fusarium verticilliodes* from the leaves of UAM 09 1051-1		

6.	*Macrophomina phaseolina* from the leaves of IT 99k 573-1-1		
7.	*Lasiodiplodia theobromae* from the leaves of IT99k-573-1-1		
8.	*Aspergillus flavus* from the leaves of IT99k-573-1-1		
9.	*Aspergillus niger* from the leaves of IT99k-573-1-1		

10.	*Pythium* sp from the leaves of IT99k-573-1-1		
11.	*Macrophomina phaseolina* from the leaves of UAM 09 1051		
12.	*Fusarium verticilliodes* from the seed of IT99k-573-1-1		
13.	*Phoma* sp from the leaves of IT99k-573-1-1		

14.	*Fusarium solani* from the seeds of IT99k-573-1-1		

The incidence of leaf spot disease on five varieties of cowpea in Makurdi and Otobi at three growth stages is presented in Table 2.In Makurdi, Cowpea variety UAM 09 1055-6 was highly susceptible to leaf spot disease recording significantly higher (P≤ 0.05) leaf spot incidence of 97.50% at the vegetative stage compared with leaf spot incidence of IT 99k-573-1-1(16.70%), IT 90k-277-2 (10.80%) and IT 99k-573-2-1(9.20%). The leaf spot incidence of UAM 09 1055-6 and UAM 09 1051-1 progressed significantlyto 100% and 99.20% respectively at flowering and pod stages in both locations. On the average, cowpea variety IT 99k-573-1-1, IT 99k-573-2-1 and IT 90k-277-2 were classified as moderately resistant in Makurdi with a mean incidence of 22.23%,16.97% and 16.67% respectively which was significantly (P≤ 0.05) lower compared with the mean leaf spot incidence recorded on UAM 09 1051-1 (41.67%) classified as Moderately susceptible and UAM 09 1055-6 (99.17%) classified as Highly susceptible. In Otobi, all the cowpea varieties screened were classified as Susceptible to leaf spot incidence with the exception of variety IT 90k-277-2 which was classified as Moderately susceptible to leaf spot disease.

Table 2: Incidence of leaf spot disease on five varieties of cowpea in Makurdi and Otobi

Cowpea Varieties	Vegetative stage		Flowering stage		Pod stage		Mean Reaction class	
	Makurdi	Otobi	Makurdi	Otobi	Makurdi	Otobi	Makurdi	Otobi
IT 99k-573-1-1	16.70	30.80	18.30	48.30	31.70	80.80	22.23MR	53.30S
IT 90k-277-2	10.80	26.70	12.50	40.00	26.70	67.50	16.67MR	44.73MS
UAM 09 1051-1	12.50	43.50	12.50	62.30	100.00	99.20	41.67MS	68.33S
UAM 09 1055-6	97.50	44.20	100.00	61.70	100.00	100.00	99.17HS	68.63S
IT 99k- 573-2-1	9.20	30.00	11.70	40.80	30.00	82.50	16.97MR	51.10S
FLSD (0.05)	9.22	12.76	9.20	12.88	16.10	10.49		

MR= Moderately resistance, MS= Moderately susceptible, HS= Highly susceptible S= Susceptible

Table 3 shows the pooled emergence and growth characteristics of Cowpea varieties screened for leaf spot disease in Makurdi and Otobi in 2018 cropping season. Variety UAM 09 1051-1 had significantly higher(P≤ 0.05) emergence of 94.45% compared with all other cowpea varieties screened at both locations. VaVariety IT 90K 277-2 and IT 99K-573-1-1 recorded 8282.42 % and 80.20 % emergence while variety UAM 0909 1055-6 recorded the least emergence percentage ofof 61.99 %. Cowpea variety IT 90K 277-2 had significantly

higher number of branches and number of leaves compared with all other cowpea varieties screened. Cowpea variety UAM 09 1055-6 attained 50% flowering earliest in 42.50 days while variety UAM 09 1051-1 attained 50 % flowering in 48.16 days. Variety IT 99K-573-1-1 was significantly taller (67.29cm) compared with other cowpea varieties screened while variety IT 90K 277-2 was the shortest with 60.54cm.

