

Appraisal of fungi leaf spots of groundnut (*Arachis hypogaea*) and control of *Cylindrocladium* blight disease using biocontrol, botanical, and chemical measures

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Abstract

Groundnut is infected by 55 pathogens which adversely contribute to the low yield of groundnuts. Three sub-trials were conceived to proffer solutions to groundnut *Cylindrocladium parasiticum* disease. These sub-trials (using biocontrol, synthetic and botanical measures) were set up *in vitro* using completely randomized design with each treatment replicated thrice. Firstly, all biocontrol (*Trichoderma*) isolates showed a steady linear increase in the inhibition of the growth of the pathogen. There was no significant difference ($P \leq 0.05$) between the isolates of *Trichoderma harzianum* at 96 h after inoculation (HAI). Secondly, the synthetic pesticide Mancozeb® 100% significantly inhibited the pathogen more than all other synthetic chemical treatments, followed by Mancozeb 50%, Tandem® 100% and Tandem 50% (which were at par (at 144 HAI)). Thirdly, the botanical *Parkia* sp. 100% was significantly superior to other botanicals (192 HAI results). Likewise, *Casuarina* sp. 100% and *Parkia* sp. 50% were at par but performed better than other treatments except for *Parkia* sp. 100%. Inhibition by *Casuarina* sp. 50% was at par with *Parkia* sp. 50% but it was significantly superior to other treatments (except for *Parkia* sp. 100%, *Casuarina* sp. 100%, and *Parkia* sp. 50%). Finally, all trials showed that Mancozeb 100% performed better than other treatments.

Introduction

Groundnut (*Arachis hypogaea* L.) is an oil-seed and grain legume crop cultivated in most areas of the tropics and subtropics (latitudes 40°N-40°S) (Pattee & Young, 1982). El-Sherbeny et al. (2020) reported that global annual groundnut yields were up to 45.3 million metric tons per annum. Groundnut seeds contain 50% oil, 25-30% protein, 20% carbohydrate, 5% fibre/ash (Fageria et al., 1997; Naab et al., 2005; Tsigbey et al., 2004). Groundnut seeds are boiled or roasted and consumed as a snack, and soup thickener. While the hay/haulms are fed to livestock. Moreover, this crop fixes atmospheric nitrogen in its nodules thus enhancing soil fertility (Hassain et al., 2005).

Nonetheless, its production and yields are constrained by various pathogens (viruses, bacteria, fungi, and insects) and abiotic factors like drought, salinity, and unfavourable temperature (El-Sherbeny et al., 2020). Globally groundnut is attacked by more than 55 pathogens which is one of the most important factors causing the low yield of groundnuts (Muthukumar et al., 2014).

Some fungi-induced foliar diseases of groundnut include *Macrophomina*, *Choanephora*, *Colletotrichum*, *Cylindrocladium*, *Drechslera*, *Pestalotiopsis*, *Phomopsis*, *Phyllosticta*, zonate, early leaf spot, and late leaf spot.

Besides these, *Alternaria*, *Botrytis*, *Myrothecium*, *Phoma*, and *Phomopsis* leaf blights as well as powdery mildew, rust, web blotch (net blotch), *Alternaria* vein necrosis, and anthracnose attack the crop ([Onyishi, 2010](#); [Porter, 1993](#); [Shaza et al., 2004](#)).

Early leaf spots (induced by *Cercospora arachidicola* Hori.: its teleomorph is *Mycosphaerella arachidicola* W.A. Jenkins) and late leaf spot (induced by *Phaeoisariopsis personata* (Berk. & Curt.) V. Arx.: its teleomorph is *Mycosphaerella berkeleyi* W.A. Jenkins) coupled with groundnut rust (*Puccinia arachidis* L.) have been reported to be able to cause severe economic yield loss in groundnut globally ([Anco et al., 2016](#); [Damicone, 2017](#); [Ghewande, 2009](#); [Nutsugah et al., 2007](#); [Subramanyam et al., 1991](#); [Waliyar et al., 2000](#); [Woodward et al., 2013](#)). Generally, leaf spots can cause between 50-70% yield losses in West Africa ([Naab et al., 2004](#); [Waliyar et al., 2000](#)) and up to 50% yield loss worldwide ([Desai & Bagwan, 2005](#); [Koita et al., 2017](#); [McDonald et al., 1985](#); [Pal et al., 2014](#); [Sangoyomi & Alabi, 2016](#)).

