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IN VITRO FUNGICIDAL ACTIVITY OF TWO PLANT EXTRACTS AGAINST FIVE PHYTOPATHOGENIC FUNGI OF CUCUMBER (*CUCUMIS SATIVUS* L.) FRUIT

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ABSTRACT

The ethanolic extracts of *Chromolaena odorata* L and *Moringa oleifera* Lam were tested at concentrations of 20, 40, 60, 80 and 100 mg/ml for their *in vitro* fungicidal activities against five phytopathogenic fungi isolated from diseased Cucumber fruits. The pathogens were *Fusarium oxysporum* Schlecht, *Aspergillus niger* Van Tiegh, *Rhizopus stolonifer Ehrenb*. ex. Fr, *Geotrichum candidum* Link and *Mucor micheli* ex Staint – Amans as confirmed by pathogenicity tests. The inhibitory effects of the extracts increased with increase in concentrations. Some of the concentrations reduced the mycelial growth of the pathogens to a significant ($P > 0.05$) level. Very strong fungicidal activity was produced by extracts of *M. oleifera* at 100 mg/ml against all the fungi. The inhibitory effects of *C. odorata* extracts at 20, 40 and 60 mg/ml were greater than those of *M. oleifera* on *A. niger, F. oxysporum* and *M. micheli.* The results of the investigation indicated that plant extracts possess antifungal activity that can be exploited as an ideal treatment for future plant disease management.

KEYWORDS: Phytopathogenic Fungi, Fungicidal Activity, Cucumber Fruit, Plant Extracts

INTRODUCTION

Cucumber (*Cucumis sativus* L) fruits are borne on indeterminate, tendril-bearing vines of subtropical and tropical origin (Robinson and Decker-Walters, 1997). *Cucumis sativus,* originated from northern India. It is a member of the Curcubitaceae family. Valenzuela *et al*. (2005) showed that the crop is widely grown because of the nutritional composition of the fruit.

Cucumber is one of the most popular salad vegetables. It is served and eaten at home or in restaurants especially fast food establishments. Aboloma *et al*. (2009) stated that the seed kernels are occasionally eaten and yield edible oil. The young leaves are cooked as spinach in Indonesia and Malaysia (Anonymous, 2010). Cucumber gives relief from heart burn, acid stomach, gastritis and even ulcer. Bates *et al.* (1990) showed that cucumber fruits are beneficial for those suffering from lung, stomach and chest problems.

Economic losses due to post-harvest diseases may be up to 50 % or even higher in developing countries (Park *et al*., 2008). Cucumber fruits grown for the production and processing industries are seriously affected by post harvest diseases which can cause losses of up to 30 % of the total yield of crops (Agrios, 2005). Attempts have been made to control phytopathogenic fungi through the use of synthetic fungicides. Although synthetic fungicides are effective, the continued use of them has disrupted biological control by natural enemies and led to outbreaks of diseases, widespread development of resistance to various types of fungicides (Park *et al*., 2008), toxicity to non-target organisms and environmental problems (Hayes and Laws, 1991).

Nwankiti *et al.* (1990) highlighted that protective fungicides have been found to be effective in keeping down rot diseases but remarked that the chemicals were expensive and not usually easily available at prices that most farmers in Nigeria could afford. There is also the problem of lack of expertise in the safe handling of the fungicides among most of

the farmers. The use of bio fungicides of plant origin has been suggested by some workers as alternative to use of chemicals in order to counter the potential hazards and pollution problems associated with the use of synthetic chemicals (Amadioha, 2000; Chiejina, 2006). Plant extracts are biodegradable, cheap, readily available and environmentally safer than synthetic chemicals. Fungicidal properties of *Chromolaena odorata* and *Moringa oleifera* on phytopathogens of post harvest fungal rot of cucumber fruits are therefore aimed at in this research. This is to serve as a relative alternative to the use of synthetic chemicals so as to extend the shelf life of cucumber fruits and reduce or eliminate loss due to post harvest rot in a non-toxic way.

MATERIALS AND METHODS

Sources of Materials

The cucumber fruits with symptoms of rot were randomly collected from three different markets in Enugu State, Nigeria. The markets were Nsukka main market, Ogbete main market, Enugu and Artisan market Enugu. The leaves of *M. oleifera* and *C. odorata* were collected within the vicinity of University of Nigeria, Nsukka, while benlate was collected from the department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Identification of the plants was confirmed by Mr. A. Ozioko of the Biodiversity and Conservation Programme, Nsukka.

