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RESEARCH PAPER

Mycotoxicity of leaf extract of *Tabernaemontana pachysiphon* Stapf against *Fusarium oxysporum* Schlecht, a postharvest fungal rot pathogen of taro

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ABSTRACT

Taro, otherwise known as cocoyam, is one of the major sources of calories in the tropics. However, losses of its corm during storage are high, partly due to pathogenic diseases. *Fusarium oxysporum* is one of the principal fungal pathogens causing about 50% of postharvest losses of taro corms in the tropics. The rise in pesticide-resistant fungal pathogens, amongst other factors, has necessitated the recent search for alternatives to synthetic pesticides. This work aimed to evaluate the effects of six concentrations (0%, 10%, 20%, 30%, 40%, and 50%) of aqueous leaf extract of *T. pachysiphon* on spore germination, mycelial growth and reproduction of *F. oxysporum* both *in vitro* and *in vivo*. The *in vitro* experiment was conducted using the poison bait technique, while the *in vivo* experiment involved 21 corms (200 g) artificially infected with *F. oxysporum* and treated with the respective concentrations of the test extracts or griseofulvin. The control consisted of fungus-infected corms only. Generally, the experiments consisted of 7 treatments replicated 3 times and were laid out in a completely randomized design. Results of the cultures studies showed that all aqueous extract of *T. pachysiphon* significantly ($P< 0.05$) and to varying degrees impeded spore germination (56.38–70.24%) and retarded radial growth of the pathogen (68.27–82.01%). The percentage rot expression in the living tissues treated with the extracts ranged between 19.4% and 40.4%. All results were statistically ($P< 0.05$) superior to the control but comparable to the results (75.06%, 86.41%, and 17.2%) obtained with griseofulvin for these features, respectively. The inhibitions were linearly correlated with the dose of application of the biotoxicant both *in vitro* and *in vivo*.

Key words: *Tabernaemontana pachysiphon*, *Fusarium oxysporum*, taro, cocoyam, rot disease, postharvest storage

INTRODUCTION

Fusarium oxysporum is a cosmopolitan, soil-borne filamentous fungus. It causes wilts characterized by leaf drooping, chlorosis, and defoliation in a wide array of field crops such as eggplants, tomatoes, chilli pepper, and leads to the deterioration of tuber crops such as cassava, yams, and taro (Sumana et al., 2011; Ye et al., 2020). These are some of the major sources of calories for tropical natives (Pandukur & Amienyo, 2016; Paul et al., 2021). Attacks on these crops result in serious yield reductions. In Indonesia, up to 70% on-farm yield loss of taro caused by attacks by Fusarium species has been documented (Widodo & Mulyadisastra, 2011). The fungus has also been implicated in postharvest rots and deteriorations. Recently, a 70% incidence of rot disease on stored taro corms was reported in China (Ye et al., 2020). In Nigeria, the fungus is the principal mycotic rot agent, contributing to about 40–50% of postharvest losses in sweet potato, yams, and cocoyam (Agu et al., 2015; Agu et al., 2016a, Agu et al., 2016b; Ano, 2019; Paul et al., 2021). Affected corms develop soft, round, and slightly sunken mycelia-laden lesions, leading to significant reductions in overall produce quality and nutrient depletion (Meyer et al., 2001; Enyiukwu et al., 2021a; Enyiukwu et al., 2021c).

Besides contaminating edible agricultural products with harmful toxins (Enyiukwu et al., 2018a), the organism has become an emergent and the second most frequent mycotic agent of fatal localized mycosis and invasive life-threatening fusarioses (Dananjaya et al., 2017; Batista et al., 2020; Shamrah et al., 2020), especially in immunocompromised humans (Esnakula et al., 2013; Enyiukwu et al., 2018b).

Control of the disease in affected crops revolves around breeding for resistance through the modification of the host's genetic makeup, altering environmental conditions to culturally disfavor the growth of the organism, and using chemical antifungals such as

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amphotericin B, prothioconazole, tebuconazole, thiabendazole, and benomyl, among other azoles. However, despite the numerous advantages of chemical pesticides in modern agriculture, their use in recent times have been constrained by several factors, including growing antifungal resistance to common systemic antifungals, especially azoles. Additional concerns include the effect of pesticides on the ecosystem and the food safety of treated crops due to chemical residues, which have been implicated in CNS (central nervous system) and mammalian organ dysfunctions. These drawbacks have spurred the search for botanical agents as viable potentiates or complements to known azoles (Likhayo et al., 2014; Dananjaya et al., 2017; Yadav et al., 2021). These plant extracts are generally less toxic to humans and the environment, easy to use, and cost-effective for low-input tropical farmers (Amadioha et al., 2019).

Tabernaemontana pachysiphon (Apocynaceae) is an important medicinal plant in eastern Nigeria. In ethnobotany, decoctions or infusions of the roots, stems, and leaves of members of this genus are used to treat scabies, headaches, stomachaches, ulcers, diabetes, breast inflammations, and microbial infections (Uwumarongie et al., 2007; Uwumarongie et al., 2018; Duru & Mbata, 2010). Tropical higher plants, including *T. pachysiphon,* contain a variety of natural products such as alkaloids, polyphenolics, tannins, saponins, and anthraquinones (Duru et al., 2015), which exhibit fungitoxic and antimicrobial activities (Cárdenas-Laverde et al., 2021; Adeogun et al., 2023).

Antimycotic activities of tissue extracts from members of the Apocynaecea family, such as *T. alternifolia*, *T. stapfiana,* and *T. pachysiphon,* against *Candida albicans*, *Aspergillus niger*, *Penicilium notatum,* and *Colletotrichum destructivum,* which cause field and storage diseases of crop plants in sub-Saharan countries, have been reported (Ruttoh et al., 2009; Marathe et al., 2013; Duru et al., 2015; Enyiukwu et al., 2021a). However, to the best of our knowledge, information on the potential activity of leaf extracts of the plant against *Fusarium oxysporum* is not available. Therefore, the aim of this work was to assess the antimycotic activity of aqueous leaf extracts of *T. pachysiphon* against *F. oxysporum,* a postharvest fungal pathogen causing rots of taro corms in storage.