[Pal et al. \(2014\)](#) reported that effective control of fungi diseases in groundnut has not been achievable to date despite the availability of different kinds of fungicides. They pointed out that the situation is going to deteriorate due to climatic change accompanied by the emergence of new virulent strains of pathogens. [Grahame \(2014\)](#) reported that resistance to several classes of fungicides used in groundnut production has been reported for some leaf spot pathogens. For instance, resistance to tebuconazole (triazole fungicides) was reported in the United States. [Kishore et al. \(2005\)](#) reported that host plant resistance to leaf spot diseases is very rare in groundnut cultivars hence use of synthetic pesticides has persisted.

[Anco et al. \(2016\)](#) reported that pest control programs in South Carolina, USA, mainly target late leaf spots which consistently cause economic yield loss. [Nutsugah et al. \(2007\)](#) reported that fungicides (thiophanate methyl, benomyl and tebuconazole) suppressed groundnut leaf spot diseases and increased biomass and pod yields in Ghana. Tebuconazole was the most effective fungicide in reducing leaf spot severity, and increasing biomass and pod yield. Neem seed extract, *Alata samina* extracts, thiophanate-methyl, carbendazim (Bavistin®), and tebuconazole (Folicur®) mixed with azoxystrobin (Abound®) reduced leaf spot severity and increased peanut yield in northern Ghana ([Tsigbey et al., 2000, 2001](#)).

So far chlorothalonil is the most reliable pesticide for the control of leaf spots and rust on groundnuts ([Andrews, 1992](#); [Kishore et al., 2005](#); [Tsatsia & Grahame, 2013](#); [Woodward et al., 2013](#)). [Kishore et al. \(2005\)](#) reported that at more than 250 µg mL⁻¹ chlorothalonil alone or in the presence of *Pseudomonas aeruginosa* GSE 18 and GSE 19, (100 µg mL⁻¹), completely controlled *Phaeoisariopsis personata*. However, [Podile and Kishore \(2002\)](#) revealed that the performance of mycoparasitic fungi (like *Verticillium*

lecanii, *Dicyma pulvinata*, and *Acremonium obclavatum*) in the field is highly variable.

[Kiran \(2012\)](#) reported that collar rot or seedling blight or crown rot (by *Aspergillus niger* and *A. pulverulentum*) could be controlled using seed treatment with *Trichoderma viride* or *T. harzianum* (4 g/kg seeds) preferably in combination with organic amendments such as castor, neem or mustard cakes (500 kg/ha). These researchers confirmed that treatment of the seeds with thiram (75% WP), captan (80% WP), mancozeb (75% WP) or carbendazim (50% WP) effectively controlled seed-borne infections.

[Frances et al. \(2002\)](#) and [Buck \(2004\)](#) stated that another promising direction of research is to utilize fungicide-tolerant biocontrol agents with synthetic fungicides thereby reducing the amount of fungicides required. This form of integration was more effective against damping-off of tomato, *Rhizoctonia* root rot and take-all of spring wheat and postharvest diseases of fruits than using each method of control alone.

Several research findings have proven that plants that contain antimicrobial biochemical metabolites, when applied against microbes are less detrimental to the environment than synthetic pesticides ([Hashim & Devi, 2003](#)). [Koita et al. \(2017\)](#) reported that aqueous extracts of *Lippia multiflora* Moldenke and *Ziziphus mucronata* Wild were most effective and significantly increased groundnut pod yield on a susceptible groundnut variety (TS32-1) in Burkina Faso compared to other treatments.

[Awurum and Uwajimgba \(2013\)](#) reported that plant extracts of *Dennettia tripetala* and benomyl significantly reduced the disease severity of *Fusarium oxysporium* wilt compared to plant extracts of *Spondias mombin* in the screenhouse. [Soumya and Bindu \(2012\)](#) effectively controlled groundnut seed pathogens (*Aspergillus niger*, *A. flavus*, *Penicillium* sp. and *Rhizopus* sp.) using *Capsicum frutescens* extracts.

