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ANTIFUNGAL ACTIVITIES OF SELECTED PLANT EXTRACTS IN IN-VITRO CONTROL OF ANTHRACNOSE AND ROOT ROT DISEASES ON CUCUMBER (*Cucumis sativus B.*)

Wuraola Funmi Ogundipe[1+]
Ayotunde Ajoke Pitan[2]
Oluwafemi Michael Adedire[3]
Adekunle Opeyemi Farinu[4]
Barry Oyeyemi Oyewole[5]

[1]*Federal College of Agriculture, Moor Plantation Ibadan, Nigeria.*
[1]*Email: ogundipewuraola@gmail.com Tel: +2348085295695*
[2,5]*National Horticultural Research Institute, Ibadan, Nigeria.*
[2]*Email: pitanayotunde@yahoo.com Tel: +2348062143983*
[3]*Email: fecky09@gmail.com Tel: +2348034771628*
[3,4]*Federal College of Agriculture, Moor Plantation, Ibadan, Nigeria.*
[4]*Email: wisdom365.ww@gmail.com Tel: +2348147636777*
[5]*Email: oyeebarry@gmail.com Tel: +2348052730539*

(+ Corresponding author)

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ABSTRACT

An *in-vitro* experiment was conducted at National Centre for Genetic Resources and Biotechnology and the Nigeria Agricultural Quarantine Service, Moor plantation, Ibadan. The experiment was carried out to test the antifungal efficacy of some plant extracts (*Ageratum conyzoides*, *Azadirachta indica*, *Morinda lucida*, and *Chromolaena odorata*) and a chemical fungicide (mancozeb). The mycelial growth inhibition potentials of five concentrations of aqueous plant extracts were assayed at different incubation periods on the growth of *Colletotrichum orbiculare* and *Lasiodiplodia theobromae*. The experiment was carried out in a Completely Randomized Design (CRD) with five replications. A 3 mm mycelial disc of each test fungus was placed at the center of a 9 cm Petri dish containing 5, 10, 15, 20, and 25 g of the plant extracts or 0.25g/100mL of mancozeb (synthetic fungicide) in Potato Dextrose Agar. The results obtained revealed that all the plant extracts, at all concentrations, significantly inhibited the growth of these mycopathogens, with 25g *C. odorata* having the highest percentage inhibition of 70.78% and 73.68% at 48 and 96 hours of incubation on *C. orbiculare* and *L. theobromae* respectively. All the selected aqueous extracts inhibited more than 50% *C. orbiculare* mycelial growth. Antifungal extracts recovered from the selected plants could be further purified to improve and characterize their fungicidal activities in controlling plant diseases. Extracts of plant materials, which are readily available to the farmers, are better alternatives to the commonly used hazardous, synthetic fungicides.

Contribution/Originality: This paper's primary contribution is finding that all the plant extracts used against cucumber pathogens had significant inhibitory properties inhibited at different concentrations and incubation periods respectively.

1. INTRODUCTION

Cucumber in Nigeria is fast becoming one of the important vegetables produced, being a source of proteins, carbohydrates, minerals, vitamins, and fibers (Abbey, Nwachoko, & Ikiroma, 2017). Despite its importance, fungal diseases are one of the factors that affect its production. Many pathogens are known to attack cucumber but *Colletotrichum orbiculare* and *Lasiodiplodia theobromae* are among the most destructive pathogens that attack this vegetable crop. In recent times, a lot of investigations were done on the use of extracts of medicinal plants for controlling many phytopathogens (Bobbarala, Katikala, Naidu, & Penumajji, 2009). Most plant constituents such as alkaloids, glycosides, terpenes, terpenoids, and different flavonoids have been reported to show high potential effects against plant diseases which were extracted from plants such as *Laurus camphora* L., *Peganum harmala* L., *Zygophyllum coccineum* L. and *Cymbopogon nardus* C. (Abeer, Hoballah, Abdel-Halim, & Sanaa, 2017). Chemical fungicides have been employed over the years for the control of fungal diseases to increase crop production. Unfortunately, its overzealous and indiscriminate use has created different environmental and toxicological problems and the occurrence of resistant strains of pests (Gurjar, Ali, Akhtar, & Singh, 2012). This led to the development and utilization of newer approaches, including the use of botanical pesticides which are eco-friendly. Pesticides obtained from plants are effective in the management of plant pathogens and biodegradable, thereby supporting both crop production and the environment than synthetic fungicides (Utobo, Ekwu, Nwogbaga, & Nwanchor, 2016). Phytochemicals present in these botanical pesticides have proved to have inhibitory effects on all types of phytopathogens (Gurjar et al., 2012). Therefore, this present study was designed to investigate the efficacy of aqueous extract of different plant materials at various concentrations and incubation periods on the *in-vitro* control of *Colletotrichum orbiculare* and *Lasiodiplodia theobromae.*