MATERIALS AND METHODS

Experimental Site and Location. This experiment was carried out at the Plant Health Laboratory of Michael Okpara University of Agriculture, Umudike, Nigeria, during the cocoyam harvesting season from October to November 2022. Umudike is located in the warm, humid tropics at an altitude of 122 meters above sea level, with a mean annual temperature of 28 ± 2.0 ºC, rainfall of 2388.87 mm and relative humidity of about 80%.

Source of Plant Material. *Tabernaemontana pachysiphon* Stapf leaves were obtained from a courtyard in Ekebedi Oboro Ancient Kingdom in Ikwuano Council Area of Abia State, Nigeria. The ancient kingdom is located at a latitude of 5.3821º N and a longitude of 7.5803º E, with an elevation of 111 meters. Fresh young leaves of the plant were collected and authenticated by Mr. Nmeregini at the Department of Forestry, College of Natural Resources and Environmental Management (CNREM) of Michael Okpara University of Agriculture, Umudike.

Preparation of *Tabernaemontana pachysiphon* Leaf Extracts. The leaves of *T. pachysiphon* were washed in tap water, rinsed twice with sterile distilled water, and air-dried on the laboratory bench for 2 hours. They were then shredded and ground into a paste using a Philip blender (Model: HR-20565) to obtain 250 g of ground paste. Six 200 mL beakers sterilized with 70% ethanol were each packed separately with 0 g, 10 g, 20 g, 30 g, 40 g, and 50 g of the ground paste. To each beaker, 100 mL of sterile distilled water was added, and the mixtures were allowed to stand for 8 hours. After the extraction period, the contents were separately strained through two layers of cheesecloth into another set of six 200 mL beakers to obtain 0%, 10%, 20%, 30%, 40%, and 50% filtrates of the test plants, which were stored in a refrigerator until needed for the trial (Nwaogu et al., 2022).

Isolation of Causal Fungus. Rotted cocoyam, also known as taro (*Colocasia esculenta* L.), corms were collected from grocers at Ndoru market in Ikwuano Local Government Area of Abia State, Nigeria. The corms were washed in tap water, and the advancing edges of the rotted portions were cut into 6 mm bits with a surgical blade. The cut tissues were sterilized in 3.5% sodium hypochlorte (Hypo™) for 1 min, rinsed in sterile distilled water, and dried on Whatman No. 1 filter papers. Thereafter, they were plated on moistened filter paper in Petri dishes and incubated in an incubation chamber for 5 days to allow the pathogens to grow. Culture medium made from dehydrated potato dextrose agar (PDA) (Oxoid™ Thermo

Scientific Product, England, UK) was prepared by reconstituting 39.5g of dehydrated PDA in 1000 mL of sterile distilled water and autoclaved at 15 psi for 15 min (Nwaogu et al., 2022). Bits of the organisms that grew out of the plated cocoyam corms were aseptically transferred onto solidified PDA (20 mL) in Petri dishes and repeatedly sub-cultured until pure cultures of the organisms were obtained (Amadioha & Markson, 2007a; Amadioha & Markson, 2007b). Slides of the organisms were prepared and mounted on a compound microscope, and their preliminary identity was determined by comparing their colony and spore characteristics with monographs by Barnett & Hunter (1998). The organism whose colony and spore characteristics matched the descriptions of *F. oxysporum* was used for the experiment.

Pathogenicity Test. Three relatively healthy taro corms, each weighing about 200 g, were thoroughly washed in tap water and sterilized with a cotton swab dipped in 70% ethanol. A 6 mm hole was bored into each corm using a 6 mm cork borer and then plugged with a 6 mm core cut from a 9-day-old culture of the test fungus. The holes was covered with tissues removed from the respective taro corms and smeared with petroleum jelly. The corms were then incubated at room temperature in a humid chamber for 14 days. After this period, the corms were cut longitudinally and observed for rot lesions and mycelial growth, which corresponded to those seen in the naturally infected specimens. Using a sterile needle, a bit of the mycelium was transferred onto PDA medium and incubated for 7 days The culture was re-examined under a microscope, and its identity was confirmed to match the *F. oxysporum* isolate from the naturally infected corms.

PCR Identification of Causal Fungus. The genomic DNA of the test mycoflora from a 9-day-old culture was extracted using a hexadecyltrimethylammonium bromide (CTAB)-based method (Nygren et al., 2008). DNA concentration was determined spectrophotometrically using a NanoDrop (Thermo Scientific, Wilmington, DE). PCR amplification of the highly conserved fungal internal transcribed spacer (ITS) region of the genomic DNA was performed using the primer pairs ITS1F and ITS4, as described in previous studies (White et al., 1990). The amplicons were purified using ethanol-sodium acetate precipitation protocol and sequenced at Macrogen in Europe (Amsterdam, The Netherland). Sequences obtained were used for BLASTx searches at the National Center for Biotechnology Information (NCBI-www.ncbi.gov).