[Asama and Channya \(2018\)](#) reported the occurrence of *Cylindrocarpon lichenicola*, *Aspergillus niger* (*brasilensis*), *Aspergillus flavus*, *Penicillium chrysogenum*, *Rhizopus stolonifer*, *Paecilomyces lilacinus*, *Pseudallescheria boydii*, and *Scedosporium prolificans* on groundnut seeds in North-eastern Nigeria. *Cylindrocarpon* spp. have been reported to be capable of producing mycotoxins especially aflatoxins, zearalenone and trichothecenes and Deoxynivalenol (i.e. D.O.N.).

[Nutsugah et al. \(2007\)](#) reported that farmers in northern Ghana perceive shoot defoliation as an indicator of plant maturity and not as a disease. This is a common view held by farmers in Nigeria as well. In West Africa use of fungicides in groundnut production is rare due to constraints like lack of credit, low yields, and non-availability of fungicides ([McDonald et al., 1985](#)). In this region, the application of fungicides can reduce the severity of leaf spots and increase yields ([Kannaiyan & Haciwa, 1990](#); [Waliyar et al., 2000](#)). Based on the foregoing information this research was conceived to

appraise the status quo of leaf spot diseases in groundnuts and proffer some solutions to these plant diseases.

Materials and Methods

Site of the study

This research was carried out at the Faculty of Agriculture Laboratories in Alex Ekwueme Federal University Ndufu-Alike, Ikwo Local Government Area of Ebonyi State. The University is located in Ikwo (6.069°N by 8.199°E) about 21 kilometres from Abakaliki; the State capital. Ebonyi State is in the derived savanna zone of Nigeria with a humid tropical climate.

Isolation and identification of the fungi associated with groundnut leaf spots

The infected groundnut leaves utilized for this research were obtained from farms in Ebonyi State. The fungi were isolated using potato dextrose agar (PDA) medium which was autoclaved at 120°C and 15 psi for 15 min according to the manufacturer's (LifeSave Biotech Inc.) instructions. 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 fungi isolates were stored in the freezer for use later in determining possible management techniques.

Subtrial 1: Effects of *Trichoderma* isolates as a biocontrol agent on *Cylindrocladium parasiticum* associated with groundnut leaf spots

The fungus (*Cylindrocladium parasiticum* Crous, M.J. Wingfield & Alfenas (syn. *C. crotalariae* (Loos) Bell & Sobers) isolate utilized in this sub-trial was isolated from infected groundnut plants as described above. While the *Trichoderma* isolates were isolated from the Bambara groundnut seeds, mushrooms, crop seeds and farmland soils. The experiment was laid out in Petri dishes using completely randomized design and each treatment was replicated three times.

The treatment set consisted of 6 *Trichoderma harzianum* isolates (*T. harzianum* AIBN, *T. harzianum* BGMP, *T. harzianum* ZXMZ, *T. harzianum* BGMZ3, *T. harzianum* BGMZM, and *T. harzianum* BGMZ4), a check (Mancozeb® at 2000 g/ha) and a control. The control was inoculated with the pathogenic fungi (*C. parasiticum*) isolate alone. The agar medium was inoculated with a 2-mm disc (of the pathogen or biological control agent), placed at the edge of the plate.

Subtrial 2: Effects of synthetic pesticides on *C. parasiticum* associated with groundnut leaf spots

The experiment was carried out using Petri dishes. It was laid out in the laboratory using a completely randomized design (CRD) with 5 treatments. Each treatment was replicated three times. The treatment set included control, Mancozeb 100%, Mancozeb 50%,

Tandem 100% and Tandem 50%. Mancozeb® (2000 g/ha - it is a contact fungicide) and Tandem® (wetable powder of Cu (I) O (60%) + Metalaxyl (12%) recommended at 800 g/ha, it is a systemic fungicide) were utilized to compose the treatments. Each treatment consisted of three levels (0.0, 50 and 100% concentrations).

Subtrial 3: Effects of plant extracts on *C. parasiticum* associated with groundnut leaf spots

The experiment was carried out using Petri dishes. It was laid out in the laboratory using a completely randomized design (CRD) with 8 treatments. Each treatment was replicated three times. The treatment set included control, bark of African locust bean (*Parkia biglobosa* (Jacq.) R. Br. ex G. Don), shoot of Australian pine (also called beef-wood, iron-wood, she-oak, whistling pine or horse-tail tree (i.e. *Casuarina equisetifolia*), and shoots/branches of purple-leaved spiderwort (also called Moses-in-the-cradle or oyster plant i.e. *Rhoeo spathacea* (Sw.) Stearn). Each plant tissue was weighed at the rate of 333.3 g tissues/L distilled water for 100% concentration. Each treatment consisted of three levels (0.0, 50 and 100% concentrations).

