

# Influence of Cropping System on the Incidence and Severity of Leaf Spot Disease of *Telfairia occidentalis* Hook f. Caused by *Phoma sorghina*

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**Abstract:** Fungi infections are responsible for the major diseases that attack the fluted pumpkin (*Telfairia occidentalis* Hook f.) in most areas where the crop is grown in Cameroon. This study aimed to inter-cropped *Telfairia occidentalis* Hook f. with the Giant French variety of plantain and compared it with sole cropping to determine the effect of the cropping system on the incidence and severity of leaf spot disease caused by *Phoma sorghina*. We used different planting distances of fluted pumpkins in the two cropping systems. We conducted the study between September 2017 and November 2018 at the Faculty of Agronomy and Agricultural Sciences (FASA), University of Dschang, West Region, Cameroon. We used Randomized Complete Block Design (RCBD), with three replications consisting of six plots per replicate (a total of 18 plots per treatment). Fluted pumpkin was intercropped with the Giant French variety of plantain at a distance of both 1 m x 3 m and 1 m x 1.5 m apart for the sole cropping. We found a significant ( $P \leq 0.05$ ) difference in the incidence and severity of leaf spot disease associated with *Phoma* sp on the leaves and leaflets of fluted pumpkin in the two cultural practices using the pairwise comparison. Sole cropping of fluted pumpkin reduced the disease incidence and severity of leaf spot disease than fluted pumpkin inter-cropped Giant French variety of plantain. Therefore, the sole cropping of fluted pumpkins compared to intercropping with giant crops enhanced the production of the leaves and leaflets of fluted pumpkins. Additionally, the sole cropping of fluted pumpkin reduced the incidence and severity of leaf sport disease, including an improvement in the disease outcome.

**Keywords:** *Telfairia occidentalis*, Cropping Systems, Incidence, Severity, Leaf Spot Disease

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## 1. Introduction

*Telfairia occidentalis* Hook F., commonly known as fluted pumpkin, is a tropical vine grown in Cameroon and the West African coast as a leafy vegetable and for its edible seeds. The common local names for this tropical vine in Nigeria are "ugu" in the Igbo language, "edikangkong" in Efik language (Calabar), "egusiuroko" in Yoruba and "uwmenkhen" in Benin [1]. Meanwhile, in the southern part of Cameroon, it is called

"okonghobong." The plant is highly consumed in west Africa because of its unique characteristics and benefits. Firstly, it is a drought-tolerant dioecious perennial crop usually grown as trellised [2]. Secondly, it is a good source of organic acids, mineral salts, oils, vitamins, proteins, and carbohydrates [3]. Thirdly, the high oil content of the seed makes the crop a potential raw material for the chemical and pharmaceutical industries [4]. Fourthly, the crop has been reported to have some medicinal values, including being a blood purifier and a

therapy for some diseases [5]. For example, when the young leaves are sliced and mixed with coconut water and stored in a bottle, the concoction could be used to treat convulsion, malaria, and anemia. Also, the leaf extracts from this plant can be used to manage cholesterolemia, liver problems, including the management of people with impaired immunity [6].

The most important disease of fluted pumpkin is leaf spot disease, which was reported to reduce the productivity and market value of the crop. According to Nwufo [7], spraying the plant with benlate, captan, and the anvil at two weeks intervals will significantly reduce the leaf spot disease of fluted pumpkin in the field. Fluted pumpkin can be cultivated as a sole crop (mono-cropped) or intercropped with other crops [8, 9]. When comparing the benefits of these cropping systems to disease incidence and severity, Kannaiyan and colleagues [10] reported that there was no significant difference between the sole cropping of groundnuts and intercropping with maize, sorghum, pigeon pea, sunflower, or cotton. Also, Nwufo and Ihejirika [8] reported that removing diseased leaves and the intercropping of fluted pumpkin with cassava, maize, and yam could reduce the incidence and severity of leaf spot disease in the field. With the rising trend of antimicrobial resistance, chemical control of diseases is hazardous to humans, other microorganisms, and the environment [3]. In Cameroon, there is a lack of information on the control of leaf spot disease of the fluted pumpkin using cultural practices.

Therefore, we aimed to discover how different cropping systems influence the incidence and severity of leaf spot disease of *Telfairia occidentalis*.

