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Inhibitory efficacy of selected botanical, microbial and synthetic pesticides against *Colletotrichum alatae,* causing water yam anthracnose disease

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Abstract. *Ndifon EM. 2023. Inhibitory efficacy of selected botanical, microbial and synthetic pesticides against* Colletotrichum alatae*, causing water yam anthracnose disease. Cell Biol Dev 7: 41-46.* Yam (*Dioscorea* spp.) ranks first followed by cassava and sweet potato among root and tuber crops in sub-Sahara Africa. However, yam production is constrained mainly by diseases caused by fungi, bacteria, and nematodes. The aim of this research was to propose some practicable botanical, chemical, and biocontrol measures appropriate to the management of *Colletotrichum alatae,* causing tuber rot disease in water yam. All the experiments were conducted using a completely randomized design. The results revealed that Carbendazim+Mancozeb (50% concentration), Mancozeb+Cu(I)O+Metalaxyl (100% concentration), and Mancozeb (100% concentration) significantly ($P \leq 0.05$) inhibited the pathogen compared to the other treatments. *Trichoderma harzianum* isolate AIM16 showed significantly ($P \leq 0.05$) better inhibition of *C. alatae*. *T. harzianum* isolate NSBM, *T. harzianum* isolate BGMZ4, and *T. harzianum* isolate AIM3 were significantly ($P \leq 0.05$) better that the control. Extract of *Eucalyptus globulus* L. (100% concentration) showed the best inhibitory ability and was significantly different ($P \leq 0.05$) from other treatments, followed by *E. globulus* (50%), *Guieria senegalensis* JF Gmel. (100%), *Bauhinia purpurea* L. (100%), *Cymbopogon citratus* (D.C.) Stapf. (100%). A 50% concentration of *Guieria*, *Bauhinia*, and *Cymbopogon* species also effectively controlled the pathogen. All plant extracts inhibited *C. alatae* significantly ($P \leq 0.05$) more than the control. It was concluded that *C. alatae* can be successfully managed using any of the three strategies.

Keywords: Anthracnose, antifungal activity, *Colletoticrum alatae*, plant extracts, *Trichoderma* species, water yam

INTRODUCTION

West Africa (mainly Benin Republic, Ghana, Ivory Coast, Nigeria, and Togo) is responsible for approximately 72.39 million metric tonnes of yam tubers (95%) out of 72.6 million metric tonnes of yam tubers produced per annum globally (FAOSTAT 2018). Among root and tuber crops in sub-Saharan Africa, yam ranks first, followed by cassava and sweet potato (Amienyo and Ataga 2007; FAOSTAT 2008). Moreover, the production of water yam or *Dioscorea alata* L. (which is one of the top three most important yam species) is quite profitable; for instance, its production resulted in a gross margin and net farm income of ₦1 893 114 and ₦1 705 965, respectively in Nigeria (Nwike et al. 2017). Nigeria is the world's leading yam producer, accounting for more than 47.19 million metric tonnes of yam tubers (65%) out of 72.6 million metric tonnes of global yam tuber yield per annum (FAOSTAT 2018).

Dioscorea rotundata Poir. and *D. alata* are the most widely cultivated yam species in the world. The production of water yam is worthwhile because it does well in inferior soils compared to *D. rotundata* and actually produces more than half the quantity of yam tubers produced by white yam in such dismal situations. White yam has about 78% carbohydrates, and water yam contains about 75.65% carbohydrates (Asiedu et al. 2003; Ezeocha and Jimelukwe 2012).

Water yam has a low glycemic index and no fat, so it is often recommended for diabetic patients and those wishing to lose weight (Nestor et al. 2009; Itam et al. 2012). Furthermore, water yam exhibits ease of propagation (through the production of bulbils and reliability of sprouting), early vigor for weed suppression, and good storage ability (Asiedu et al. 2003). However, water yam's market value is lower than white yam's, particularly due to its less desirable pounding features. But water yam can be used for porridge, yam fritters, pounded yam "fufu" (i.e., yam loaves), grated water yams, and yam mixed with vegetables, especially in Nigeria. The main disadvantage of water yam is that it takes ten months to mature compared to the four to six months required to grow white yam (Oko and Famurewa 2015). This may reduce the areas where water yam can be grown.

