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

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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 25 g 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, Nwogbagha, & 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).

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PGL = \frac{R - R_1}{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 ≤ 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 62.93% 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 Billebeck, 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 flavonoids 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 |6.39e |
|                      | 10g                  | 29.65c                           | 29.64c|33.60c |26.30c |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                           | 33.76b|41.75b |36.63b |31.44b|
|                      | 10g                  | 35.96c                           | 36.34b|42.35b |40.22b |35.28b|
|                      | 15g                  | 36.91c                           | 38.91b|43.74b |41.20b |35.95b|
|                      | 20g                  | 40.06b                           | 41.50b|45.73b |44.53b |38.63b|
|                      | 25g                  | 52.68b                           | 51.80b|46.92b |48.19b |39.30b|
| *Ageratum conyzoides*| 5g                   | 36.91c                           | 31.19c|34.39c |23.70c |11.37c|
|                      | 10g                  | 40.06b                           | 34.28c|37.97c |25.49c |20.69c|
|                      | 15g                  | 43.22c                           | 36.08c|39.76c |39.50c |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 |34.44b |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                           | 50.06b|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                           | 44.39b|41.39b|45.56b|48.09b|
| *Azadirachta indica* | 5g                   | 15.14ab                          | 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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