



Research article

Managing *Phoma* Leaf Blight of Fluted Pumpkin Using Diverse Agents

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A B S T R A C T

Fluted pumpkin (*Telfairia occidentalis*), a key vegetable crop in Africa, faces significant production challenges due to various diseases, particularly *Phoma* blight. Known pathogens affecting this crop include fungal diseases (*Fusarium* and *Verticillium* wilts, leaf blight, downy mildew, leaf spot, anthracnose, and postharvest storage fungi), viral infections (*Telfairia* mosaic virus, cucumber mosaic virus, and pepper mottle virus), bacterial soft rot, and root-knot nematodes. This study evaluated the in vitro efficacy of five *Trichoderma* isolates and three botanical extracts against *Phoma sorghina* using a completely randomized design with three replications per treatment. Inhibition rates by *Trichoderma* ranged from 14% to 58%, with *T. virens* (MZ2) showing the highest efficacy, followed by *T. harzianum* (AIBN3, BGMZ3, ZXMZ6) and *T. harmatum* (ZXGV5). Among botanicals, *Parkia biglobosa* at 100% concentration demonstrated the strongest inhibitory effect, followed by *P. biglobosa* at 50% and *Juniperus chinensis* at 100%, which were statistically similar, while *Monoon longifolium* at 100% was least effective. These findings highlight the potential of biocontrol agents and botanicals as sustainable alternatives for managing *Phoma* blight in fluted pumpkin cultivation, though further research is needed to explore integrated disease management strategies and assess the safety and interactions of these control measures in vegetable production systems.

1. INTRODUCTION

Telfairia occidentalis Hook F. (Family: Cucurbitaceae) is principally cultivated in the forest zones of West and Central Africa (especially in the Benin Republic, Nigeria, Sierra Leone, and Cameroon), as well as in Angola, Uganda, Asia, and the Americas (Odiaka, 2001; Schippers, 2002; Oluwatoyin & Abdurashied, 2014; Tobih, 2015; Bassey & Opara, 2016). *T. occidentalis* is stereotypically called fluted pumpkin, fluted gourd, 'ukongubong' or 'Ugu' (Time and Chikezie, 2016; Opara & Uduak, 2019). Bassey and Opara (2016) expounded that the largest species diversity of fluted pumpkin is found in

southeastern Nigeria. Tobih (2015) affirmed that the crop is produced mainly in southeastern Nigeria in Anambra, Imo, Cross Rivers, Enugu, Edo, and Delta States.

Time and Chikezie (2016), Opara and Uduak (2019) testified that the average yields of fluted pumpkin (leaf and fruits) in Nigeria were low because its production was being constrained by biotic and abiotic stresses. Biazzo and Mabiunas (2000) and Tobih (2015) further enumerated some of these major constraints, including insect pests, pathogenic diseases, and weeds. However, earlier researchers, Shoyinka and Thottappilly (1998) specified that fluted pumpkin production in Nigeria is mainly limited by virus-related diseases, which include *Telfairia* mosaic

potyvirus (TeMV), cucumber mosaic cucumovirus - Y-strain (CMV-Y), and pepper veinal mottle potyvirus - strain TYVC (PVMV-TYVC). Anno-Nyako (2008) stated that it was possible to effectively spread TeMV through seeds of four highly susceptible cultivars of fluted pumpkin during a screen-house trial.

Nonetheless, this view by Shoyinka and Thottappilly (1998) has been strongly disputed. Mbong et al. (2021) pointed out that fungi-induced diseases constituted the most important constraint to the production of this crop in Cameroon. Bassey and Opara (2016) supported this observation of fungi being more important by claiming that the major fungal diseases in Nigeria (such as leaf spot induced by *Phoma sorghina* and anthracnose caused by *Colletotrichum* sp.) could significantly reduce the yield of fluted pumpkin.

Godwin-Egein et al. (2015) and Osai et al. (2017) also emphasized that fungi leaf spot diseases are most prevalent among the pathogens of fluted pumpkin. Maduewesi (1977) showed that the isolation rate of *Phoma sorghina* was very high from leaf spots obtained in the field and from a controlled experiment in Nigeria. Telfairia occidentalis (no date) reported that fungi may cause up to 95% yield loss and bacteria about 5% yield loss in long-term fruit storage. To reduce drudgery in this mini assessment, a detailed chronicle of pathogens recorded on fluted pumpkin was expediently reduced and presented in Table 1. Osai et al. (2017) reported that in Nigeria, leaf spot and blight constituted major problems in fluted pumpkin production; thus, an urgent need exists for control of these diseases.