Yam production is constrained mainly by diseases, pests, the cost of seed yams, labor, and difficulty of breeding (Sangoyomi 2004; Onuh et al. (2015). For instance, fungi, bacteria, and nematode storage rots cause the greatest yam yield losses. Ikotun (1983) reported that fungi pathogens caused 80% of storage rots of yam tubers in the West Indies and 57-77% in Nigeria. Among the diseases, *Colletotrichum gloeosporioides* causes anthracnose disease on water yam. It was reported that 95%

of the yam farmers in Ghana suffered poor yield of water yam due to anthracnose disease. The *D. alata* is the most susceptible yam species to anthracnose disease (Amusa 1997). Meanwhile, in West Africa, yam anthracnose is caused by *Colletotrichum alatae* (in *C. gloeosporioides* species complex), and it results in about 50-90% tuber yield loss (Emehute et al. 1998; Weir et al. 2012; Bhattacharjee et al. 2018; Ntui et al. 2021).

Therefore, to ensure profitable cultivation of water yam, research on control of these diseases, especially anthracnose, is highly sort after. Okigbo (2005) reported that yam anthracnose is controlled mostly with chemical fungicides, including benomyl (benlate), maneb, chlorothalonil, and mancozeb. In comparison, the combinations of copper fungicides and other synthetic fungicides like mancozeb are very effective (Johnson and Hofman 2009). But, a few years ago, Bhattacharjee et al. (2018) lamented that there are no economically feasible solutions to these disease problems, as control of diseases through chemical pesticides or cultural practices is often not convenient for small-holder resource-poor farmers. However, chemical pesticide use is still the most effective for most farmers. Onuh et al. (2015) used aqueous extracts of nine plants (fresh and dry plant materials) to control diseases in yam. Generally, fresh plant extracts showed more antimicrobial activity than dry plant extracts. Also, Okigbo (2005) reported that yam tubers inoculated with *Bacillus subtilis* showed no rot, while those inoculated with *Aspergillus niger, Botryodiploidia theobromae,* or *Penicillium oxalicum* showed considerable rot. He reported that inoculating the yam with *B. subtilis* (antagonist) and pathogens simultaneously resulted in rot symptoms. In contrast, those tubers that received *B. subtilis* a day before the fungal pathogen was inoculated resulted in completely rot-free tubers.

Okigbo and Emeka (2010) reported that *Trichoderma harzianum, Pseudomonas syringae,* and *Pseudomonas chlororaphis* reduced rot caused by *Botryodiplodia theobromae* and *Fusarium solani* by 53.5-84.0% and 59.6-87.1%, respectively. The research on managing water yam diseases has barely taken off among yam species. This research proposed some practicable botanical, chemical, and biocontrol measures appropriate to managing *C. alatae* tuber rot disease of water yam.

MATERIALS AND METHODS

Study site

The research was conducted at the Faculty of Agriculture Laboratories in Alex Ekwueme Federal University, Ndufu-Alike at Abakalik, Nigeria (6.069°N, 8.199°E). Yam (including water yam) production is a major agricultural activity in South-Eastern Nigeria, including Abakaliki.

Procedures

Sub-procedure-1: Isolation and identification of the fungi

Infected yam tubers were obtained from the main market of Abakaliki. Yam tubers were surface sterilized using 1.0% sodium hypochlorite solution for five minutes and then washed three times in sterile distilled water. Yam tubers were cut into 1 cm pieces (both infected and healthy tissues together), placed in a Petri dish containing PDA medium, and then incubated at 28°C for five days.

Trichoderma isolates were obtained from mushrooms, crop seeds, and farmland soils collected from south-eastern Nigeria and West Cameroons. Pure cultures of isolated *Trichoderma* spp. and *C. alatae* were maintained on dehydrated commercial Potato Dextrose Agar (PDA) medium.

The pure cultures were used to identify the fungi with the aid of literature on fungi morphology (Barnett and Hunter 1972).

Sub-procedure-2: Control of C. alatae *using* T. harzianum *isolates* in vitro

The experiment was laid out in Petri dishes using a completely randomized design, and each treatment was replicated three times. The treatment set consisted of *T. harzianum* isolate NSBM, *T. harzianum* isolate AIM3, *T. harzianum* isolate AIM16, *T. harzianum* isolate BGMZ4, and the control. The Control treatment was inoculated with *C. alatae* isolate alone. A 2-mm disc of the pathogen and a 2-mm disc of the biological control agent were placed at the edge of the plate according to the layout.