Spore Germination and Radial Growth Inhibition Studies. A spore suspension of the fungus was obtained by irrigating an 8-day-old culture of *F. oxysporum* with sterile water and filtering it through two layers of cheesecloth. The suspension was standardized to 1×10^5 spores/mL of distilled water. Approximately 1 drop of this suspension was placed separately on 3 different sterile slides, to which an equal volume of various concentrations (10%, 20%, 30%, 40%, and 50%) of the aqueous extract of *T. pachysiphon* was added, and incubated in a humid chamber for 24 hours. Controls were prepared in a similar manner, but the extracts were replaced with 1 mL of sterile water or griseofulvin. Spore germination was halted by adding 0.05 mL of lactophenol cotton blue to each slide preparation. The spore germination inhibition effects of the plant tissue extract on the test fungus was determined by examining 100 randomly selected spores of the pathogen under a microscope. Records of the number of germinated spores for each treatment and replicate were taken. The percentage inhibition of spore germination of the pathogen compared to the control was calculated using the formula adopted by Enyiukwu et al. (2021b) as follows:

$$I = \frac{(m - n)}{m} \times 100\%$$

I = Inhibition of spore germination;

m = Average number of germinated spores of the test fungus with control;

n = Average number of germinated spores of the test fungus with treatment.

Similarly, about 1 mL of different concentrations of the aqueous plant extract was smeared separately on the surface of solidified PDA in Petri dishes by gentle swirling motion (Amadioha & Markson, 2007a; Amadioha & Markson, 2007b). A 3-mm disc of a 10-day-old culture of the pathogen was transferred to the center of the solidified PDA-extract medium in the Petri dishes, which had been marked underneath with two perpendicular lines intersecting at the center. The dishes were covered and incubated at 27 ºC for 7 days. The control was set up in the same way, but the growth medium was treated with either griseofulvin or sterile distilled water instead. The radial growth of the pathogen was measured along the perpendicular lines with a ruler 7 days after incubation.

The percentage mycelial growth inhibition of

the fungus was used as an indication of the toxicity of the *T. pachysiphon* extract and was calculated using the formula adopted by Enyiukwu et al. (2021b) as:

$$R = \frac{(x - y)}{x} \times 100\%$$

R = Radial growth inhibition;
x = Average diameter of fungal colony with control;
y = Average diameter of fungal colony with treatment.

***In vivo* evaluation.** Twenty-one cocoyam corms, each weighing about 200 g, were thoroughly washed in tap water, rinsed in two changes of sterile distilled water, and sterilized by dipping them in a 3.5% sodium hypochlorite (Hypo™) solution for about 30 sec. The corms were then air-dried on the laboratory bench for 8 hours. Subsequently, each corm was transversely cut into two halves from the center using a sterile surgical knife. Each half of the 3 cut corms was then sprayed separately with the respective test extracts (10, 20, 30, 40, or 50% concentration) and allowed to stand for 2 min. Afterwards, one half of each cut corms was inoculated at the center of the open face with 1 mL (1.0×10^5 spores/mL of sterile distilled water) of the fungal inoculum. The halves were gently rejoined, and the cut zone was sealed with petroleum jelly; the two halves were then held tightly together with a rubber band. The control was treated similarly, with either griseofulvin or the fungal inoculum only. All the corms were incubated in a humid chamber for 15 days. The experiment consisted of 7 treatments, each replicated 3 times, and laid out in a completely randomized design. The test corms were visually assessed at the end of the incubation period using an 8-point descriptive scale adopted from Amadioha (2004), modified as follows:

1 = No rots;
2 = Slightly rotted;
4 = Moderately rotted;
6 = Heavily rotted;
8 = Completely rotted.

The mycotoxicity of *T. pachysiphon* extracts was measured as a reduction in rot severity in the treated corms compared to the untreated controls.

Data Analysis. Data generated from this study were analyzed by analysis of variance (ANOVA) using the Genstat computer program version 12.0. Means were separated and compared using LSD at a 5% level of probability.

RESULTS AND DISCUSSION

PCR Identification of Causal Fungus. Results presented in Table 1 based on molecular sequence analysis of the causal fungus of taro corm rot in this study is *F. oxysporum* (Accession number FJ6095.1).

Effect of Extracts of *T. pachysiphon* on Spore Germination and Radial Growth of *F. oxysporum*. Results presented in Figure 1 indicate that the incorporation of varying concentrations of *T. pachysiphon* in the culture medium had significant effects on the germination of spores and the radial growth of the test pathogen in a concentration-dependent manner. The fungus was most sensitive to the 50% concentration of the plant extract, which demonstrated 70.24% and 82.01% inhibitions of spore germination and radial growth of the fungus, respectively. Exposure of the fungus to 40% strength of *T. pachsiphon* extract in the growth medium inhibited spore germination by 67.19% and radial growth by 79.01%. This was closely followed by 64.52% and 75.12% inhibitions observed for the respective attributes at the 30% concentration of the extract. The 10% extract concentration, which showed inhibition values of 56.38% and 68.22% for spore germination and radial growth of the pathogen, was the least effective (Figure 1). Although the inhibition effects recorded from griseofulvin were statistically ($P < 0.05$) superior to those obtained for all levels of the plant extract, the inhibition effects from all the plant extracts compared favorably with it. All concentrations of *T. pachysiphon* tissue extracts demonstrated significantly ($P < 0.05$) greater potential than the control in curbing germination and fungal growth *in vitro*.

Distortion of appressoria and hyphae tips, as well as sticking and thinning of fungal sporangia, were observed in Petri plates impregnated with varying concentrations of the test extracts. This observation aligns with the findings of Lengai et al. (2020). Inhibition of spore germination and retardation

Tabel 1. Fungal species isolated from taro samples

Fungal isolate	Query coverage (%)	Sequence identity (%)
Fusarium oxysporum	100	85.9

of mycelia elongation have been reported by many researchers as effective mechanisms of action of phytotoxicants against plant pathogenic fungi (Sumana et al., 2011; Amadioha et al., 2019). Dananjaya et al. (2017) reported that the incorporation of *Lawsonia inermis* extracts in vitro caused significant loss of viability and germination capacity of *F. oxysporum* conidia in a trial. Similarly, cinnamon oil demonstrated potent *in vitro* suppression of conidial germination of *F. oxysporium* f. sp. *fragariae,* which causes Fusarium wilt in strawberry (Park et al., 2017). Additionally, Cárdenas-Laverde et al. (2021) reported that isolates derived from *Piper elongatum* effectively impeded the conidial germination of *F. oxysporium* in a culture study. The findings of present study, where extracts of *T. pachysiphon* inhibited spore germination of *F. oxysporum,* are consistent with the reports of these researchers.