Table 1 revealed that more fungal pathogens are associated with fluted pumpkin than any other type of pathogens. Thus, the view by Shoyinka and Thottappilly (1998) may no longer be valid if the damage they cause is directly proportional to the number of kinds of pathogens involved. Also, fungi, viruses, bacteria, nematodes, and insects may infect fluted pumpkins.

Telfairia occidentalis (no date) reported that fluted pumpkin is remarkably resistant to root knot nematodes (*Meloidogyne* spp.). However, the findings of Soala et al. (2018) showed that this crop is susceptible to attack by root knot nematodes. Oluwatoyin and Abdulrasheed (2014) reported that marketable *T. occidentalis* leaves were observed to be contaminated with pathogens of human diseases, like *Enterobacter agglomerans* (25.9%), *Proteus vulgaris* (24.9%), and *Klebsiella* spp. (2.6%) and *Serratia liquefaciens* (2.1%). Other bacterial isolates they recovered included: *Staphylococcus* spp. (33.7%), *Bacillus* spp. (8.3%) and *Pseudomonas fluorescens* (2.6%).

The uses of fluted pumpkin leaves and seeds include food, feed, and medicinal purposes (this may be related to the rich composition of the leaves and seeds). Kuku et al. (2014) reported that fluted pumpkin seeds are prepared by roasting and boiling for thickening of soup (Osai et al., 2017). Furthermore, the seed oil is used for cooking, making marmalades, confectionery, soaps, paints, and varnishes (Giami & Barber, 2004; Horsfall & Spiff, 2005; Nworgu et al., 2007).

While the fermented seeds are used in the production of an indigenous custard (i.e., 'Ogiri ugu') (Christian, 2007), the seed cake residue may be fed to animals (Soala et al., 2018). The sale of these products yields a lot of the much-needed income to participants involved in fluted pumpkin production, distribution, processing, and marketing.

In relation to the management of diseases of fluted pumpkin, Osai et al. (2017) recounted the advantage of using plant extracts, which are often rich in secondary metabolites, cost less, are easily accessible, and require low-input technologies. These advantages make such extracts appealing to resource-poor farmers since the extracts are highly affordable. Furthermore, they perceived that the incidence and severity of leaf spot disease were lower in monocropped fluted pumpkins than in intercropped fluted pumpkins within plantain farms.

Lum et al. (2019) assessed the anti-fungal activity of aqueous and methanol extracts of *Eleusine indica*, *Diospyros crassiflora*, *Fagara heitzii*, and *Milicia excelsa* for the management of *Fusarium oxysporum* f.sp. elaeidis. The results revealed that at three, seven, and ten days after exposure, the fungicide and extracts of most of the plants inhibited mycelial growth of the fungus significantly ($P \leq 0.05$) compared to the control. Rajeswari and Kannabiran (2011) reported that the production of conidia of *Fusarium oxysporum* was significantly inhibited by *Trichoderma viride* (86.6%), *Trichoderma harzianum* (84.0%), and *Pseudomonas fluorescens* (60.0%). The percentage inhibition of conidial germination was as follows: *T. viride* (89.4%), followed by *T. harzianum* (85.7%), and then *P. fluorescens* (83.2%).

From the foregoing mini appraisal, it is clear that fluted pumpkin is a very important vegetable crop that has not received the attention it deserves in terms of pest and pathogen management. This study aims to document the genetic basis of diseases affecting fluted pumpkin and evaluate the effectiveness of biocontrol and botanical agents in managing *Phoma* blight disease.

2. MATERIALS AND METHODS

2.1. Site of the study

This research was carried out at the Faculty of Agriculture Laboratories in Alex Ekwueme Federal University, Ndufu-Alike, Abakaliki (6.069°N by 8.199°E at an altitude of 57 m above sea level), about 21 kilometres from the Abakaliki metropolitan area (i.e., the State capital). Abakaliki is located in Ebonyi State, which is in the derived savanna zone of Nigeria, and has a humid tropical climate. The region is covered by lush green tropical vegetation. The State inhabitants practice cultivation of many field crops like fluted pumpkin, maize, yams, sweet potatoes, cassava, *Amaranthus* spp., cocoyams, and groundnuts.