## 2. Materials and Methods

### 2.1. The Study Site

The research was conducted at the research farm of the University of Dschang, Menoua Division, Western Region of Cameroon. The area lies at latitude 5°26'38" N and longitude 10°03'11" E. It is 1345 m above sea level. The Dschang university area is characterized by an average temperature of 27°C, relative humidity of 78%, and an average rainfall of 2500-4000 mm. Like many other areas in the tropics, Dschang has two main seasons, the dry and rainy seasons. The dry season sometimes begins in mid-November and ends in late February. February is the hottest month in Dschang, with an average temperature of 21°C, while July is the coldest month, with an average temperature of 18°C. The wettest month is September with an average rainfall of 244.6 mm. Menoua Division is a significant crop-producing area in the Western Highlands agro-ecological zone of Cameroon, where intensive agriculture is carried out, despite soil fertility constraints [11].

### 2.2. Experimental Design

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each experimental site had a total of 18 plots. In the sole cropped system, each experimental plot measured 3 m x 3 m with a distance of 30 cm, separating the experimental units (Figure 1). In the intercropped experimental plot, there were equally 18 plots, measuring 3 m x 2 m. The distance separating the plots was 3 m (Figure 2).

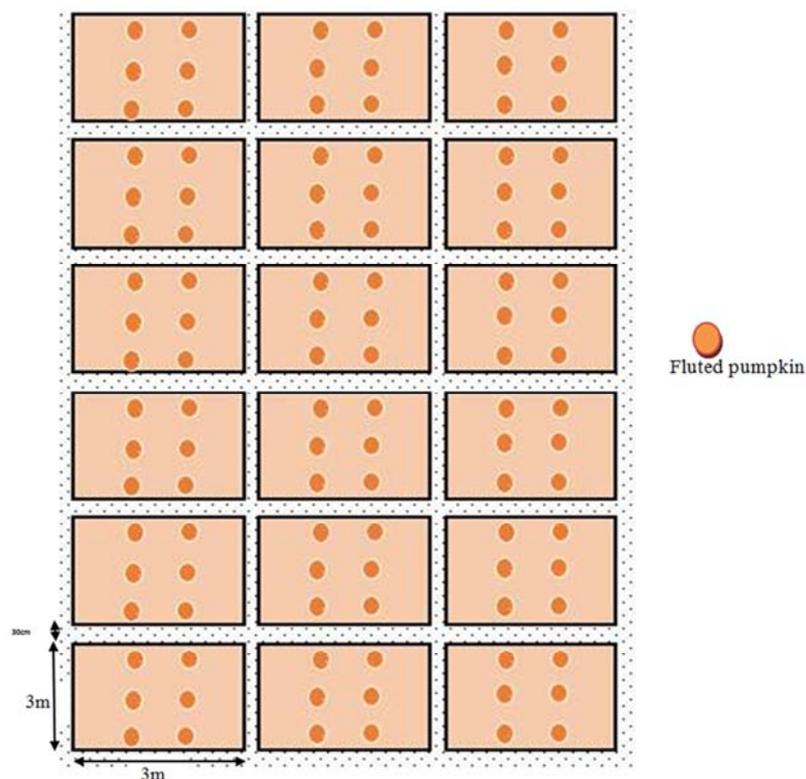


Figure 1. Experimental layout of fluted pumpkin cultivated solely.

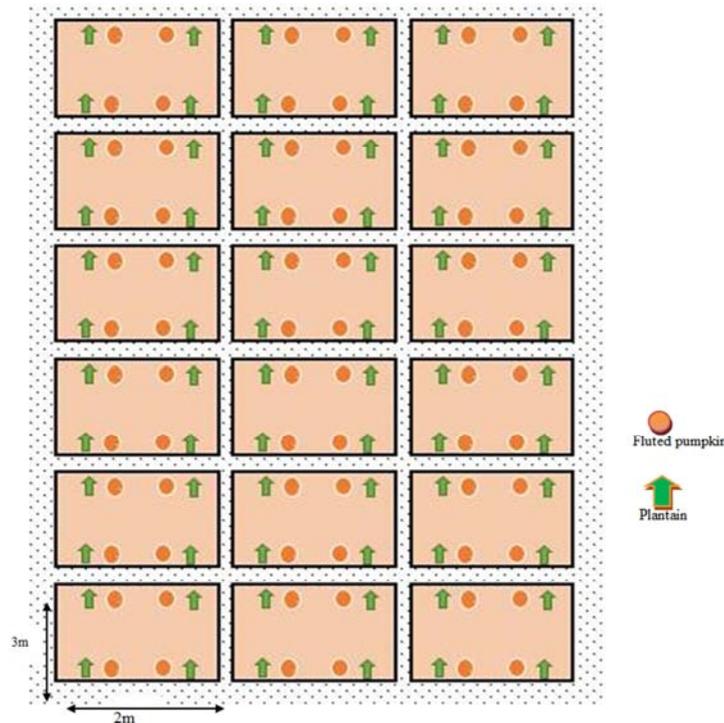


Figure 2. Experimental layout of fluted pumpkin intercropped with Giant French variety of Plantain.