Data collection used for the subtrials

The radius of *C. parasiticum* colony was measured using a transparent ruler at 24 h intervals starting from day 1 until each sub-trial was terminated. The percentage inhibition of the pathogen was calculated using:

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

Where

PI= Percent inhibition of growth of the fungus

C= Perpendicular radius of fungus colony in the control plate

T= Perpendicular radius of the fungus colony in treated plate

Data analysis used for the subtrials

The data were subjected to analysis of variance (ANOVA) and the means were separated using Student Newman-Keuls' (SNK) method. Genstat® 2nd edition discovery statistical package was used to carry out ANOVA test. Descriptive statistics were used to illustrate the trends in the growth of the pathogen and its management.

Results

The results of the trial on inhibition of the growth of *C. parasiticum* from groundnut by three botanical pesticides are presented in Figure 1. It was observed that *Casuarina* and *Rhoeo* spp. (at 50% and 100% concentrations) exhibited a similar pattern of inhibition of *C. parasiticum* whereby the level of inhibition dropped to the right and then resurged as time passed till the trial was terminated. On the contrary, both

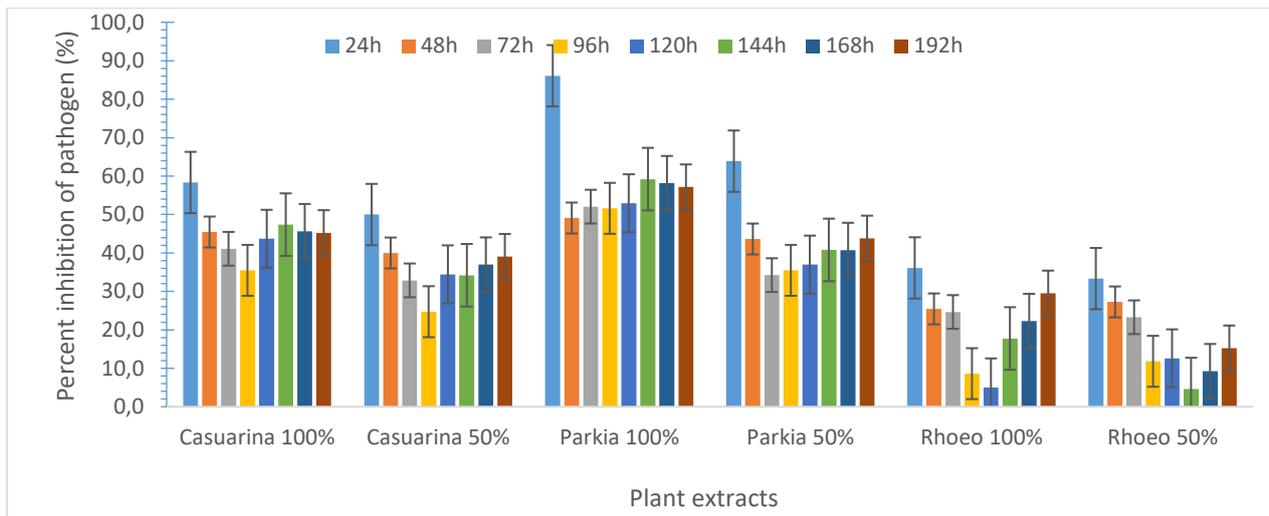


Figure 1. Inhibition of the growth of leaf spot pathogen isolated from groundnut caused by the application of three botanical pesticides.

concentrations of *Parkia* sp. showed an unswerving increase in inhibition of the pathogen all through till the termination of the trial.

The means separation (based on 192 HAI data) showed that the inhibition by *Parkia* 100% was significantly superior ($P \leq 0.05$) to all treatments and control. Means of *Casuarina* 100% and *Parkia* 50% were at par statistically but performed better than all other treatments except *Parkia* 100%. The inhibition by *Casuarina* 50% was at par with *Parkia* 50% but it was significantly superior to all other treatments (excepting *Parkia* 100%, *Casuarina* 100%, and *Parkia* 50%). The inhibition by both extracts of *Rhoeo* sp. was significantly superior to that of the control only. In fact, inhibition by all the plant extracts was significantly higher compared to the control.