Sub-procedure-3: Effect of synthetic pesticides on the growth of Colletotrichum alatae in vitro

The experiment was carried out using Petri dishes. It was laid out in the laboratory using a Completely Randomized Design (CRD) with seven treatments, and each treatment was replicated three times. The treatment set included the control, mancozeb (at 100% concentration), mancozeb (at 50% concentration), Carbendazim+Mancozeb (at 100% concentration) and Carbendazim+Mancozeb (at 50% concentration), Mancozeb+Cu(I)O+Metalaxyl (at 100% concentration), Mancozeb+Cu(I)O+Metalaxyl (at 50% concentration) (Ndifon and Lum 2021; Ndifon 2022).

The Carbendazim+Mancozeb (at 50% concentration) and Mancozeb+Cu(I)O+Metalaxyl (at 50% concentration), and Mancozeb (at 50% concentration) treatments were composed by combining half the recommended rate of each pesticide.

The formulation of the synthetic fungicides was carried out thus. For Mancozeb+Cu(I)O+Metalaxyl (at 50% concentration), where there are three pesticides and mancozeb at 50%; it should be 33.33% on average of each agent. Also, at Carbendazim+Mancozeb (at 100% concentration), there are two pesticides thus 50.0% of each agenty was used, while mancozeb agent alone as a treatment at 50% concentration it was 50% of the agent.

Each treatment consisted of three levels (i.e. control 0.0, 50, and 100% concentrations) and was applied to the Petri dishes according to the layout. The in vitro rates were drawn (after obtaining the standard recommended field fungicide quantities) at 0, 50, or 100 µL per Petri dish as required.

Sub-procedure-4: Effect of plant extracts on the growth of Colletotrichum alatae in vitro

The experiment was carried out using Petri dishes. It was laid out in the laboratory using a Completely Randomized Design (CRD) with seven treatments, and each treatment was replicated three times. The treatment set included the control, *Eucalyptus globulus* L. leaves (at 50 and 100% concentrations), *Guieria senegalensis* JF Gmel. leaves (at 50 and 100% concentrations), *Bauhinia purpurea* L. leaves (at 50 and 100% concentrations), and *Cymbopogon citratus* (D.C.) Stapf. leaves (at 50 and 100% concentrations). The plant leaves utilized were weighed at 333.3 g plant leaves per L of distilled water to make 100% concentration. The 50% concentration of plant leaf extract was made by diluting one liter of 100% plant extract with one liter of distilled water. Each treatment had three levels (i.e. control 0.0, 50, and 100% concentrations). The plants were identified at National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Zaria, Nigeria.

Data collection

The radius of the fungal colony was measured using a transparent rule at 24-hour intervals starting from Day 1 (i.e., 24 Hours After Inoculation (HAI)) to Day 8. The percentage inhibition of the pathogen was calculated using the equation:

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

Where,

PI = Percentage inhibition of the growth of the fungus pathogen

C = Perpendicular* radius of the colony of the fungal pathogen in the control plate

T = Perpendicular radius of the colony of the fungal pathogen in the treated plate

*Perpendicular refers to the 'right angle' distance through the center of the dish because other radii could be obtained, especially the longest radius away from the source/front of inhibition.

Data analysis

The data were subjected to Analysis of Variance (ANOVA), and the means were separated using Student Newmann Keul's (SNK) method (as obtainable with Genstat® Discovery (Second Edition) statistical package). Descriptive statistics were used to illustrate the trends in the growth of the pathogen and its management (as obtainable with IBM Statistical Package for Social Sciences (version 25).

RESULTS AND DISCUSSION

Results

The inhibitory effect of synthetic fungicides on *C. alatae* in vitro is shown in Figure 1. The result shows that mancozeb (at 100% concentration) for the first two days had better inhibition of *C. alatae* followed by Mancozeb+Cu(I)O+Metalaxyl (at 50% concentration), Carbendazim+Mancozeb (at 50% concentration), and mancozeb (at 50% concentration). All the fungicides inhibited *C. alatae, ranging* between 8.0-76.0% inhibition.