2. MATERIAL AND METHODS

2.1. Sample Collection and Isolation of Fungal Isolates

The plants used in this study (*Ageratum conyzoides*, *Azadirachta indica*, *Morinda lucida*, and *Chromolaena odorata*) were obtained from the Federal College of Agriculture, Ibadan. Cucumber leaves showing various disease symptoms of anthracnose were obtained from the cucumber field at Abeere, Ede North local government, and Isale Osun, Osogbo local government area of Osun State, Nigeria. The infected leaves were collected in sterile bags and taken to the laboratory for isolation and identification of pathogens. Infected leaves were dissected with a sterile scalpel at the interphase between the healthy and necrotic portions of the leaves, surface sterilized with 2 mL sodium hypochlorite solution for 2 min, and rinsed in four successive changes of sterile distilled water (SDW) (Gwa & Nwankiti, 2017). Disinfected tissues were blotted with sterile filter paper for 2-3 min in the laminar airflow cabinet to dry, aseptically plated on Petri dishes containing acidified sterile potato dextrose agar (PDA), and incubated at room temperature for 7 days.

2.2. Characterization and Identification of Fungal Isolates

Pure cultures were obtained following the sub-culturing of the growing fungi after 7 days of incubation. Microscopic examination and morphological characteristics were noted and compared with existing authorities (Burgess, Knight, Tesoriero, & Phan, 2008).

2.3. Pathogenicity Test

The experiment was carried out according to Dania, Fadina, Ayodele, and Kumar (2015) using, completely randomized design, replicated five times. Planting pots with the dimension 21.5 cm x 27 cm were filled with 7 kg of sterilized soil; three seeds of each variety of cucumber were sown in each pot and later thinned to 2 seedlings per pot. After three weeks of planting, 6.0 x 10^6 spore/mL suspension of the fungal isolates was sprayed on cucumber seedlings. Six days after inoculation, plants that showed symptoms of infection were collected and taken to the laboratory for further evaluation.

2.4. Preparation of Plant Extracts

The plant extracts were prepared according to the methods described by Tohamy, Aly, Abd-El-Moity, Atia, and Abed-Rl-Moneim (2002). *Azadirachta indica*, *Ageratum conyzoides*, *Morinda lucida*, and *Chromolaena odorata* leaves were washed thoroughly with cold running tap water, air-dried to constant weight at room temperature, and separately grounded into a fine powder using a Warring blender. Different weights of each plant powder (5 g, 10 g, 15 g, 20 g, and 25 g) were soaked in 100 mL of sterilized distilled water for 24 hours and subsequently filtered through a four-fold of sterile cheese-cloth and 1.25 mm Whatman filter paper. The filtrates were used as the plant extracts in the experiment. Also, 0.25 g of Mancozeb (fungicide) was added to 100 mL of distilled water. Thereafter, 1 mL of each concentration of the extracts or chemical fungicide was added to sterilize PDA.