### 2.3. Land Preparation and Establishment of the Crop

We cleared the land with cutlasses and prepared the ridges with hoes in September. The fluted pumpkin seeds were planted in mid-October 2017 for proper establishment before the onset of the dry season. The sustainability of the crop in the field during the dry season was ensured by watering weekly. The seeds were planted directly at a depth of 3 cm.

In the inter-crop experiment, the fluted pumpkins were intercropped in an already established farm of Giant French variety of plantains. The site was adapted to form three replications, with six plots per replicate, thus totalling 18 plots. One fluted pumpkin seed was planted in each planting spot, giving a total of 4 grains per plot with a planting distance of 1 m x 3 m.

In the sole cropping, the experimental plots were also replicated three times with six plots per replicate giving a total of 18 plots with each measuring 9 m. The fluted pumpkin seeds were planted one per hole giving a total of six fluted pumpkin seeds per plot and a planting distance of 1.5 m x 1 m. A total of 108 fluted pumpkin seeds were planted in the experimental site. Seeds emergence took two weeks after planting.

The proportion of percentage emergence and survival rate was calculated using the formulae below:

$$\text{survival} = \frac{\% \text{ Emergence and } \text{number of seeds that emerged and survived}}{\text{total number of seeds planted}} \times 100$$

### 2.4. Cultural Activities

Mulching and weeding were carried out bi-weekly for the proper establishment of the crop. Throughout the dry season,

the crops were watered weekly. Staking was done five weeks after planting in the sole cropping experiment to direct the crop growth vertically. The stakes measured 1.5 m, and by the time the crop attained a certain height, trellises were constructed to enable the herbaceous stems to climb appropriately, supporting themselves using helically twisting tendrils. Trestle-like structures were erected for each replication in March to allow the climbing vine to spread. A total of eighteen trellises were constructed to provide support to the climbing plant. Within this period, the vine length had attained a height of about 1.5 m. In the intercropped experiment, we did not erect trestle-like structures. Instead, the fluted pumpkin stands were supported with pegs measuring 1.5 m in height and directed to crawl around the plantain stems as ultimate support, within this height so that the evolution and progress of the leaf spot disease could be conveniently monitored.

### 2.5. Determination of Disease Incidence and Severity

Data on disease incidence and severity was spanned over a period of six weeks, from first appearance of symptoms. The disease assessment was done by visual observation. The recording of disease incidence was in weeks after planting (17, 19, 20, and 22 weeks) after planting (WAP). All the plants in each plot for the two cropping systems were assessed for leaf spot disease. Infected leaves of each plant were systematically counted and recorded, and the disease incidence calculated.

#### 2.5.1. Determination of Disease Incidence

Percentage of disease incidence was calculated using the formula:

$$\text{Percentage DI} = \frac{\text{Number of leaves infected}}{\text{Total number of leaves sampled}} \times 100$$

### 2.5.2. Determination of Disease Severity

Leaf spot disease severity was assessed using a modified disease severity scale [12] and the recording was done at weekly intervals as follows:

**Table 1.** Disease Severity Scale of Leaf Spot (scored at weeks after planting).

Disease Score	% Severity	Description of Symptoms
1	1	No disease symptom
2	1-20	Slight infections with small spot lesions
3	21-40	Moderate infections with spot lesions spreading on the surface of the leaves.
4	41-60	Severe infection with spot lesions found almost on most of the leaflets
5	61-80	Very severe infections with spot lesions spreading all over the leaflets and also coalescing
6	81-100	The surface of the leaves is completely covered with leaf spot disease, some lesions with hole and leaves tearing off.

## 2.6. Pathogenicity Testing of *Phomasorghina*

### 2.6.1. Collection of Fluted Pumpkin Leaf Samples



**Figure 3.** Symptoms of the white leaf spot disease on the leaves of fluted pumpkin with small circular-whitish spots.



**Figure 4.** White leaf spot on the leaves enlarges with reddish-spots that are surrounded by irregular shaped water-soaked areas.