The results of the trial on the effects of isolates of *Trichoderma* species against this pathogen (*C. parasiticum*) are presented in Figure 2. All the isolates showed a fairly steady linear increase in inhibition of the

pathogen throughout. Mancozeb 100% was consistently superior (24 HAI) compared to the biocontrol agents as time passed. The means separation (96 HAI data) showed no significant difference ($P \leq 0.05$) between the isolates of *T. harzianum* utilized but they were significantly different ($P \leq 0.05$) from the check which was significantly superior to them.

The results of the trial on the effects of synthetic fungicides against *C. parasiticum* are presented in Figure 3. It shows that both concentrations of mancozeb maintained a linear decline in efficiency as time passed. However, both concentrations of tandem had a steady improvement in the efficacy of inhibiting the pathogen as time passed. The means separation showed significant differences ($P \leq 0.05$) between the inhibition caused by chemicals utilized to control the pathogen. Mancozeb 100% was significantly ($P \leq 0.05$) superior to all the treatments and control followed by Mancozeb 50%, Tandem 100% and Tandem 50% (which were at par).

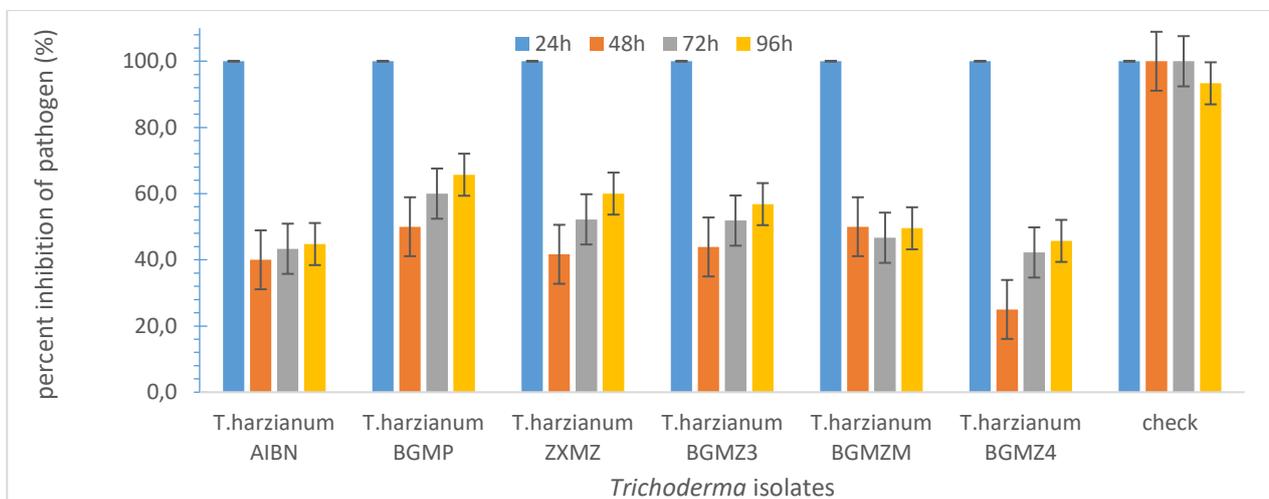


Figure 2. Inhibition of the growth of leaf spot pathogen isolated from groundnut due to the application of six *Trichoderma* isolates.