2.5. Effect of Plant Extracts on Mycelial Growth of Fungal Isolates

To evaluate the fungi toxic effect of the plant extracts and the chemical fungicide on fungal mycelial growth, cool (about 45ºC), molten PDA medium with the plant extracts were mixed and allowed to solidify before the inoculation of fungi (Gwa & Nwankiti, 2017). The experiment was carried out using a completely randomized design with 5 replications. To create a point of intersection that would indicate the center of the plates, two perpendicular lines were drawn at the bottom of the Petri dishes to generate four equal sections on each plate (Amadioha & Obi, 1999). Three milliliters of each plant extract and chemical fungicide, at different levels of concentrations, were poured into Petri dishes containing 9 mL of the sterilized PDA, properly swirled, and allowed to solidify (Nene & Thapilyal, 2002). Five - millimeter mycelial disc of the test fungi (pure cultures) were thereafter inoculated at the point of intersection drawn at the bottom of the plate. Sterile PDA without plant extracts and chemical fungicide served as the control treatment. The inoculations were incubated at room temperature (25±2ºC) and the growth was monitored. The mycelial radial growth of the fungal isolates was recorded at 48, 72, 96, 120, and 144 hours after inoculation.

Fungal inhibition was determined as percentage growth inhibition (PGI) according to the method described by Iwuagwu, Onejeme, Ononuju, Umechuruba, and Nwogbaga (2018).

$$PGI = \frac{R - R1}{R} \times 100$$

Where,

PGI = Percent Growth Inhibition.

R = the distance (measured in mm) from the point of inoculation to the colony margin in the control plate.

R1 = the distance of fungal growth from the point of inoculation to the colony margin in the treated plate.

2.6. Data Analysis

Data collected were analyzed using the analysis of variance (ANOVA) at $P \leq 0.05$ and mean separation was done using Duncan multiple range test.

3. RESULTS

Results of the inhibitory effect of different concentrations of selected plant extracts on cucumber pathogens revealed that mancozeb, the synthetic chemical used in this study, completely inhibited the growth of the pathogens. All the plant extracts (*C. odorata*, *M. lucida*, *A. conyzoides*, and *A.* indica) significantly exhibited antifungal properties against the fungi. The results revealed that the higher the concentration of the plant extracts, the higher the rate of inhibition of the mycelia growth of these pathogens. Table 1 shows the results of different plant extracts on mycelia growth of *C. orbiculare* at different concentrations. At 96 hours of incubation, the result revealed that 25 g of *C. odorata* had the highest percentage inhibition of the fungus (70.78%) while 5 g at 144 hours incubation had the lowest percentage inhibition (6.69%). Also, at 48 hours of incubation, 25 g of *M. lucida*, *A. conyzoides*, and *A. indica* highly inhibited the growth of the fungus at 52.68%, 63.09%, and 62.33% respectively. This observation was not significantly different from the effect of 20 g extract of *C. orbiculare*. However, 73.68% of 25 g *C. odorata* had the highest growth inhibition effect against *L. theobromae* at 48 hours of incubation, while 6.25% of 5 g extract of the same plant had the lowest growth inhibition Table 2. Also, 25 g of *M. lucida* and *A. conyzoides* at 144 hours of incubation, as well as 25 g of *A. indica* at 72 hours incubation gave the highest growth inhibition of 64.71%, 48.09%, and 41.04%, respectively.

4. DISCUSSION

This study showed that aqueous extract of the *C. odorata*, *M. lucida*, *A. conyzoides*, and *A. indica*, as well as the synthetic chemical (mancozeb), had significant effects on the radial growth of *C. orbiculare* and *L. theobromae* causing anthracnose and root-rot of cucumber. Several reports have documented the effects of plant extracts in controlling many phytopathogenic fungi (Abd-El-Khair & Haggag, 2007; Perez-Sanchez, Infante, Galvez, & Ubera, 2007). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides, and many pharmaceutical drugs (De Billerbeck, Roques, Bessière, Fonvieille, & Dargent, 2001). The efficacy of the inhibitory activities of selected plant extracts was enhanced with an increase in the concentration of extracts. This finding agrees with the report of Mares, Tosi, Poli, Andreotti, and Romagnoli (2004) that a higher concentration of antimicrobial substance showed an increase in growth inhibition. The observation was also in line with the earlier findings of Sidra and Bashir (2012) who reported that a higher concentration of plant extracts induced maximum inhibition on fungal growth. The minimum inhibitory concentration values of the plant extracts, in their report, against the test organism, showed that fungi vary widely in the degree of their susceptibility to antifungal agents.