In the same vein, aqueous and organic extracts of cinnamon, *Allium sativum*, *Sargassum dentifolium* retarded *in vitro* hyphal elongation and growth of *F. oxysporum* f. sp. *lycopersici* in a manner comparable to carbendazim, a synthetic fungicide (Ohunakin & Bolanle, 2017; Carmello et al., 2022; Mostafa et al., 2022). Other in vitro studies conducted globally have also demonstrated that aqueous and organic extracts of, or isolates from, *Solanum indicum*, *Azadirachta indica, Oxalis latifolia*, *Eucalyptus globulus*, *Sizygium* sp., *T. elegans,* and *T. pachysiphon* inhibited radial growth of *Drechslera halodes, Alternaria alternata, Rhizoctonia solani, Verticillium lateritium, Aspergillus niger,* and *Fusarium oxysporum* (Duru et al., 2015, Naidoo et al., 2021; Vengadaramena & Laxmija, 2021; Cárdenas-Laverde et al., 2021). The findings of this study, where the radial elongation of *F. oxysporum* was significantly retarded by aqueous extracts derived from the leaves of *T. pachysiphon,* corroborate the observations of these researchers. Inhibition of protein, DNA, and ATP synthesis, disruption of cell wall integrity and permeability, causing leakage of radicals or denaturation of specific enzyme due to terpenes, tannins, and other phenolic components of the test extracts, may explain the observed retardation of fungal mycelia in this study (Lengai et al., 2020).

Effect of Extracts of *T. pachysiphon* on *Fusarium oxysporum*-Induced Rot Development. Results presented in Figure 2 indicate that the extracts of *T. pachysiphon* showed varying degrees of inhibition of the initiation and advancement of rot caused by *F. oxysporum* in the living tissues of cocoyam. Rot development was least in corms treated with 50% concentration of *T. pachysiphon* (19.4%), but highest in those treated with 10% concentration (40.2%). Corms treated with 30% and 40% concentration of the test extract expressed moderate rot severity (Figure 2). Griseofulvin (10 mL/L), which had a rot severity of 17.2% was statistically ($p< 0.05$) better at reducing rot development in the corms than the botanicals but yielded similar results to the 50% concentration of *T. pachysiphon*. As shown in Figure 1, the severity of rot development had a linear relationship with the concentration of the test botanical agent. The higher the concentration of *T. pachysiphon* applied, the lower the degree of rot expression observed on the test corms. However, all the assayed concentrations of the plant extract outperformed the control experiment, which had up to 68.7% rot severity.

Similarly, results presented in Figure 2 showed that extracts of *T. pachysiphon* retarded the development, spread, and rot expression caused by *F. oxysporum* in the living tissues of cocoyam. This result aligns with the reports of Amadioha (2004) and Amadioha et al. (2019), where extracts of *A. indica* and *Acacia* sp.

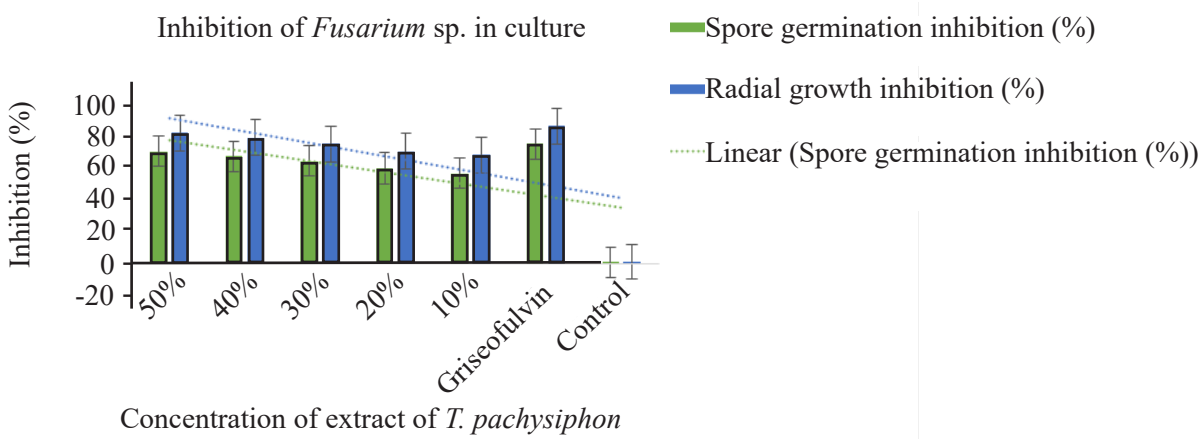

Figure 1. Effect of extracts of *T. pachysiphon* on spore germination and radial growth of *F. oxysporum.*

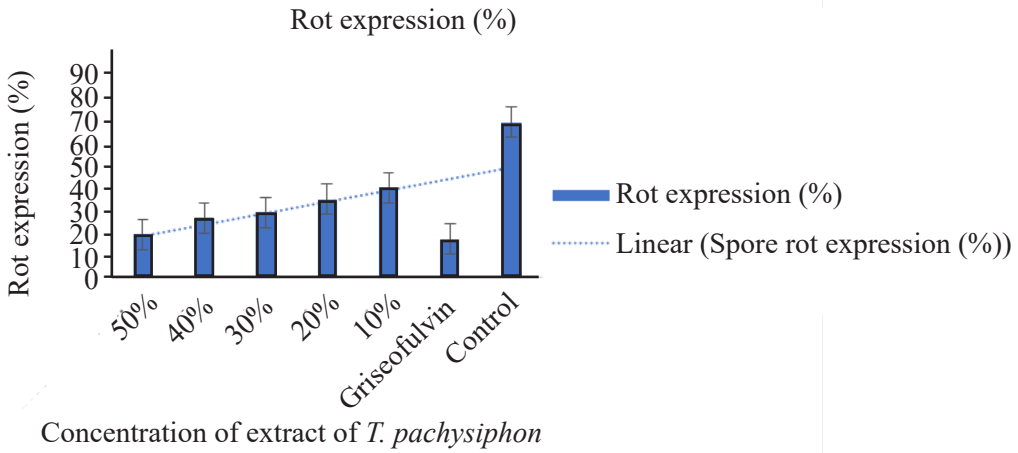

Figure 2. Effect of extracts of *T. pachysiphon* on *F. oxysporum*-induced rot development.