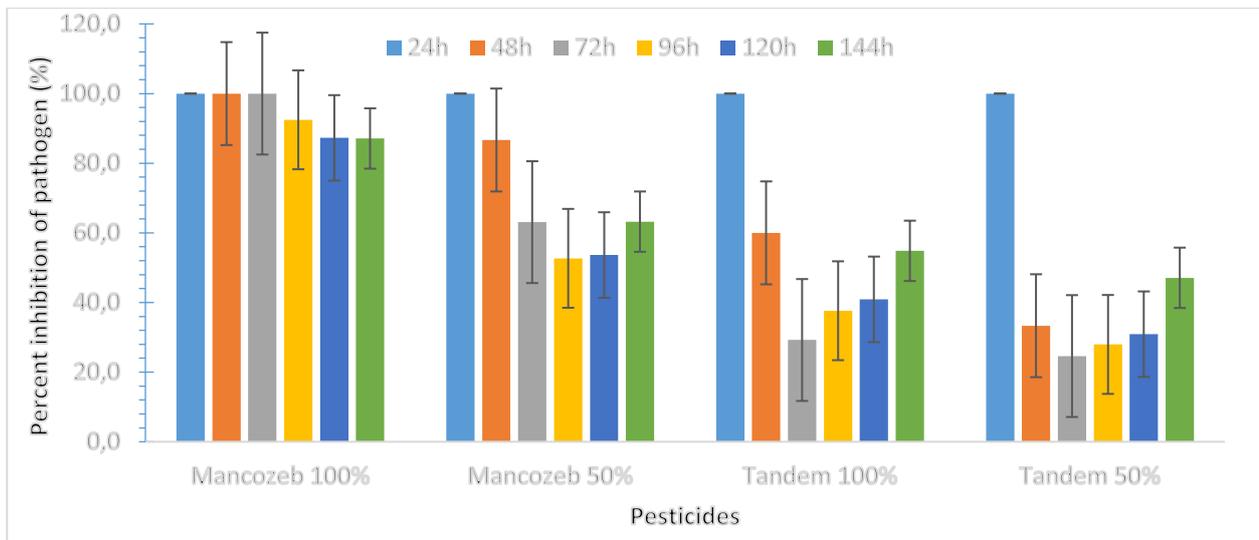


Figure 3. Inhibition of the growth of leaf spot pathogen isolated from groundnut as influenced by the application of two synthetic pesticides.

Discussion

The inhibition of the growth of *C. parasiticum* by *Trichoderma harzianum* isolates corroborated the findings of [Kiran \(2012\)](#) who reported that collar rot or seedling blight or crown rot of groundnut (induced by *Aspergillus niger* and *A. pulverulentum*) could be controlled using seed treatment applications of either *Trichoderma viride* or *T. harzianum* (4 g/kg seeds) preferably in combination with organic amendments such as castor, neem or mustard cakes (500 kg/ha).

Likewise, [Ndifon \(2022\)](#) utilized biocontrol agents (i.e. *Trichoderma* and *Cladosporium* spp.) to inhibit the mycelial growth of *Globisporangium ultimum*; (the causal agent of groundnut pod rot) which corroborated the findings of this present study. Also, [Apet et al. \(2015\)](#) reported that *Ceratocystis paradoxa* was controlled using *Trichoderma viride*, *T. harzianum*, and *T. harmatum*.

Meanwhile, [Zandoná et al. \(2019\)](#) recommended that fludioxonil combined with *Trichoderma* spp. and Biozyme® may be combined and used for treating soybean seeds. While, [Vargas-Inciarte et al. \(2019\)](#) reported that *T. koningiopsis*, *T. virens*, *T. harzianum*, and *T. spirale* effectively controlled *Fusarium* wilt *in vivo* in the greenhouse.

Thus the findings of [Ndifon \(2022\)](#), [Apet et al. \(2015\)](#), [Zandoná et al. \(2019\)](#), and [Vargas-Inciarte et al. \(2019\)](#) affirmed the findings in this study based on application of biocontrol agents against *C. parasiticum*.

Mancozeb 100% (contact fungicide) and Tandem® (i.e. Cu (I) O + Metalaxyl, it is a systemic fungicide) both produced significantly higher inhibition of *C. parasiticum* growth than the control. These findings confirmed the findings of [Ndifon \(2022\)](#) who reported that a commercial fungicide (i.e. mancozeb+carbendazim) inhibited the mycelial growth of *Globisporangium ultimum* more than Mancozeb (at 50% and 100% concentrations). These findings also affirmed those of

[Kiran \(2012\)](#) reiterated that tikka disease and rust disease should be controlled using foliar sprays of mancozeb. While *Alternaria* leaf spots should be managed using foliar sprays of copper oxychloride and mancozeb.

In the current study, copper (I) oxide plus metalaxyl caused less inhibition of *C. parasiticum* compared to the percentage inhibition of growth caused by mancozeb. This corroborated the findings of [Ndifon and Lum \(2021\)](#) who reported that the level of inhibition of the pathogen's growth was more when Mancozeb® was applied compared to application of Tandem®. Moreover, [Apet et al. \(2015\)](#) reported that *Ceratocystis paradoxa* infecting sugarcane was controlled using systemic fungicides (viz Carbendazim, Propiconazole and Hexaconazole) which caused higher average mycelial growth inhibition followed by non-systemic fungicides (viz Thiram and Captan). These numerous chemical pesticides should be used when farming to delay/avoid development of resistance to over-used chemicals.