The results revealed that all the plant extracts inhibited the growth of both cucumber pathogens. This could be as a result of the presence of phytochemical compounds such as mycotoxigenic proteins, steroids, terpenoids, anthraquinones, flavonoids, saponins, tannins, glycosides, glucosinulate, phenols, and alkaloids as reported by Gwa and Nwankiti (2017). *C. odorata* extract produced a higher inhibitory effect on radial growth of the fungal isolates in culture. The findings from the results revealed that all the plant extracts inhibited over 50% growth of *C. orbiculare Chromolaena odorata*, been the plant extract that gave the highest growth inhibition of the isolates, has also been reported by Ngane et al. (2006). They investigated the leaves and some of their fractions against yeasts and filamentous fungi through dilution methods on solid and liquid media, and observed extract from the plant possess significant antifungal properties. The authors reported that both extract and fractions can inhibit *in vitro* growth of *Cryptococcus neoformans*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. This inhibitory property was attributed to the presence of biologically active constituents such as coumarins, flavonoids, phenols, tannins, and sterols through chemical analysis of the crude extract and its fractions (Onifade, 2000). As an allelopathic plant, extracts from all vegetative parts of *Ageratum* conyzoides including leaves have been reported to inhibit the growth of plant pathogens both *in-vitro* and in-vivo. The inhibitory effects observed in this study are also in line with the report of Sidra and Bashir (2012) who observed that the leaf extract of *A. conyzoides* inhibited the growth of *Fusarium solani* by 72% at the highest concentration of 6%. Extracts from this plant have also been documented to possess pharmacological and biocidal activity. It is evident from the present findings that the leaf extract of *A. conyzoides* possesses fungi toxic potentials worth exploiting against diseases caused by plant pathogens. The leaves, bark, and root extract of *Azadirachta indica* has been reported to control *Colletotrichum lindemuthianum* of cowpea recording a 100% inhibition of spore germination and mycelia growth (Gwa & Nwankiti, 2017; Onifade, 2000). Biu, Yusufu, and Rabo (2009) also revealed in their investigation, that the presence of anti-nutrients like saponins, tannins, glycosides, alkaloids, terpenes, and flavenoids in the aqueous extracts of the leaves of *A. indica* is responsible for its inhibitory potentials against mycelia growth of pathogens. Akinbode (2010) also reported that at higher concentrations, the mycelia growth of *Curvularia lunata* was inhibited by the extracts of *Morinda lucida* compared to the control plates.

Table-1. Antifungal activity of plant extracts on the growth inhibition (%) of *Colletotrichum orbiculare* of cucumber at various incubation durations.

Plant Extracts	Concentrations (g/L)	Period of incubation (Hours)				
		48	72	96	120	144
Chromolaena odorata	5g	28.39c	15.47d	13.12d	7.90d	6.69e
	10g	29.65c	29.64c	33.60c	26.39c	24.25d
	15g	34.70c	36.86c	42.74c	30.52c	29.77d
	20g	42.59c	37.37c	44.33c	44.88c	45.99c
	25g	67.51b	69.85b	70.78b	67.68b	65.39b
Morinda lucida	5g	28.39c	33.76bc	41.75b	36.63b	31.44b
	10g	35.96c	36.34bc	42.35b	40.22b	35.28b
	15g	36.91c	38.91bc	43.74b	41.29b	35.95b
	20g	40.06b	41.50b	45.73b	44.35b	38.63b
	25g	52.68b	51.80b	46.92b	48.19b	39.30b
Ageratum conyzoides	5g	36.91c	31.19c	34.39c	23.70c	11.37d
	10g	40.06c	34.28c	37.97c	25.49c	26.09c
	15g	43.22c	36.08c	39.76c	39.50bc	35.95bc
	20g	62.15b	54.90b	47.32b	48.83b	46.49b
	25g	63.09b	57.47b	54.87b	51.89b	48.66b
Azadirachta indica	5g	30.11c	29.10c	31.32c	19.50c	10.17d
	10g	38.23c	31.46c	32.11c	20.95c	20.99c
	15g	43.12c	34.08c	36.15c	38.44b	31.00bc
	20g	60.15b	51.00b	47.10b	42.93b	44.62b
	25g	62.33b	55.40b	49.89b	48.21b	46.10b
Mancozeb	0.25g/100mL	100a	100a	100a	100a	100a