The effect of synthetic fungicides on the growth rate of fungi is presented in Figure 2. The first day of the control shows an erratic trend, whereby some synthetic fungicides did not inhibit the pathogen more than the control. However, synthetic fungicides showed better inhibition of C. alatae than the control during the second and third growth intervals. The trend was virtually the same in the fourth growth interval, except that mancozeb (at 50% concentration) was less efficient at this stage. Based on the means separation using SNK, Carbendazim+Mancozeb (at 50% concentration), Mancozeb+Cu(I)O+Metalaxyl (at 100% concentration), mancozeb (at 100% concentration) were significantly ($P \leq 0.05$) better than all the other treatments at inhibition of the pathogen. Mancozeb (at 50% concentration) was significantly superior to Mancozeb+Cu(I)O+Metalaxyl (at 50% concentration), Carbendazim+Mancozeb (at 100% concentration), and the control.

The inhibitory effect of four *Trichoderma* isolates on *C. alatae* in vitro is shown in Figure 3. It was observed that *T. harzianum* isolate BGMZ4 was more consistent and stable in inhibiting *C. alatae*. On the first day, *T. harzianum* AIM 3 was the best isolate, while on the second day, *T. harzianum* NSBM was the best isolate, and isolate AIM 16 was the best isolate on Day 3. The results of using isolates of *T. harzianum* against *C. alatae* ranged between 16-52% inhibition. Results revealed that *T. harzianum* isolate AIM3 could not inhibit the pathogen better than the control during the first growth interval. At the same time, *T. harzianum* isolate NSBM also could not inhibit the pathogen sufficiently. During the third and fourth growth intervals (for all the isolates), the bio-control agents could inhibit the pathogen more than the Control (Figure 4).

The means separation shows that *T. harzianum* AIM16 exhibited significantly ($P \leq 0.05$) better in controlling the pathogen, followed by *T. harzianum* NSBM, *T. harzianum* BGMZ4, and *T. harzianum* AIM3. The inhibitory effect of plant extracts on *C. alatae* in vitro is shown in Figure 5. The result shows that *E. globulus* (at 50 and 100% concentration) followed by *G. senegalensis* (at 100% concentration) caused better inhibition of *C. alatae* compared to the other treatments. Generally, plant extracts showed acceptable inhibition of *C. alatae,* ranging between 8.0-96.0%. All plant extracts showed inhibitory efficacy against *C. alatae* compared to the control.

The effect of plant extracts on the growth rate of *C. alatae* is presented in Figure 6. The *E. globulus* (at 50 and 100% concentrations) effectively reduced the growth rate of *C. alatae*, followed by *G. senegalensis* (at 100% concentration). The means separation shows that the extract of *E. globulus* 100% was significantly different ($P \leq 0.05$) from the other treatments and showed the best inhibitory potential, followed by *E. globulus* (at 50% concentration), 100% concentrations of *G. senegalensis*, *B. purpurea*, and *C. citratus*. However, all plant extracts (at 50 and 100% concentrations) were significantly different ($P \leq 0.05$) compared to the control.

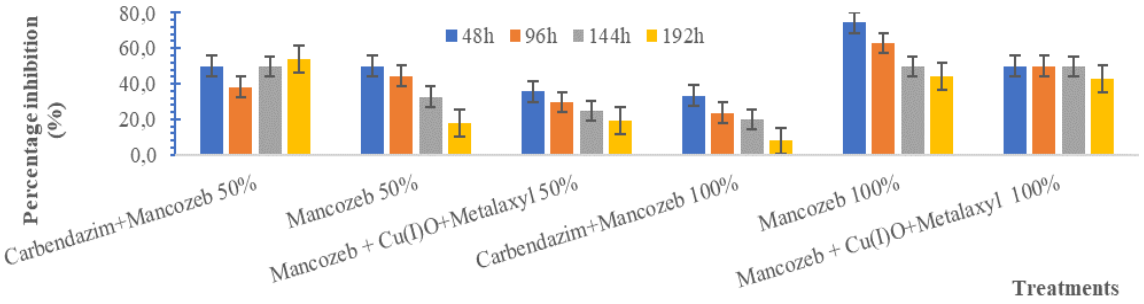

Figure 1. The inhibitory effect of synthetic fungicides on *Colletotrichum alatae*