Isolation and Identification of Fungal Pathogens

Diseased cucumber fruits were randomly collected from each of the markets. The Chiejina (2008) isolation method was used. Thin sections (2mm diameter) were cut from the periphery of diseased cucumber fruits and surface sterilized in 0.1% mercuric chloride for 2–3 mins, after which they were rinsed in three changes of sterile distilled water. The sections were plated in water agar and mycelium was transferred into clean PDA plates. The plates were incubated at room temperature (27 ± 2^0C) for 6–7 days. Subcultures were made aseptically from the plates into similar clean PDA plates and were incubated under similar conditions until pure cultures were obtained. The identification of the isolated fungi was done macroscopically and microscopically. Macroscopic identification was based on observed culture growth patterns and mycelial colour. Small portions of the fungal cultures were teased and mounted in lactophenol in cotton blue on clean slides, covered with clean cover slips and then viewed microscopically. Fungal identification was confirmed with the aid of books by Barnett and Hunter (1999), Alexopoulos *et al*. (2002), Agrios (2005) and Ellis *et al*. (2007).

Pathogenicity Test

Each of the fungal isolates obtained from the diseased cucumber fruits were tested for their ability to cause the same disease condition previously observed in healthy cucumber fruits. Healthy cucumber fruits were washed in sterile distilled water and surface sterilized by dipping into 0.1% mercuric chloride and with the aid of a sterile cork borer, cylindrical cores were removed from each of the cucumber fruit. Pure cultures of the isolates were introduced into the open cores and the cores were replaced and sealed with sterile petroleum jelly. The fruits were kept at room temperature for 7 – 10 days. On establishment of disease condition, inocula were taken from the infected cucumber fruits and cultured. The organisms were re-isolated and identified as the previously isolated organisms. This was taken as evidence that they incited the disease.

Preparation of Plant Materials

The leaves of *M. oleifera and C.odorata* were dried under a favourable room temperature of (27 ± 2^oC). The drying was done by spreading the leaves on the floor for some days until the texture became brittle. The dried leaves were ground into fine powder and kept in a sealed container before the extraction.

Extraction of Active Principles

A modified Nwosu and Okafor (1995) extraction method was used. Fifty grammes each of the powdered plant materials were soaked in 500 ml of absolute ethanol and allowed to stand for 2 – 3 days on a laboratory bench. The suspension was filtered through No 1 Whatman filter paper. The filtrates were poured into saucers and placed under a ceiling fan which evaporated the solvents leaving behind the crude extracts. The crude extracts were put into sterile sealed bottles, labeled accordingly and stored in the refrigerator throughout the experiments. The plant extracts were each dissolved in 50 % concentration of dimethyl sulphoxide (DMSO; $(CH_3)_2SO$) in the ratio of 1:10 (1 g of crude extract dissolved in 10 ml of DMSO) to give a concentration of 100 mg/ml. Dilutions of 80 mg/ml, 60 mg/ml, 40 mg/ml, 20 mg/ml were made from the stock concentration.

Effect of Plant Extracts on *in vitro* Inhibition of Mycelial Growth of Fungal Isolates

Onyeke and Ugwoke (2011) method of inoculation was used. Two milliliters each of the plant extract concentrations were aseptically dispensed into sterile Petridishes. Eighteen millilitre of molten streptomycin sulphate – modified PDA was poured into each of the Petridishes containing plant extracts. The Petridishes were swirled on a laboratory bench to mix the extracts and the PDA thoroughly before solidifying. A 3 mm diameter cork borer was used to cut discs from each 5 day – old actively growing cultures of the pathogens. Each disc was inoculated face down in the centre of the plant extract-PDA Petridishes. Petridishes without extracts served as the controls. Benlate, a standard fungicide, at a concentration of 20 mg/ml was used to assess the efficacy of the plant extracts, Owolade and Osikanlu (1999) modified method. The experiment was laid out in completely randomized design (CRD) in three replicates. The fungitoxicity of the plant extracts on mycelial growth was determined after five days using the Onuh *et al.* (2005) formula:

$$Fp = \frac{F_1 - F_2}{F_1} \times \frac{100.}{1}$$

Where: Fp = percentage inhibition of fungal growth; F_1 = fungal growth in control Petridishes and F_2 = fungal growth in treatment Petridishes.

RESULTS

A total of five fungal pathogens were isolated from the diseased cucumber fruits. They were identified as follows: *Fusarium oxysporum* Schlecht, *Aspergillus niger* Van Tiegh, *Rhizopus stolonifer* Ehrenb. ex. Fr, *Geotrichum candidum* Link and *Mucor micheli* ex Staint – Amans. Pathogenicity tests proved them as the causal agents of the disease.

The results on the effect of ethanolic plant extracts on *in vitro* mycelial growth of *Aspergillus niger* are shown in Table 1.