The fresh leaf samples of fluted pumpkin infected with leaf spots disease were randomly harvested from the field of the experiment for both sole cropping and intercropping, using a sharp knife. Harvesting was done in the morning, just at sunrise, when the pathogen is active and during which they were void of surface moisture. Grossly infected diseased samples were not used for isolation as the pathogens would probably not be viable and saprotrophic organisms would have colonized necrotic tissues. To prevent any form of damage, samples were carefully packed in a bag made of fiber, not plastic, so they would not be crushed and undergo condensation, where surface moisture would have encouraged the growth of any saprotrophs present. The leaves from both experimental treatments were taken to the Laboratory of Phytopathology and Agricultural Zoology

(LAPHYZA) at FASA, the University of Dschang in separate bags for isolation and identification of pathogens.

### 2.6.2. Pathogen Isolation and Identification

One hundred milliliters (100 ml) of the culture medium, potato dextrose agar (PDA), was initially prepared from Irish potato (*Solanum tuberosum*) agar and amended with chloramphenicol. In the laboratory exercise, 200 g of Irish potato (weighed with the aid of a scale balance) was washed with tap water, peeled with a knife, chopped into slices, and washed again with tap water. The potato juice was extracted by boiling the slices for 20 minutes in a pot filled with 500 ml of water. 20 g of agar were then added to the juice to solidify the medium. At the same time, four capsules (250 mg) of chloramphenicol were added to the medium (to inhibit the growth of any bacteria). The preparation was warmed for 15 minutes to obtain 1000 ml of the PDA. The medium was then kept inside a heating chamber (an oven) to kill any bacteria that might have contaminated it. The fluted pumpkin leaves were initially rinsed under a flowing tap in the laboratory and later sliced into sections at the interface between the infected and healthy portions with a sterile knife. The cut-leaf sections were placed on two Petri dishes, and each Petri dish labeled with each cropping system.

Cut-leaf sections were surface-sterilized three times in 70% sodium hypochlorite (bleach) solution for 5, 10, and 15 minutes respectively and rinsed in quick succession in 3-changes of sterilized distilled water. The cut-leaf sections were then blotted dry on tissue paper and plated on Petri dishes on the freshly prepared PDA medium containing 20 ml PDA medium. A total of 16 Petri dishes containing prepared samples for culture, 8 for each set of cut-leaf sections representing fluted pumpkin leaves from the two cropping systems, were employed. Each of the Petri dishes was covered and sealed with paraffin tape and incubated at  $24 \pm 2$  for five days, during which visible fungi growth was noticed. After five days, the different healthy isolates were sub-cultured on freshly prepared PDA plates and incubated in ambient conditions for another five days to obtain the fungi's pure cultures.

Microscopic examination of the fungi isolates permitted the identification of the microflora, while the fungi species were identified using the micromorphological characteristics and conidia structure. The Isolated fungi were identified using the International Mycological Institute Kew, Hunter, and Barnett guides [13].

**2.6.3. Artificial Culture of Fungi Isolates**

To confirm whether the isolated pathogens from the pure culture could produce symptoms of the disease as observed on the field, suspensions of all pure culture isolates were prepared under aseptic conditions with distilled water. A drop of twin 20 solutions was prepared and added to each plate to homogenize the suspension. Conidial suspensions obtained were filtered through a double layer of filter paper to remove leaf debris. Healthy and fresh fluted pumpkin leaves were harvested with the aid of a knife from each of the cropping systems and taken to the laboratory. Twenty-one (21) leaflets were cut off from the leaves and sterilized for two minutes with hypochlorite solution and then rinsed in distilled water for five minutes.

We place within each Petri dish one leaflet. Using hypodermic syringes with a micropipette fitted at one end, 50  $\mu$ m of each fungi suspension was inoculated onto the leaflets. Each fungi suspension was replicated three times for the leaflets from both treatments. A buffer (cotton moistened with sterilized distilled water) was used to maintain humidity within each experimental setup, and all the Petri dishes were carefully covered. The setups were incubated at ambient temperatures for five days. Developing lesions/spots were compared with those observed in the field. The characteristics of the re-isolated pathogens were compared with their original isolates. In the control experiment, the leaflets were not inoculated.

**2.7. Statistical Analysis**

All the data collected from the two cropping systems were inputted into Microsoft Excel and the data was analysed using SPSS version 23. An analysis of variance (ANOVA) was conducted and the treatment means were separated using the least significant difference (LSD) for statistical significance

at 95% confidence interval and P value  $\leq$  0.05.