Table 3: Emergence and Growth characteristics of Cowpea varieties screened for leaf spot disease in Makurdi and Otobi in 2018 cropping season

Cowpea varieties	**Emergence percentage**	**Number of branches**	**Number of leaves**	**Days to 50% flowering**	**Plant height (cm)**
IT 99k-573-1-1	81.58	21.87	76.93	45.50	67.29
IT 90k-277-2	82.42	25.24	92.33	44.66	63.59
UAM 09 1051-1	94.45	23.55	85.00	48.16	64.56
UAM 09 1055-6	61.99	21.72	75.15	42.50	64.01
IT 99k- 573-2-1	80.20	22.79	82.50	43.66	60.54
FLSD(0.05)	4.33	0.02	0.11	0.01	0.01

The yield characteristics of five cowpea varieties screened for leaf spot disease in Makurdi and Otobi is presented in Table 4. One hundred seed weight was not significantly different among the five cowpea varieties in Makurdi. However, one hundred seeds of cowpea variety IT 99k-573-1-1 planted in Otobi weighed significantly (P≤ 0.05) higher (19.63g) than one hundred seeds of all other cowpea varieties in the same location. Pods of cowpea variety UAM 09 1055-6 in Makurdi weighed significantly (P≤ 0.05) higher (2.66g) while the pods of variety IT 99K-573-1-1 had the least weight of 2.07g. There was no significant difference in the pod weight of all cowpea varieties in Otobi. Variety IT 99K-573-2-1 and IT 99K-277-2 produced significantly higher number of seeds per pod while UAM 09 1051-1 had the least number of seeds at both locations.

Table 4: Yield characteristics of Cowpea varieties screened for leaf spot disease in Makurdi and Otobi in 2018 cropping season

Cowpea Varieties	100 seed weight(g)		Pod weight(g)		Number of pods		Number of seeds per pod		Yield (Tons/ha)	
	Makurdi	Otobi	Makurdi	Otobi	Makurdi	Otobi	Makurdi	Otobi	Makurdi	Otobi
IT 99k-573-1-1	15.70	19.63	2.07	2.53	29.77	12.37	12.00	12.27	1.10	0.53
IT 90k-277-2	16.85	15.27	2.55	2.37	30.17	13.20	13.50	13.30	0.80	0.75
UAM 09 1051-1	15.60	15.53	2.27	2.23	28.83	11.87	9.50	10.80	1.17	0.19
UAM 09 1055-6	15.35	14.97	2.66	2.63	25.07	12.53	13.50	12.17	0.80	0.44
IT 99k- 573-2-1	15.85	15.20	2.34	1.97	30.23	12.80	16.00	12.43	1.43	0.61
FLSD(0.05)	NS	1.27	0.04	NS	1.27	1.43	0.52	1.29	0.76	0.30

Discussion

Four out of the five cowpea varieties screened had emergence percentage higher than the standard emergence percentage of 75% indicating a high viability. The study showed seed to field transmission of some cowpea fungi. Anjorin and Mohammed (2014) reported that watermelon seeds with higher fungi inoculum recorded lower germination. Cowpea varieties