significantly arrested rot initiation and spread in both Irish potato and sweet cassava *in vivo*. This finding is also consistent with the reports of Rongai et al. (2015) and Seepe et al. (2020), which found that some tropical flora extracts impeded the development and spread of *F. oxysporum in vivo*. Mycotoxicity trends in the study (Figure 1 and 2) indicate a linear relationship between concentration of application and percentage fungal inhibition, a view supported by Nwaneri et al. (2020). Toxicity trials revealed that aqueous root bark extract of *T. pachysiphon* did not cause death or any observable toxicity in laboratory animals (Uwumarongie & Onwukaeme, 2011), implying that the use of the plant extract as a pesticide on corms is non-toxic to mammals.

Our findings from both *in vitro* and *in vivo* evaluations also showed that the sensitivity of *F. oxysporum* to aqueous extract of *T. pachysiphon* was inferior compared with griseofulvin, a standard fungicide. This is consistent with the report of Nwaogu et al. (2022), who found aqueous extracts of plantains and bananas inferior to griseofulvin in controlling attacks of *V. longisporum, A. niger* and *P. chrysopeum,* causal agents of leaf spot disease in orange-fleshed sweet potato. On the other hand, our findings are inconsistent with reports from other studies where extracts of *Piper guineense, Xylopia aethiopica, Azadirachta indica* and essential oil of *Chenopodium ambrosioides* were significantly superior to benomyl, ketoconazole, aluminum phosphide, and ethylene dibromide in protecting yam tubers and legumes against *Colletotrichum destructivum* and *Fusarium* sp. in storage and the field (Pandey et al., 2013; Awurum et al., 2014; Ezeonu et al., 2018). The lower performance of *T. pachysiphon* in this study compared with griseofulvin may be due to factors such as the plant's age, environmental conditions, the plant part used, or the solvent employed in the evaluation (Enyiukwu et al., 2016).

Tropical higher plants are sources of bioactive substances (Piana et al. 2014; Edeoga et al., 2015; Khadija et al. 2021), including alkaloids, flavonoids, polyphenols, various fatty acids, and tannins (Uwumarongie et al., 2007; Duru & Mbata, 2010; Piana et al. 2014), which exhibit a wide range of biological activities, including fungitoxicity (Okwu & Njoku, 2009; Enyiukwu & Awurum AN, 2013; Duru et al., 2015). High levels of total phenolic compounds in medicinal plants have been linked to significant inhibition of *F. proliferata* and *F. oxysporum* (Seepe et al., 2020; Mostafa et al., 2022). The high presence of phenolic compounds (Duru et al., 2015) in the test extracts could explain the increased inhibition observed in this study against the test fungus with increasing concentration of *T. pachysiphon.*

Storage fungi macerate the cementing components of cell walls and invade tuber tissues, degrading stored starch granules and nutrients using powerful hydrolytic enzymes (Amadioha et al., 2019). Dananjaya et al. (2017) noted that the mechanism of action of botanical agents against *F. oxysporum* includes the induction of high levels of reactive oxygen species in fungal spores and filaments, leading to oxidative stress, which results in cell damage, loss of plasma membrane integrity, and cell death. Fatty acids such as palmitic, oleic, and lauric acids are richly present in *T. pachysiphon* leaves. These compounds have been implicated in the disruption of fungal cell wall integrity in various studies (Enyiukwu et al 2021b). The observed rot inhibitions in this study may

also be attributed to these compounds.

Additionally, it may be due to the inhibitions of extracellular enzymes that play crucial roles in fungal nutrition and growth (Premjanu & Jaynthy, 2014). It appears that inhibition of certain enzymes, such as reductases, hydrolases, amylases, or cell proliferation, might partly explain the ability of *T. pachysiphon* to retard the growth of *F. oxysporum* in culture and in the living tissues of cocoyam.

However, serious drawbacks to the adoption of plant-derived pesticides include their sensitivity to high temperatures, humidity, sunlight and air. They are reported to be easily degraded or oxidized by these factors, which could render them ineffective, especially if not properly stored in dark, airtight containers away from direct sunlight (Lengai et al., 2020), or if they are not formulated to meet local application needs (Ngegba et al., 2022).

CONCLUSION

The results of this study showed that the aqueous extract of *T. pachysiphon* strongly inhibited spore germination and mycelial growth of *F. oxysporum in vitro,* as well as the development and spread of rot in the living tissues of cocoyam. Based on our findings, inhibition of rot increased with concentration. Therefore, we recommend using 50% or 40% extract concentrations of the plant to effectively protect corms in storage. Since the botanical is easily available in local tropical settings and is relatively safe to mammals, it should be explored for possible adoption by tropical farmers on a wide scale.

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AUTHORS' CONTRIBUTIONS

NDE and ACA designed the experiment. INE identified the causal fungus. DNE and INE were involved in collecting, analyzing, and interpreting the results. ACA arranged the manuscript, while DNE edited the final version. All authors have read and approved the manuscript for publication.