2.2. Isolation and identification of the fungi associated with blighted fluted pumpkin leaves and *Trichoderma* isolates

The pathogen-infected (blighted) fluted pumpkin leaves were obtained from the Faculty of Agriculture Research and Teaching Farm of the University. The *Trichoderma* isolates were obtained from mushrooms, beans, maize, orchard, and farmland soils in West Cameroon and southeastern Nigeria. The fungi were isolated using potato dextrose agar (PDA) medium, which was autoclaved at 120°C, 15 psi for 15 minutes. The isolated fungi were sub-cultured to obtain pure cultures, which were used to identify the fungi with the aid of literature on fungi morphology (Barnett & Hunter, 1972).

The fungus found causing the blighting of fluted pumpkin leaves was identified as *Phoma sorghina* (Sacc.) Boerema, Dorenb. & Kesteren. It was observed that in Table 1, this pathogen was said to cause white leaf spot disease instead of leaf blight. This may be due to the disease severity in this area, or it may be linked to the fact that the disease samples were obtained from seedlings, or it could be linked to problems usually associated with common names. The fungi isolates were stored in the freezer for use later.

Subtrial 1: Control of *Phoma* blight using *Trichoderma* isolates

The subtrial was laid out in Petri dishes using a completely randomized design, and each treatment was replicated three times. The medium utilized to set up the trial was Acetate Differential Agar enriched with Dextrose (ADAD), which was autoclaved at 120°C, 15 psi for 15 minutes according to the manufacturer's instructions. The treatment set consisted of 5 *Trichoderma* isolates (three *Trichoderma harzianum*, one *Trichoderma harmatum* and one *Trichoderma virens* isolates) and a control. The control

was inoculated with the blight pathogen isolate alone. The agar medium was inoculated with a 2-mm disc of the pathogen, and then the biological control agent was placed at the edge of the plate (i.e., a dual culture trial). The data were collected as shown below.

2.3. Subtrial 2: Control of *Phoma* leaf blight of fluted pumpkin with plant extracts

The experiment was carried out using Petri dishes. This poison-food technique trial was set up using PDA medium, which was autoclaved at 120°C, 15 psi for 15 minutes according to the manufacturer's instructions. It was laid out in the laboratory using a completely randomized design (CRD) with seven treatments, each replicated three times. The treatment set included shoots of juniper tree (*Juniperus chinensis* L.), leaves of masquerade tree (*Monoon longifolium* (King) B.Xue & RMK Saunders), and bark of African locust bean (*Parkia biglobosa* (Jacq.) R. Br. ex G. Don). The 100% concentrations were made using 333.3 g plant organ / 1000 ml H₂O. The other concentrations were constituted by diluting the 100% concentration using distilled water. Each treatment had three levels (0.0, 50, and 100% concentrations). The data were collected as shown below.

2.4. Data collection used for both subtrials 1 and 2

The radius of the fungus colony was measured using a transparent measuring rule at 24-hour intervals from day 1 through day 6. The percentage inhibition of the pathogen was calculated using this equation.

$$PI = ((C - T) / C) \times 100\% \quad (1)$$

Where

PI= Percent inhibition of growth of the fungus

C= Perpendicular radius of fungus colony in control plates

T= Perpendicular radius of the fungus colony in treated plates

2.5. Data analysis used for both trials

Descriptive statistics were used to illustrate the trends in growth of the pathogen and its management. The data were subjected to analysis of variance (ANOVA) and the means separated using the Student-Newman-Keuls test (as accessible with IBM SPSS® version 25 statistical package).

3. RESULTS AND DISCUSSIONS

The pathogens associated with fluted pumpkins are presented in Table 1. The Table was actually presented in the introduction earlier.

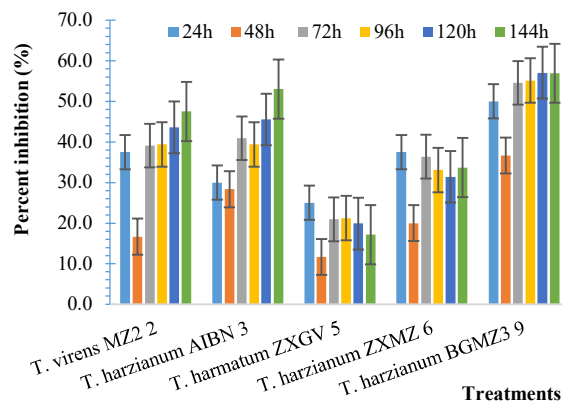
Table 1. Plant Pathogens Associated with Fluted Pumpkin Globally