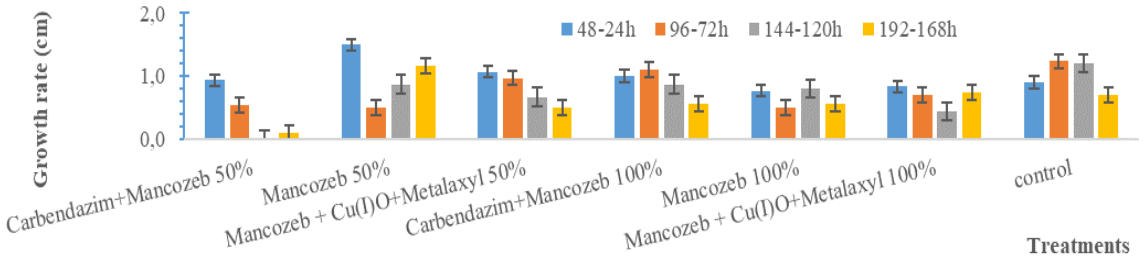

Figure 2. The effect of synthetic fungicides on the growth rate of *Colletotrichum alatae* in vitro

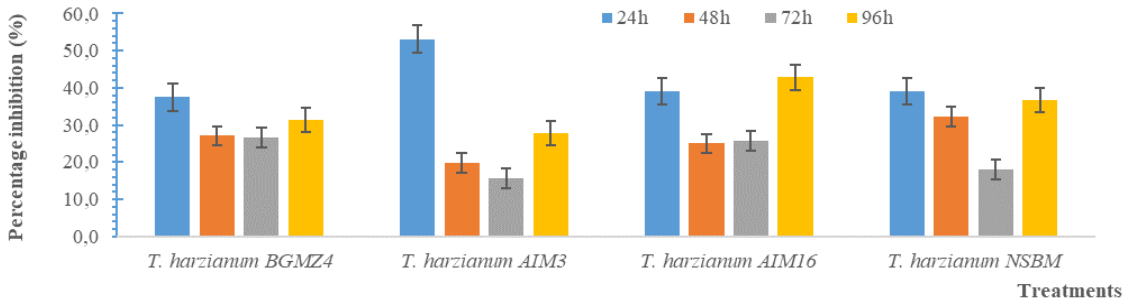

Figure 3. The inhibitory effect of *Trichoderma* isolates on *Colletotrichum alatae* in vitro

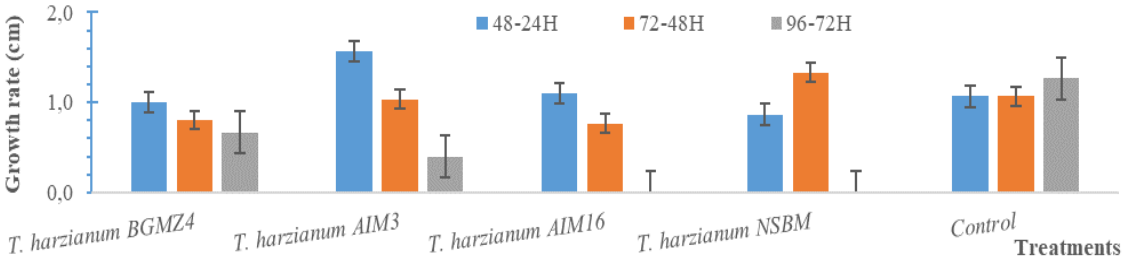

Figure 4. The effect of *Trichoderma* isolates on the growth rate of *Colletotrichum alatae* in vitro

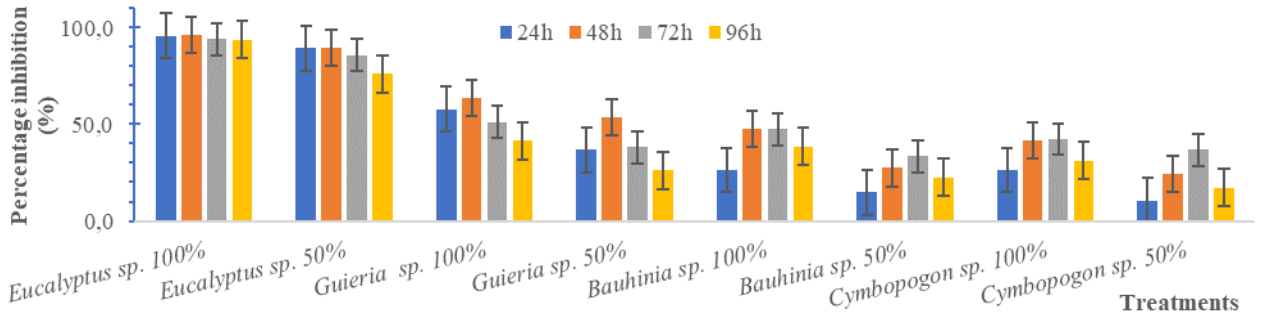

Figure 5. The inhibitory effect of plant extracts on the mycelia of *Colletotrichum alatae* in vitro