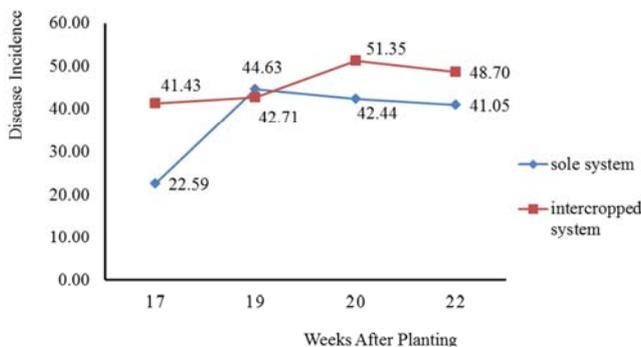
**3. Results and Discussions**

**3.1. Disease Incidence on the Leaves of Fluted Pumpkin in Weeks After Planting (WAP)**

The disease incidence on the leaves of fluted pumpkin was generally high in the intercropping system compared to sole cropping (Table 2, Figure 5). Comparing the disease incidence on the leaves of fluted pumpkin intercropped, it was observed that the incidence at 20 WAP was higher (with a standard deviation (SD) of 15.90 and mean of 51.35) compared to 17 WAP, 19 WAP, and 22 WAP respectively. Sole cropping showed the highest disease incidence at 19 WAP (with SD of 25.46 and mean of 44.63) and the least at 17 WAP with SD=6.77 and mean=22.59 (Table 2, Figure 5). Variation in the disease incidence on the leaves of fluted pumpkin in the different cropping systems could be due to differences in the microclimate. The shading within the fluted pumpkin's intercropping with the giant French variety of plantains could provide a humid condition that favored the rate of sporulation and spore germination of the pathogen. This was in agreement with Bassey and Opara who reported that lesions or spots develop rapidly under humid conditions [14]. In addition, the absence of direct penetration of solar radiation had the effect of limiting the rate of photosynthetic activity, consequently bringing about reduced vegetative growth of the crop, and in some cases, the complete absence of side branches, thereby leading to low yield. This ties with the works of Orji, Webster and Wilson, and Agboola and Fayemi who intimated that some of the disadvantages of intercropping systems are decrease yield of one of the component crops [12, 15, 16].

*Table 2. Disease Incidence on the leaves of Fluted Pumpkin in Weeks after planting (WAP).*

Cropping Systems	Weeks After planting (WAP)	Standard Deviation (SD)	Mean
Intercrop	17 WAP	16.89	41.43
Sole		6.77	22.59
Intercrop	19 WAP	16.49	42.71
Sole		25.46	44.63
Intercrop	20 WAP	15.90	51.35
Sole		9.18	42.44
Intercrop	22 WAP	9.96	48.70
Sole		9.02	41.05



**Figure 5.** Variation of disease incidence on the leaves of fluted pumpkin in weeks after planting.

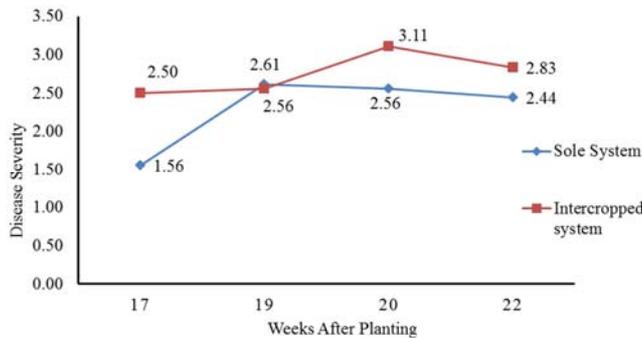
**3.2. Leaf Spot Disease Severity of Fluted Pumpkin Leaves in Weeks After Planting**

The leaf spot disease severity of fluted pumpkin leaves of the intercropping system was generally higher than that of the sole cropping. Still, some variations were observed at the 19 WAP with sole cropping having a higher disease severity than the intercropping (SD=0.85, mean=2.61 and SD=0.86, Mean=2.56), respectively (Table 3, Figure 6). The disease severity in the different intercropping systems showed that the highest disease severity on the leaves of fluted pumpkin was observed at 20 WAP (SD=0.90, Mean=3.11) compared to 17 WAP, 19 WAP, and 22 WAP respectively. For sole cropping, the highest disease severity on the leaves of fluted pumpkin

was observed at 19 WAP (SD=0.85, Mean=2.61) compared to 17 WAP, 20 WAP 22 WAP, respectively (Table 3, Figure 6). The high severity of leaf spot disease in the intercropping system could also be attributed to changes in microclimate, shading, and high humidity, which provided favorable conditions for spore germination, development, and the rapid multiplication of the pathogen increases infection. Nwufu and Ihejirika reported that high humidity favor disease condition when fluted pumpkin is intercropped with plantain than when cultivated with other crops such as cassava, yam, and maize