Common name of pathogen or disease	Binomial name	Sources
Wilt	<i>Fusarium oxysporium</i>	Learn Today (2020)
Downy mildew	<i>Pseudoperonospora</i> spp.	Learn Today (2020)
Leaf blight or brown spot	<i>Alternaria cucumerina</i>	Learn Today (2020)
White leaf spot disease	<i>Phoma sorghina</i>	<i>Telfairia occidentalis</i> (no date), Maduewesi (1977), Nwufu and Atu (1987)
Wilt	<i>Fusarium moniliforme</i>	<i>Telfairia occidentalis</i> (no date),
Leaf soft rot	<i>Erwinia aroideae</i>	<i>Telfairia occidentalis</i> (no date),
<i>Telfairia</i> mosaic virus	<i>Telfairia</i> mosaic potyvirus (TeMV)	<i>Telfairia occidentalis</i> (no date), Anno-Nyako (1988); Shoyinka et al. (1987)
Storage diseases	<i>Rhizopus stolonifer</i>	<i>Telfairia occidentalis</i> (no date),
Storage diseases	<i>Aspergillus niger</i>	<i>Telfairia occidentalis</i> (no date),
Storage diseases	<i>Botryodiplodia theobromae</i>	<i>Telfairia occidentalis</i> (no date),
Anthraxnose	<i>Colletotrichum</i> sp.	Bassey and Opara (2016)
Cucumber mosaic cucumovirus	cucumber mosaic cucumovirus (CMV-Y)	Atiri (1986)
Pepper veinal mottle virus	pepper veinal mottle potyvirus (PVMV-TYVC)	Atiri (1986)

3.1. Bio-Control of *Phoma* Leaf Blight of Fluted Pumpkin

The percentage inhibition of the *Phoma* blight pathogen by *Trichoderma* isolates is presented in Figure 1. The amount of control of the blight pathogen by all the isolates of *Trichoderma* ranged between 14-58% (i.e, inhibition of mycelial growth). The percentage inhibition was observed to be stable from the second day after inoculating the biocontrol and pathogenic agents. The effects of the *Trichoderma* isolates did not cause the pathogen's death; instead, the secondary metabolites involved in this interaction resulted in biostatic inhibition of the pathogen.

The data (after 144 hours of biocontrol) were subjected to ANOVA, and it was observed that significant differences ($P \leq 0.05$) existed between the treatments. The treatments that were most preferred, based on the means ranking, were *T. virens* isolate MZ2, *T. harzianum* isolate AIBN3, and *T. harzianum* isolate BGMZ3, followed by *T. harzianum* isolate ZXMZ6 and finally *T. harmatum* isolate ZXGV5.

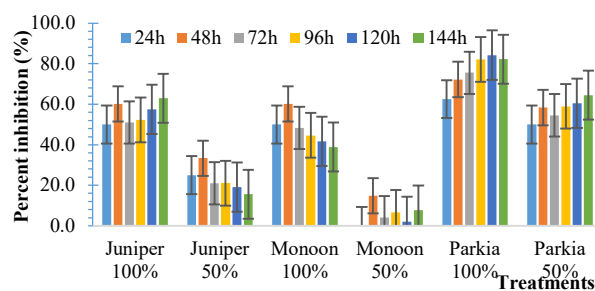


NB: The error bars were calculated using standard error

Figure 1. Percentage Inhibition of The Growth of *P. Sorghina* Using *Trichoderma* Isolates In Vitro

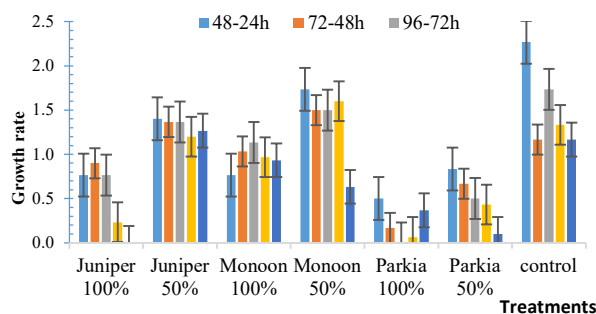
3.2. Control of *Phoma* Leaf Blight of Fluted Pumpkin with Plant Extracts

The percentage inhibition of the pathogen using plant extracts is presented in Figure 2. The data (management with botanicals at 144 hours) were subjected to ANOVA, and it was observed that significant differences ($P \leq 0.05$) existed between the treatments. Based on the ranking of the means, the best treatment was *P. biglobosa* (100% concentration), followed equally by *P. biglobosa* 50% and *J. chinensis* 100%, and then *M. longifolium* 100% and the least preferred treatments were *J. chinensis* 50% and *M. longifolium* 50% which at 144 hours were not significantly different from the control. The effects of plant extracts on the pathogen were mainly related to growth reduction, whereby the growth rate was reduced depending on the type of plant extract and its concentration (Figure 3).