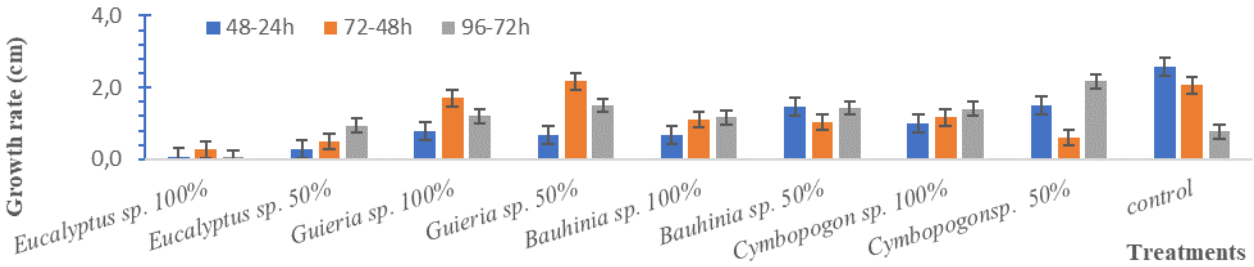

Figure 6. The effect of plant extracts on the growth rate of *Colletotrichum alatae* in vitro.

Discussion

Globally the production of *D. alata* is quite profitable, especially in Nigeria, the world's leading producer of yams (65% of 72.6 million metric tonnes of yam tubers produced per annum globally). Crop production is hampered by pests and diseases, including anthracnose in yam caused by *C. alatae*. The results of the sub-trial on the use of low rates of synthetic pesticides revealed that they could reduce the growth of *C. alatae,* which is in line with the findings of Ndifon and Lum (2021) and Ndifon (2022). Mancozeb alone showed better control potential than other fungicide treatments; this corroborated the findings of Ndifon and Lum (2021). Furthermore, Ndifon (2022) observed that the inhibition level of A. niger and *Athelia rolfsii* was more when mancozeb was applied than Mancozeb+Cu(I)O+Metalaxyl.

The result demonstrated that applying biocontrol agents against *C. alatae* was effective. These results followed the findings of Ndifon (2022), who successfully inhibited pathogenic *A. rolfsii* using *Trichoderma* and *Cladosporium* spp. Okigbo and Emeka (2010) also reported that biocontrol agents (such as *Trichoderma* and *Pseudomonas* spp.) showed promising potential against fungal pathogens.

In this present experiment, all four plant extracts (*E. globulus, G. senegalensis, B. purpurea*, and *C. citratus*) effectively inhibited the growth of *C. alatae*. Among them, *Eucalyptus* sp. plant extract was very effective against the growth of *C. alatae*. This finding is consistent with the results of Ndifon and Lum (2021), who reported that leaf extracts (i.e., *E. globulus, Melaleuca cajuputi* Powell, *Andrographis paniculata* (Burm.fil.) Nees, and *Azadirachta indica* A. Juss.) and the extract of shoots (i.e., *Euphorbia hirta* L.) inhibited the growth of *A. niger* significantly; Onuh et al. (2015) also successfully utilized plant extracts to control pathogenic fungi. Dania et al. (2014) reported that (in vitro and in vivo*)* plant extracts of *Oryza sativa* L. and *Quercus phillyraeoides* A. Gray were able to control *Lasiodiplodia theobromae, A. niger, Rhizoctonia solani, P. oxalicum, Sclerotium rolfsii,* and *Fusarium oxysporum* associated with white yam.

The results of the present study concluded that *C. alatae* was successfully inhibited in vitro using the four *Trichoderma* isolates (AIM 3, AIM 16, BGMZ4, and NSBM). The *C. alatae* was also effectively inhibited using synthetic fungicides like Mancozeb, Carbendazim+ Mancozeb, and Mancozeb+Copper(I) oxide+Metalaxyl. All the plant extracts (*E. globulus, G. senegalensis, B. purpurea,* and *C. citratus*) effectively controlled *C. alatae*. Thus the selected fungicides, plant extracts, and biocontrol agents could control the growth of *C. alatae.* Further research needs to be carried out to determine the effects of these control strategies in vivo.

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