were infected by *Fusarium verticillioides, Curvularia lunata* and *Trichoderma viride.* Cowpea variety UAM 09 1055-6 had the highest incidence and severity of leaf spot while variety IT 90K-277-2 was the least susceptible to leaf spot. The isolation of *Fusarium sp* from the infected cowpea leaves suggests the possibility of seed to field transmission of the fungus. Ekhuemelo and Ekefan (2013) reported the possibility of pepper seedling disease from infected seeds. The low incidence of *A. niger* in this study is in contrast with the report of Dania and Arambi (2015) in which A*. niger* was reported as the predominant fungi of cowpea stored between 5^{o} C and -20^{o}C at IITA Ibadan South West Nigeria. The study revealed the presence of *M. phaseolina* a soil and seed borne pathogen that causes damping off, root rot and charcoal rot reported to cause yield losses in Cowpea (Ndiaye *et al*., 2015). *Curvularia sp*, *A. niger*, *A. Flavus* have been reported to reduce nutritional quality and viability of seeds. *Curvularia sp*. is also reported to be associated with leaf spots (Ellis and Galvez) and may lead to abnormal seedling emergence (Muhammad *et al*.**,** 2012). *Aspergillus niger* causes seed storage rot while *L. theobromae* causes seed decay in dry beans (Ellis and Galvez). Khan and Kumar (1992) also isolated *Curvularia lunata, Fusarium spp*. from wheat seed. *Lasiodiplodia theobromae* is reported to cause 95% fruit rot in fluted pumpkin (Grubben, 2004). The isolation of *A. tamarii* in this study is in line with the report of Chuku (2011) which confirmed *A. tamari* as causal agent of leaf spot of *Duranta repens*.

The cowpea varieties screened showed different levels of resistance and susceptibility in the two locations. Cowpea variety UAM 09 1055-6 recorded the highest incidence and severity of leaf spot on the field at the various growth stages while variety IT 90K-277-2 had the lowest incidence with variety IT99K-573-1-1 and IT99K-573-2-1 having the least severity, indicating that the cowpea varieties have different levels of resistance to leaf spot disease. This may be due to the inherent genetic make-up of the varieties to resist the disease at different levels (Allerd *et al*., 1992; Sinsiri *et al*., 2006). Omoigui *et al*. (2018) reported IT99K-573-1-1 as resistant to *Cercospora* leaf spot and UAM09-1055-6 as susceptible to *Cercospora* leaf spot in Makurdi. The isolation of these fungi from cowpea leaves suggests that several other fungi could be responsible for leaf spot disease in cowpea. This indicates a suspicion of new emerging fungi initiating leaf spot on cowpea apart from *Pseudocercospora cruenta* causal agent of *Cercospora* leaf spot in the study area. Chuku (2011) earlier observed the incidence of leaf spot through the formation of a disease complex on *Duranta repens.*

Leaf spot disease had a significant effect on the yield parameters of cowpea. The higher grain yield recorded by variety IT99k-573-2-1 corroborates the findings of Musa *et al* (2018) which recorded higher grain yield from IT99k-573-2-1 and IT99k-573-1-1 than the yield of UAM 09-1051-1 at Kano. This study showed significant difference in number of pod, pod length and number of seeds per pod among cowpea varieties screened. This is in contrast with the report of Adetumbi *et al*. (2011) in which pod length and number of seed/pod were not significantly different for two cowpea varieties. The Otobi location recorded more vegetative growth and lower seed yield. Karungi *et al*. (2000) reported similar trend on cowpea in eastern Uganda and attributed it to heavy rainfall. Chuku (2011) also reported higher incidence of leaf spots in *Duranta repens* L. due to high humidity during the rainy season in Rivers State Nigeria. Basaran *et al*. (2011) also reported significant differences in the yield and yield parameters of cowpea genotypes assessed in different locations in Turkey due to differences in temperature.

Conclusion

The study concludes that cowpeavariety UAM 09 1051-1 had the most viable seeds as it had the highest emergence percentage on the field. Fungi infecting the cowpea seedlings were *Fusarium verticillioides, Curvularia lunata*, *Aspergillus tamarii* Kite, *Lasiodiplodia theobromae, Aspergillus flavus* Link and *Aspergillus niger* van Tiegh, *Pythium sp, Fusarium solani, Macrophomina phaseolina* and *Phoma sp*. Variety UAM 09 1055-6 had the highest incidence and severity of leaf spot while variety IT 90K-277-2 was the least susceptible to leaf spot disease.

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