NB: The error bars were calculated using standard error

Figure 2: Percentage Inhibition of the Growth of the *Phoma* Blight Pathogen Using Botanicals In Vitro



NB: The error bars were calculated using standard error

Figure 3. Effects of Plant Extracts on The Growth Rate of *P. Sorghina* In Vitro

The biocontrol results have furnished evidence that corroborates the findings of Rajeswari and Kannabiran (2011), which revealed that *Trichoderma* and *Pseudomonas* isolates significantly inhibited plant pathogenic fungi. In fact, the findings revealed that the biocontrol agents and extracts of plants inhibited mycelial growth of the fungus, which corroborated the findings of these trials as reported herein.

Opara and Uduak (2019) reported that agricultural effluents (from rice (*Oryza sativa*), cassava (*Manihot esculenta*), corn (*Zea mays*), oil palm (*Elaeisis guinensis*) were able to reduce the incidence of bacterial leaf spot disease and increase the yield of fluted pumpkin more than the control, regardless of the concentration of the effluents used. They showed that siam weed (*Chromolena odorata* (L.), utazi leaf (*Gongronema latifolium*), bitter leaf (*Vernonia amygdalina*), and a synthetic fungicide (Forcelet®) reduced colony growth of *Phoma sorghina* (i.e., white leaf spot disease agent) *in vitro*, and curbed disease development by 26.2-51.8% under field conditions. The findings of this current study affirm the findings of Opara and Uduak.

Ndifon et al. (2015) reported that mahogany (*Kharya senegalensis* at 100% concentration), *Eucalyptus* species (*Eucalyptus globulus* at 50 and 100% concentrations), neem (*Azadirachta indica* at 100%), cashew (*Anarcadium occidentale* at 100%), *Vitalerea* sp. (at 50 and 100% concentrations), *Erythrina senegalensis* at 100%, ginger (*Zingiber officinale* at 100%), and garlic (*Allium sativum* at 100%) reduced the growth of *Fusarium oxysporum* *in vitro*. Lum et al. (2014) revealed that at seven and ten days after exposure, crude extracts of *Mitracarpus villosus* (Sw.) DC and *Ageratum conyzoides* L. (weed species) and the fungicide (Mancozan® a.i. mancozeb WP) significantly inhibited mycelia growth of leaf blight disease of taro (*Colocasia esculenta*) compared to the control ($P \leq 0.05$). These findings corroborated this study's findings that using plant extracts is a viable alternative to chemical fungicides.

Other attempts to manage fungal diseases of fluted pumpkin include the following. Godwin-Egein et al. (2015) revealed that fluted pumpkin intercropped with maize significantly reduced leaf spot disease incidence at six and 12 weeks after sowing ($P \leq 0.006$ and $P \leq 0.008$, respectively). Bassey and Opara (2016) confirmed that plant ashes (from plantain peel, oil palm kernel bunch, sawdust, firewood) and mancozeb® significantly ($P \leq 0.05$) reduced leaf spot disease on *T. occidentalis* compared to the control.

4. CONCLUSIONS

Many factors, including plant pathogenic diseases, constrain the cultivation of fluted pumpkin. This research was conceived to update information on fluted pumpkin diseases and to offer some solutions to this crop's *Phoma sorghina* leaf blight disease. It was observed that fluted pumpkin suffers from fungi, viruses, bacteria, nematodes, and many other forms of disorders. Using five *Trichoderma* isolates successfully inhibited the blight (14-58% inhibition). Using botanicals (*Parkia*, *Monoon*, and *Juniperus* species) as ecologically friendly plant extracts significantly reduced the growth of the pathogen. The importance of these findings was deemed to be related to a reduction in the use of chemical agents to control fungal pathogens on vegetables, which has been proven to be dangerous to both the environment and human health. Thus, the higher rates of the plant extracts utilized herein should be made available to farmers, while research continues on the delivery methods of *Trichoderma* species.

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