COMPETING INTEREST

All authors of this manuscript declare no potential conflict of interest regarding the publication of this work.

REFERENCES

Adeogun OO, Ebabhi AM, Adongbede EM, Oluwa OK, & Adekunle AA. 2023. Influence of leaf extract of *Lantana camara* integrated with maize-based coating on quality of fresh *Talinum triangulare* and *Telfairia occidentalis* leaves. *Caraka Tani: J. Sust. Agric*. 38(1): 85–98. https://doi.org/10.20961/carakatani.v38i1.57446

Agu KC, Awah NS, Nnadozie AC, Okeke BC, Orji MU, Iloanusi CA, Anaukwu CG, Eneite HC, Ifediegwu MC, Umeoduagu ND, & Udo EE. 2016a. Isolation, identification and pathogenicity of fungi associated with cocoyam (*Colocasia esculanta* L.) spoilage. *FS&T.* 4(5): 103–106. https://doi.org/10.13189/FST.2016.040503

Agu KC, Nweke GU, Awah NS, Okeke BC, Mgbemena LCC, Okigbo RN & Ngenegbo UC. 2015. Fungi associated with post-harvest loss of sweet potato. *IJRSB.* 3(9): 33–38.

Agu KC, Sunday AN, Okeke CB, Nwodo MT, Anucwukwu CG, Iloanusi CA, Archibong EJ, Ngenegbo U, Mgbemena IC, & Umeoduagu N. 2016b. Mycological deterioration and pathogenicity studies of post-harvest cassava. *Food Sci. Technol.* 4(2): 23–30. https://doi.org/10.13189/fst.2016.040202

Amadioha AC. 2004. Control of black rot of potato caused by *Rhizoctonia bataticola* using some plant leaf extracts. *Arch. Pytopathol. Plant Protect*. 37(2): 111–117. https://doi.org/10.1080/0323540042000202067

Amadioha AC & Markson AA. 2007a. Post-harvest control of cassava tuber rot caused by *Botrydiplodia acerina* using extracts of plant origin. *Arch. Phytopathol. Pflanzenschutz.* 40(5): 359–366. https://doi.org/10.1080/03235400600624079

Amadioha AC & Markson AA. 2007b. Control of storage rot of cassava tuber caused by *Rhizopus oryzae* using some plant extracts. *Arch. Phytopathol. Pflanzenschutz.* 40(6): 381–388. https://doi.org/10.1080/03235400500222248

Amadioha AC, Chidi KP, & Markson AA. 2019. Fungitoxic potentials of extracts of plant origin against fungal rot pathogens of cassava (*Manihot esculenta* Crantz) in storage. *Ann. Res Rev. Biol.* 31(1): 1–9. https://doi.org/10.9734/arrb/2019/v31i130036

Ano QU. 2019. Natural plant extracts and post harvest management of yams in storage: A review. *Niger. Agric. J.* 50(2): 241–246.

Awurum AN. Enyiukwu DN, & Ucheagwu PO. 2014. Effectiveness of duration of storage and phytochemicals in the management of seed-borne mycoflora of stored cowpea (*Vigna unguiculata* L. Walp.) seeds. *GJEPH*. 2(2): 22–26. https://doi.org/10.15580/GJAFH.2014.2.120113998

Batista BG, de Chaves MA, Reginatto P, Saraiva OJ, & Fuentefria, MA 2020. Human fusariosis: An emerging infection that is difficult to treat. *Rev Soc Bras Med Trop.* 53: e20200013. https://doi.org/10.1590/0037-8682-0013-2020

Barnett HL & Hunter BB. 1998. Illustrated *Genera of Imperfect Fungi 4th ed*. American Phytopathological Society, Minnesota, USA.

Carmello CR, Magri MMR, & Cardoso JC. 2022. Cinnamon extract and sodium hypochlorite in the *in vitro* control *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria alternata* from tomato. *J. Phytopathol*. 170(11-12): 802–810. https://doi.org/10.1111/jph.13143

Cárdenas-Laverde DL, Cornelio RB, & Barerra EC. 2021. Antifungal activity against *Fusarium oxysporum* of botanical end-products: An integration of chemical composition and antifungal activity datasets to identify antifungal bioactives. *Plants (Basssel)*. 10(12): 2563. https://doi.org/10.3390/plants10122563

Dananjaya SHS, Udayangani RMC, Shin SY, Edussuriya R, Nikapitiya C , Lee J, & De Zoysa M. 2017. *In vitro* and *in vivo* antifungal efficacy of plant-based lawsome against *Fusarium oxysporium* species complex. *Microbial. Res.* 201: 21–29. https://doi.org/10.1016/j.micres.2017.04.011

Duru CM & Mbata TI. 2010. The antimicrobial activities and phytochemical screening of ethanolic leaf extracts of *Hedranthera bateri* Hook and *Tabernaemontana pachysiphon* Stapf. *J. Dev. Biol. Tissue Eng.* 2(1): 1–4.

Duru CM, Anyadoh-Nwadike SO, & Okechukwu RI. 2015. Antimicrobial activity and phytochemical analysis of aqueous and ethanolic extracts of the bark of *Tabernaemontena pachysiphon* Stapf. *Sci. J. Publ. Health.* 3(5–1): 8–13. https://doi.org/10.11648/j.sjph.s.2015030501.12

Edeoga HO, Okwu DE, & Mbaebie BO. 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 4(7): 685–688. https://doi.org/10.5897/AJB2005.000-3127

Esnakula AK, Summers I, & Naab TJ. 2013. Fatal disseminated *Fusarium* infection in a human immunodefficiency virus positive patient. *Case Rep. Inf. Dis.* 2013: 379320. https://doi.org/10.1155/2013/379320

Enyiukwu DN & Awurum AN. 2013. Fungitoxic principles and *invitro* antifungal activity of extracts from *Carica papaya* and *Piper guineense* L. on *Colletotrichum destructivum*. *Continental J. Biol. Sci.* 6(1): 29–37.