**Table 3.** Disease Severity on the leaves of Fluted Pumpkin in Weeks after planting (WAP).

Cropping System	Weeks After Planting (WAP)	Standard. Deviation	Mean
Intercrop	17 WAP	0.86	2.50
Sole		0.51	1.56
Intercrop	19 WAP	0.86	2.56
Sole		0.85	2.61
Intercrop	20 WAP	0.90	3.11
Sole		0.51	2.56
Intercrop	22 WAP	0.51	2.83
Sole		0.51	2.44



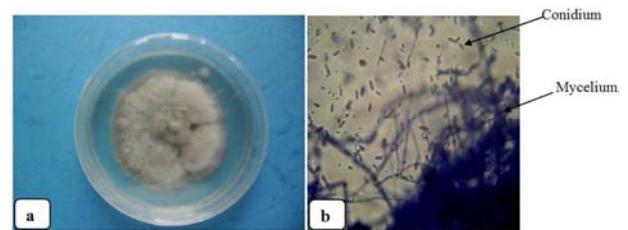
**Figure 6.** Variation of leaf spot disease severity on the leaves of fluted pumpkin in week after planting.

### 3.3. Identification and Confirmation of the Leaf Spot Pathogens

From the pathogenicity test carried out, we observed that the disease from the infected fluted pumpkin leaves had white leaf spots indicating that the causal agent is a fungus, and the pathogen is *Phoma* sp. The isolation of the fungus in an artificial culture media was consistent with that of the diseased leaves in pure culture. It produced symptoms of the disease identical to what was observed in the field in the two cropping systems. The description of the spores of *Phoma* sp. (Class Dothideomycetes, the largest class of ascomycete fungi) as observed under the microscope, was colorless and unicellular (Figure 7a). The pycnidium was black and lying within the tissues of the host. It has a mass of one-celled colorless to yellow or pink spores (conidia). The conidia are borne by inconspicuous peg-like phialides lining the inner wall of the pycnidium (Figure 7b). The prevalence of the fungus (*Phoma* sp) responsible for white spot disease in the two cropping systems confirms the report of Godwin-Egein [17] on work carried out at the University of Port Harcourt research farm, Nigeria who said that *Phoma sorghina* was the pathogen responsible for white leaf spot disease of fluted

[8]. The plantain canopy significantly prevents solar radiation from penetrating beneath, thus keeping the environment more humid and disease-prone. The variation of leaf spot disease severity in the sole cropping system compared to intercropping at 19 WAP could be attributed to high relative humidity at the onset of the rainy season, which could favor the dissemination of the fungi spore due to rain splash and windblown moisture. Osai [3] reported that the disease is rife and severe, especially in early season crops, at the onset of the rainy season.

pumpkin. This also confirms the results of Nwufu and Ihejirika (2008), and Osai and his colleagues [3, 8] who reported that *Phoma sorghina* is the pathogen responsible for white leaf spot disease of fluted pumpkin.



**Figure 7.** *Phoma* sp (a) growing in pure culture (X400), (b) *Phoma* sp showing mycelia and conidia (X400).

## 4. Conclusion

The sole cropping of fluted pumpkins was more beneficial than inter-cropping. The influence of cropping system on the incidence and severity of leaf spot disease of *Telfairia occidentalis* (fluted pumpkin) caused by *Phoma sorghina* showed that sole cropping of fluted pumpkin could reduce the disease incidence and severity of leaf spot disease compared to when fluted pumpkin is inter-cropped with the giant French variety of plantain. Thus, to enhance the production of leaves and leaflets and reduce disease incidence and severity of fluted pumpkin, sole cropping was better in mitigating leaf spot disease than intercropping with giant crops. To obtain the best growth and a substantial increase in the production of fluted pumpkin leaves, including the management of leaf spot disease of fluted pumpkins. It is therefore advisable to plant the crop solely.

## Ethical Standard

We, the authors state that this research complies with ethical standards.

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## Conflict of Interest

We the Authors declare none.

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