Table 1: Effects of Ethanolic Plant Extracts on *in vitro* Inhibition of Mycelial Growth of *Aspergillus Niger*

Plant Extracts	*Chromolaena odorata*		*Moringa oleifera*	
Concentrations	Mycelial Growth (mm)	% Inhibition	Mycelial Growth (mm)	% Inhibition
100 mg/ml	2.000	76.9	0.733	87.1
80 mg/ml	2.767	68.3	1.100	80.6
60 mg/ml	3.067	64.9	3.133	45.1
40 mg/ml	3.400	61.1	4.033	29.2
20 mg/ml	3.467	51.9	4.867	14.6
Benlate	1.033	88.2	0.000	100.0
Control	4.200	-	5.700	-
LSD (0.05)	0.7527	9.11	0.4307	7.68

The inhibitory effect of the plant extracts and benlate solution on the mycelial growth of *A. niger* was significantly different ($P < 0.05$) at the various concentrations tested. *M. oleifera* leaf extract gave the highest inhibitory effect of 87.1 % at 100 mg/ml and least inhibition of 14.6 % at 20 mg/ml. while *C. odorata* leaf extract gave 51.9 % inhibition at 20 mg/ml. However, benlate gave complete inhibition of the mycelial growth of the organism.

Table 2: Effect of Ethanolic Plant Extract on *in vitro* Mycelial Growth of *Fusarium oxysporum*

Plant Extracts	*Chromolaena odorata*		*Moringa oleifera*	
Concentrations	Mycelial Growth (mm)	% Inhibition	Mycelial Growth (mm)	% Inhibition
100 mg/ml	1.433	64.7	1.500	64.7
80 mg/ml	2.067	48.4	2.067	51.2
60 mg/ml	2.167	45.2	2.667	37.6
40 mg/ml	2.667	33.6	3.267	22.7
20 mg/ml	2.900	26.8	3.867	9.0
Benlate	3.200	19.5	3.000	29.7
Control	4.033	-	4.267	-
LSD (0.05)	0.4424	12.86	0.6054	16.20

The inhibitory effects of mycelial growth of *Fusarium oxysporum* are shown in Table 2. There was equal inhibition (64.7 %) of the mycelial growth by the two extracts at 100 mg/ml. The least effect was with *M. oleifera* that gave 9.0 % inhibition at 20 mg/ml while *C. odorata* gave 26.8 % inhibition at the same concentration.

Table 3: Effects of Ethanolic Plant Extracts on Mycelial Growth of *Geotrichum candidum*

Plant Extracts	*Chromolaena odorata*		*Moringa oleifera*	
Concentrations	Mycelial Growth (mm)	% Inhibition	Mycelial Growth (mm)	% Inhibition
100 mg/ml	1.53	58.4	1.13	79.7
80 mg/ml	2.00	46.5	1.53	72.7
60 mg/ml	2.53	32.3	2.17	60.8
40 mg/ml	2.80	24.7	2.87	48.0
20 mg/ml	3.23	13.4	4.27	23.2
Benlate	2.03	44.9	1.40	74.6
Control	3.76	-	5.53	-
LSD (0.05)	0.524	20.78	0.829	14.03

Results on the effects of ethanolic plant extracts on mycelial growth of *Geotrichum candidum* are shown in Table 3. Results revealed that both plant extracts produced significant ($P < 0.05$) levels of inhibition of mycelial growth of *G. candidum* at various concentrations. The highest (79.7 %) percentage inhibition of mycelial growth by the plant extracts was produced by *M. oleifera* at the concentration of 100 mg/ml while the least (13.4 %) percentage inhibition was recorded for *C. odorata* at the concentration of 20 mg/ml.

Table 4: Effects of Ethanolic Plant Extracts on Mycelial Growth of *Mucor micheli*

Plant Extracts	*Chromolaena odorata*		*Moringa oleifera*	
Concentrations	Mycelial Growth (mm)	% Inhibition	Mycelial Growth (mm)	% Inhibition
100 mg/ml	1.47	80.4	0.87	90.4
80 mg/ml	2.17	70.5	2.03	77.2
60 mg/ml	2.53	67.1	3.70	59.0
40 mg/ml	3.67	51.8	4.67	48.5
20 mg/ml	3.97	48.9	7.77	34.5
Benlate	0.00	100.0	0.00	100.0
Control	7.70	-	9.10	-
LSD (0.05)	1.020	8.04	0.849	20.23

Table 4 shows the effect of plant extracts on mycelial growth of *Mucor micheli*. The results also showed that both plant extracts exhibited significant ($P < 0.05$) levels of inhibition of mycelial growth of *M. micheli* at various concentrations. The highest percentage inhibition (90.4 %) was produced by *M. oleifera* and the least percentage inhibition (34.5 %) was also produced by *M. oleifera* as against 48.9 % of *C. odorata*.