Enyiukwu DN, Ononuju CC, Awurum AN, & Nwaneri JA. 2016. Modes of action of potential phyto-pesticides from tropical plants in plant health management. *IOSR J. Pharm*. 6(7): 1–17. https://doi.org/10.9790/3013-06710117

Enyiukwu DN, Ononuju CC, & Maranzu JO. 2018a. Mycotoxins in foods and indoor air: their attendant diseases and modes of injury on biological and human systems. *GJEPH.* 6(1): 034–051. https://doi.org/10.15580/GJEPH.2018.1.010818004

Enyiukwu DN, Ononuju CC, & Maranzu JO. 2018b. Plant pathogenic fungi – Novel agents of human diseases: Implications for public health. *GJEPH.* 6(1): 001–019.

Enyiukwu DN, Amadioha AC, & Ononuju CC. 2021a. Evaluation of some pesticides of plant origin for control of anthracnose (*Colletotrichum*

destructivum O'Gara) in cowpea. *Asian J. Agric.* 5(1): 4–11. https://doi.org/10.13057/asianjagric/g050102

Enyiukwu DN, Amadioha AC, & Ononuju CC. 2021b. Antifungal activities of methanol extracts of some medicinal plants against germination and growth of *Colletotrichum destructivum* O'Gara in culture. *Asian J. Nat. Prod. Biochem.* 19(1): 25–29. https://doi.org/10.13057/biofar/f190105

Enyiukwu DN, Nwaogu GA, & Bassey IN. 2021c. Association of cowpea (*Vigna unguiculata* L. Walp.) (var. Ife Brown) with *Colletorichum destructivum*: A special reference to nutrients lost by the host. *Cell Biol. Develop*. 4(2): 41–46. https://doi.org/10.13057/cellbioldev/v040201

Ezeonu CS, Imo C, Agwaranze DI, Iruka A, & Joseph A. 2018. Antifungal effects of aqueous and ethanolic extracts of neem leaves, stem bark and seeds on fungal rot disease of yam and cocoyam. *Chem. Biol. Technol. Agric*. 5: 18. https://doi.org/10.1186/s40538-018-0130-3

Khadija EO, Abdelfattah EM, Laila B, & Dalila B. 2021. Ethnobotanical study of medicinal plants used in the treatment of cancers in the city of Casablanca (West-Central Morocco). *Trop. J. Nat. Prod. Res*. 5(6): 1044–1054. https://www.tjnpr.org/index.php/home/article/view/597

Likhayo P, Olubayo F, & Ngatia C. 2014. Methyl bromide alternatives for maize storage in Kenya. *IJSR.* 3(7): 2348–2352.

Lengai GMW, Muthomi JW, & Mbega ER. 2020. Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Sci. Afr.* 7: e00239. https://doi.org/10.1016/j.sciaf.2019.e00239

Marathe NP, Rasane MH, Kumar H, Patwardhan AA, Shouche YS, & Diwanay SS. 2013. *In vitro* antibacterial activity of *Tabernaemontana alternifolia* (Roxb) stem bark aqueous extracts against clinical isolates of methicillin resistant *Staphylococcus aureus*. *Ann. Clin. Microbiol. Antimicrob*. 12: 26. https://doi.org/10.1186/1476-0711-12-26

Meyer S, Saccardy-Adji K, Rizza F, & Genty B. 2001. Inhibition of photosynthesis by *Colletotrichum lindemuthianium* in bean leaves determined by chlorophyll flourescence imaging. *Plant Cell Environ.* 24(9): 947–956. https://doi.org/10.1046/j.0016-8025.2001.00737.x

Mostafa YS, Alamri SA, Alrumman SA, Hashem M, Taher MA, & Baka ZA. 2022. *In vitro* and *in vivo* biocontrol of tomato Fusarium wilt by extracts from brown, red and green microalgae. *Agriculture.* 12(3): 345. https://doi.org/10.3390/agriculture12030345

Naidoo CM, Naidoo Y, Dewir YH, Murthy HM, El-Tendawy S, & Al-Suhaibani N. 2021. Major bioactive alkaloids and biological activities of *Tabernaemontana* species. *Plants*. 10(2): 313. https://doi.org/10.3390/plants10020313

Ngegba PM, Cui G, Khalid MZ, & Zhong G. 2022. Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. *Agriculture.* 12(5): 600. https://doi.org/10.3390/agriculture12050600

Nwaneri JA, Enyiukwu DN, Cyprain UEC, & Nicholas, JC. 2020. Response of fungal rot pathogens of sweet potato (*Ipomoea batatas* (L) Lam. in storage to aqueous extracts of some tropical plant materials and benomyl. *GJEPH.* 3(1): 18–24.

Nwaogu AG, Obani FT, Iheanocho FN, & Enyiukwu DN. 2022. *In vitro* evaluation of aqueous leaf and peel extracts of *Musa* species for low-input management of fungal leaf spots of sweet potato (*Ipomea batatas* Linn.) Cropsaver: *J. Plant Protect*. 5(1): 7–14. https://doi.org/10.24198/cropsaver.v5i1.39519

Nygren CMR, Eberhardt U, Karlsson M, Parrent JL, Lindahl BD, & Taylor AFS. 2008. Growth on nitrate and occurrence of nitrate reductase-encoding genes in a phylogenetically diverse range of ectomycorrhizal fungi. *New Phytol.* 180(4): 875–889. https://doi.org/10.1111/j.1469-8137.2008.02618.x

Ohunakin AO & Bolanle OB. 2017. In vitro antifungal activities of three aromatic plant extracts against *Fusarium oxysporum* Schlecthtend. Fr. f. sp. *lycopersici* (Sacc.) causal organism of Fusarium wilt in tomato. *Plant Sciences and Agricultural Research* 1: 1.