Finally, [Woodward et al. \(2013\)](#) reported that the following fungicides: copper dust, sulfur dust, benomyl, chlorothalonil, triazoles, strobilurins, carboximides, propiconazole, cyproconazole, tebuconazole, and propiconazole; have been utilized effectively against leaf spot diseases on groundnuts. While [Ziezold et al. \(1998\)](#) also utilized several fungicides (the best fungicides were Benlate (benomyl), Thiram (thiram), Orbit™ (propiconazole). Crown™ (carbathiin and thiabendazole), fluazinam, and UBI-2584 (tebuconazole) while the less toxic fungicides were UBI-2643 (thiabendazole), UBI-2565 (cyproconazole), and Vitaflo-280 (carbathiin and thiram) to control disappearing root rot of ginseng (*Panax quinquedius*) caused by *Cylindrocarpon destructans*.

Inhibition by all the plant extracts (in this current study) was significantly higher than that of the control which corroborated the statement by [Hashim and Devi](#)

(2003) that plants may contain antimicrobial secondary metabolites that can be used to control pathogens. For instance, [Koita et al. \(2017\)](#) in Burkina Faso obtained a significant increase in groundnut pod yield. [Awurum and Uwajimgba \(2013\)](#) reduced *Fusarium oxysporium* wilt and [Soumya and Bindu \(2012\)](#) successfully controlled groundnut seed pathogens (*Aspergillus niger*, *A. flavus*, *Penicillium* sp., and *Rhizopus* sp) using plant extracts which agreed with the findings of this current study.

The findings of the present study corroborated those of [Ndifon \(2022\)](#) who successfully utilized plant extracts (i.e. *Parkia biglobosa* (African locust bean tree), mango, shea butter tree, and pawpaw plant tissues) to control *G. ultimum*. Also, this corroborated the findings of [Ndifon and Lum \(2021\)](#) who inhibited the mycelial growth of *Aspergillus niger* using aqueous extracts from five plants (i.e., leaves of *Eucalyptus globulus*, *Melaleuca cajuputi*, *Andrographis paniculata* as well as *Azadirachta indica*, and shoots of *Euphorbia hirta* at 50 and 100% concentrations).

To cap it all, we note that initially, [Ndifon et al. \(2015\)](#) utilized *Eucalyptus* sp., cashew, shea butter leaves, *Erythrina* sp., ginger, and garlic plant extracts to control *Fusarium oxysporium* f.sp. melongenae. Similarly, [Apet et al. \(2015\)](#) reported that *Ceratocystis paradoxa* was controlled using *Allium sativum*, *Zingiber officinale*, and *Azadirachta indica*, which is in agreement with the findings reported herein that botanicals effectively controlled *C. parasiticum*.

Still, on botanicals, [Niren and Nakul \(2019\)](#) utilized aqueous extract of eight botanicals including *Eucalyptus globulus* to inhibit the growth of *Thielaviopsis paradoxa* and *Curvularia lunata* from mango and banana. While [Rongai et al. \(2015\)](#) controlled *Fusarium oxysporium* f.sp. lycopersici using 24 plant extracts, among which were *Punica granatum* and *Salvia guaranitica*. [Suranjit et al. \(2018\)](#) used nine botanicals (including garlic) to control *Sclerotium rolfsii*. These three reports above were in complete agreement with the notion that plant extracts can act as alternative pesticides against fungi and other microbes.

Conclusion

The presence of pathogenic fungi on groundnut has a detrimental effect on the production of this important oil-seed and grain legume crop. *In vitro* trials were set up to ascertain the veracity of using *Trichoderma harzianum* isolates, mancozeb and tandem as well as plant extracts from *Parkia biglobosa*, *Casuarina equisetifolia*, and *Rhoeo spathacea* to manage the *Cylindrocladium parasiticum*. It was proven that *C. parasiticum*, from groundnut can be controlled with this biocontrol, botanical and synthetic pesticides. These pesticides were therefore recommended while work continues to improve the performance of these control agents.

Conflict of Interests

There is no conflict of interests at all.

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