Table 5: Effects of Ethanolic Plant Extracts on Mycelial Growth of *Rhizopus stolonifer*

Plant Extracts	*Chromolaena odorata*		*Moringa oleifera*	
Concentrations	Mycelial Growth (mm)	% Inhibition	Mycelial Growth (mm)	% Inhibition
100 mg/ml	3.33	61.9	2.20	75.6
80 mg/ml	5.67	35.6	3.33	63.2
60 mg/ml	6.33	27.4	3.93	56.6
40 mg/ml	6.47	26.1	5.00	44.8
20 mg/ml	7.70	12.3	6.83	24.5
Benlate	2.50	71.2	1.00	88.8
Control	8.80	-	9.07	-
LSD (0.05)	1.875	23.18	0.838	10.41

Results on the effect of plant extracts on mycelial growth of *R. stolonifer* are shown in Table 5.This also showed that both plant extracts produced significant levels of inhibition of mycelial growth of *R. stolonifer* at various concentrations. The highest inhibition (75.6 %) was produced by *M. oleifera* at 100 mg/ml while the least inhibition (12.3 %) was produced by *C. odorata* at 20 mg/ml. The results showed that higher concentrations increased mycelial inhibition.

DISCUSSIONS

This research was able to associate the following fungi with post-harvest rot of cucumber fruits, *Fusarium oxysporium, Aspergillus niger, Rhizopus stolonifer, Geotrichum candidum* and *Mucor micheli*. The most virulent of these fungi was *M. micheli* which destroyed the fruit completely within five days. These fungi had been previously linked with rot of cucumber fruits (Naureen *et al*., 2009), tomato fruits (Ijato *et al*., 2010) and yam rot (Okigbo *et al*., 2010). The ability of these fungal pathogens to cause rot on different crops suggest that they are ubiquitous. Infections by pathogens are often favoured by poor production practices in the field (like excessive irrigation and nitrogen fertilization, lack of crop rotation and poor soil drainage) and wounds created during harvest, storage, and packaging (Schwartz and Gent, 2007).

Some of these pathogens like *R. stolonifer* and *M. micheli* produce numerous air-borne sessile spores that can easily land on the fruits while displayed in markets. This observation agrees with the work of Chiejina (2008) with respect to salad vegetables.

Aspergillus sp, *Fusarium* sp, and *Alternaria* sp are known to produce mycotoxins, which when ingested may cause mycotoxicosis leading to an acute or chronic disease episode like cancer (Placinta *et al*., 1999; Bryden, 2007). Kurup (2003) noted that many fungal genera such as *Aspergillus* sp, *Alternaria* sp, *Cladosporium* sp and *Penicillium* sp are capable of causing hypersensitivity in humans. Since cucumber fruits are consumed raw in order to get their full nutrient benefit, the risk of consuming them with any of these pathogens is very high and the consequences are dangerous. Since pathogenic fungi alone can cause 10-30% reduction in the yield of major food and cash crops (Agrios, 2005), this necessitates pre- and post- harvest technologies to control them (Serrano *et al.*, 2005).

Ethanolic plant extracts of *C. odorata* and *M. oleifera* have significantly reduced the mycelial growth of the isolates *in vitro*. Results of the effects of extracts of *C. odorata* and *M. oleifera* comfirmed an ealier report by Onyeke and Maduewesi (2006) on the ability of the plant extracts to inhibit the growth and development of plant pathogenic fungi *in*

vitro. This agrees with the work of Nwachukwu and Osuji (2008) in the control of *Sclerotium rolfsii* causing cocoyam cormel rot with extracts of *Cassia alata* and *Dennetia tripetala*.

The highest inhibitory effects of the extracts were usually at the highest concentration used. Enikuomehin (2005) and Okigbo *et al* (2010) used *C. odorata* to control *Cercospora* leaf spot and fungal yam tuber rot respectively indicating that the extract is a potent antifungal agent against diverse fungal pathogens, including these encountered in this work. This study also showed that *C. odorata* is a more potent antifungal agent than *M. oleifera* because the former generally gave significantly higher inhibition at the lowest concentration tested than the latter. Jabeen *et al.* (2008) and Oluduro (2012) obtained fungal inhibitory activities with extracts of *M. oleifera* as was observed in this study.

Benlate solution at 20 mg/ml was found to be more effective than the leaf extracts at the same concentration in inhibiting the mycelial growth of the pathogens but the same benlate concentration of 20 mg/ml was found more inhibitory to the pathogens: *A. niger, M. Micheli* and *R. stolonifer* than the leaf extracts at their highest concentration of 100 mg/ml. This may be as a result of the refined nature of benlate and its active ingredients being more concentrated than those of the leaf extracts.

Having shown that the extracts of *Chromolaena odorata* and *Moringa oleifera* could be used to inhibit post harvest rot fungi of cucumber fruit, their use should be encouraged since they are safer, readily available, environmentally safer and non toxic to man.

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