Okwu DE & Njoku EE. 2009. Chemical composition and *in vitro* antifungal activity screening of seed and leaf extracts from *Aframomum meleguata* and *Monodora myristica* against *Sclerotium rolfsii* of cowpea plant (*Vigna unguiculata* L.

Walp). *Pest Technol.* 3(1): 58–67.

Pandey AK, Singh P, Palni UT, & Tripathi NN. 2013. Application of *Chenopodium ambrosioides* Linn. essential oil as botanical fungicide for the management of fungal deterioration in pulses. *Biol. Agric. Hortic.* 29(3): 197–208. https://doi.org/10.1080/01448765.2013.822828

Pandukur SG, & Amienyo CA. 2016. Effects of Azadirachta indica extract on the radial growth of some fungi isolated from two varieties of cococyam (Colocasia esculenta L.) corms and cormels in some markets in Plateau State, Nigeria. *PPMJ*. 3(1): 46–59.

Park JY, Kim SH, Kim NH, Lee SW, Jeun YC, & Hong JK. 2017. Differential inhibitory activities of four plant essential oils on *in vitro* growth of *Fusarium oxysporum* f. sp. *fragariea* casing Fusarium wilt in strawberry plants. *Plant Pathol. J.* 33(6): 582–588. https://doi.org/10.5423/PPJ.OA.06.2017.0133

Paul NC, Park S, Liu H, Lee JG, Han GH, Kim H, & Sang H. 2021. Fungi associated with postharvest diseases of sweet potato storage roots and *in vitro* antifungal assay of *Trichoderma hazianum* against the diseases. *J. Fungi (Bassel).* 7(11): 927. https://doi.org/10.3390/jof7110927

Piana M, Boligon AA, Brum TFD, Zadra M, Belke BV, Froeder ALF, Frohlich JK, Nunes LT, Pappis L, Boligan AA, & Athayde ML. 2014. Phytochemical analysis and antioxidant capacity of *Tabernaemontana catharinensis* A. DC fruits and branches. *An. Acad. Bras. Cienc.* 86(2): 881–888. https://doi.org/10.1590/0001-3765201420120020

Premjanu N & Jaynthy C. 2014. Antimicrobial activity of diethyl phthalate: An insilico approach. *Asian J. Pharm. Clin. Res.* 7(4): 141–142.

Rongai D, Pulcino P, Pesce B, & Milano F. 2015. Antifungal activity of some botanical extracts on *Fusarium oxysporum*. *Open Life Sci.* 10: 409–416.

Ruttoh EK, Bli C, Tarus PK, Machocho A, Karini LK, & Okemo P. 2009. Antifungal activity of *Tabernaemontana stapfiana* Britten (Apocynaceae) organic extracts. *Phcog. Res.* 1(6): 387–391.

Seepe HA, Lodama KE, Sutherland R, Nxumalo W, & Amoo SO. 2020. *In vivo* antifungal activity of South African medicinal plant extracts against *Fusarium* pathogens and their phytotoxicity evaluation. *Plants (Bassel).* 9(12): 1668. https://doi.org/10.3390/plants9121668

Shamrah S, Swedian A, Aleshawi A, & Ayesh M. 2020. Fusarium-induced cellulitis in an immunocompetent patient with sickle cell disease: A case report. *J. Investig. Med. High Impact Case Rep.* 8. https://doi.org/10.1177/2324709620934303

Sumana K, Sreenivas SS, & Devaki NS. 2011. *In vitro* evaluation of fungicides against *Fusarium oxysporum* causing wilt in FCV tobacco. www.researchgate.net/publication/332556536. Accessed 2 June 2021.

Uwumarongie OH, Onwukaeme DN, & Obasuyi O. 2007. Antimicrobial activity of the methanolic leaf extract of *Tabernaemontana pachysiphon* Stapf. *Nig. J. Nat. Prod. Med.* 11: 23–25. https://doi.org/10.4314/njnpm.v11i1.11872

Uwumarongie OH, & Onwukaeme DN. 2011. Pharmacognostic and toxicity evaluation of the stem bark of *Tabernaemoontana pachysiphon* Stapf. (Apocynaceae). *Nig. J. Pharma. Res.* 9(1): 63–71.

Uwumarongie HO, Onwukaeme DN, & Igbe I. 2018. GC-MS metabolite profiling, antinociceptive and antipyretic activities of methanol stem bark extract of *Tabernaemontana pachysiphon* Stapf. (Apocynaceae). *Journal of Science and Practice of Pharmacy.* 5(1): 228–230.

Vengadaramena A & Laxmija S. 2021. Morphological characterization and effects of extracts of some medicinal plants on the control of *Colletorichum* isolates associated with papaya anthracnose disease. *Trop. J. Nat. Prod. Res.* 5(4): 649–655.

Widodo W & Supramana. 2011. Fusarium species associated with corm rot of taro in Bogor. *Microbiology Indonesia.* 5(3): 132–138. https://doi.org/10.5454/mi.5.3.6

White TJ, Bruns TD, Lee SB, & Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, & White TJ. (Eds). *PCR protocols: guide to methods and applications*. pp 315–322. Academic Press. San Diego.

Yadav DR, Adhikari M, Kim SW, Kim HS, & Lee YS.

2021. Suppression of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lactucae* and growth promotion on lettuce using bacterial isolates. *J. Microbiol. Biotechnol.* 31(9): 1241–1255. https://doi.org/10.4014/jmb.2104.04026

Ye Y, Liu B, Xiong G, Zhou QH, Huang YJ, Zhu QL, & Jiang JX. 2020. First report of *Fusarium oxysporum* and *F. proliferatum* causing postharvest corm rot on taro in China. *Plant Dis.* 107(5): 1261–1640. https://doi.org/10.1094/pdis-03-22-0676-pdn