Note: Mean values with similar letter(s) down the column are not significantly different at 5% level of significance by Duncan's Multiple Range Test (DMRT).

Table-2. Antifungal activity of plant extracts on the growth inhibition (%) of *Lasiodiplodia theobromae* of cucumber at various incubation durations.

Plant Extracts	Concentrations (g/L)	Period of incubation (Hours)				
		48	72	96	120	144
Chromolaena odorata	5g	7.42e	6.25d	5.85d	7.33c	11.46c
	10g	26.56cd	7.92d	9.20d	7.62c	12.37c
	15g	35.89c	12.92d	10.37d	7.63c	16.28bc
	20g	44.26c	31.25c	38.63c	32.55b	26.17b
	25g	73.68b	45.83b	55.33b	37.98b	27.08b
Morinda lucida	5g	28.42c	42.29c	38.37c	29.05c	20.59d
	10g	39.79c	50.00b	40.14bc	43.97bc	43.09c
	15g	50.13b	56.07b	50.80b	58.25b	54.85b
	20g	52.71b	56.25b	58.61b	58.25b	58.38b
	25g	56.07b	58.33b	60.92b	63.02b	64.71b
Ageratum conyzoides	5g	20.94b	17.29c	8.17d	4.29d	1.03d
	10g	27.65b	18.13c	9.95d	11.59cd	10.74ab
	15g	32.04b	25.00bc	20.60bc	15.40c	12.35c
	20g	37.99b	33.33b	31.97b	28.57b	25.44c
	25g	37.99b	44.38b	41.39b	45.56b	48.09b
Azadirachta indica	5g	15.48ab	26.46bc	20.60c	23.33bc	19.12c
	10g	17.34c	26.46bc	21.85c	23.81bc	21.62c
	15g	25.68b	29.79bc	24.16c	23.81bc	22.06c
	20g	26.63b	32.71b	27.71c	25.40bc	23.09c
	25g	31.89b	41.04b	40.85b	39.68b	36.32b
Mancozeb	0.25g/100mL	100a	100a	100a	100a	100a

Note: Mean values with similar letter(s) down the column are not significantly different at 5% level of significance by Duncan's Multiple Range Test (DMRT).

5. CONCLUSION

In replacement of synthetic chemicals that pose various side effects on humans, animals, and the environment through their application on plants, the findings from this study suggest a new pathway in developing a potent, affordable fungicidal agent from plants like *Chromolaena odorata*, *Morinda lucida*, *Ageratum conyzoides and Azadirachta indica.* The results from the present study established that all the four plant extracts contained antifungal substances which are significantly toxic to *Colletotrichum orbiculare* and *Lasiodiplodia theobromae* isolated from the infected cucumber. These can be further investigated to develop to fungicides against phytopathogens. However, *C. odorata*, *M. lucida*, *A. conyzoides*, *and A. indica* extracts appeared to be very effective against *C. orbiculare* while *C. odorata* and *M. lucida* were effective against *L. theobromae* at all selected levels of concentrations and durations. The *in vitro* inhibitory activities suggest that these plants have the potentials to control root rot disease on cucumber; it is therefore suggested that their extracts could be further purified to isolate the active components and also screened for their *in vivo* effect on cucumber plant.

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