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Effect of Three Parts of *Moringa oleifera* in the Control of Brown Blotch of Cowpea (*Vigna unguiculata*) L. WALP)

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ABSTRACT

The study was carried out to investigate the effect of *Moringa oleifera,* at different concentrations levels to control *Colletotrichum capsici,* a pathogen of Brown blotch of cowpea in the Savannah. The study was carried out at the laboratory of the Department of Plant Science Modibbo Adama University Yola. *C. capsici* was isolated on unhealthy roots tissues of cowpea obtained from infected farm in Gombe State were cut off and surface sterilized by soaking in ethanol (75%, v/v) for 40 seconds then 4 min in hypochlorite (1%, v/v) and subsequently soaking in ethanol (75%, v/v) for 30 seconds again, to remove residual hypochlorite, finally rinsed in sterile distilled water, and blot-dried on clean sterile paper to remove sterilant. The pieces were transferred with a sterile tweezer, shaking off excess water onto a plate of PDA containing 2% potato dextrose agar containing streptomycin sulphate at 50 mg/l to prevent bacterial growth. Plates were then inoculated with various plant extracts of *M. oleifera* at concentrations of 2.00/ 10mls, 2.5/ 10mls, and 3.00/ 10mls of the medium for fungal growth. A mycelia disc of *C.capsici* was placed in the centre of the petri disc, to test the inhibitory activities of various control treatments**.** The mycelia growths on the petri dish were recorded three days, six days and nine days after inoculation. *In vitro* control trial was completely randomized design (CRD) with three replications. Data obtained were analysed, using analysis of variance (ANOVA) with Genstart. Version 14 Statistical Analysis System (SAS) (PC/Window) April 2023. Mean were separated at ($p<0.05$). Results from the experiment indicated that 3.00 ml of leaves and stem extracts of *Moringa oleifera* give the best control, suppressing the mycelial growth of the fungus to 1.63 cm, followed by stem back at 2.50 and 3.00 concentrations yielding mycelial growth of 1.67cm and 1.70 cm with 3.23 and 3.20 zones of inhibition respectively compare with 4.37 cm in the control experiment. Results from *In-vitro* experimental studies revealed that the best control effect was found in using 3.00 ml of stem extract of *M. oleifera* with the highest inhibition zone of 3.27 cm on the nine day after inoculation. It may be concluded from the results that the extracts used in this research are of potent antifungal activity against *Colletotrichum capsici*. Thus, should be explored further for fungal control activities and other uses as this is important in combating the recent observed emergence of drug resistance organisms.

Keywords: *Colletotrichum capsici, Moringa oleifera,* extracts, Cowpea

INTRODUCTION

Cowpea (*Vigna unguiculata*) is a significant economic crop in many developing regions due to its rich nutrient and fiber content in all parts of the plant. Seeds are commonly boiled and consumed, while fresh leaves and immature pods are eaten in various African countries. Globally, cowpea is cultivated across tropical and subtropical regions, with an annual production of approximately 6.5 million metric tons on 14.5 million hectares. Africa, particularly Nigeria and Niger, dominates production, accounting for 96% of grain production in the region (FAOSTAT, 2020).

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High in protein, cowpea serves as a vital food source, with 52% used for human consumption, 13% as animal feed, and 10% for seed production (Dugje *et al*., 2009). Additionally, cowpea plays a crucial role in soil nitrogen enrichment through nitrogen fixation, allowing it to thrive in poor soil conditions. The grains, rich in lysine and tryptophan, serve as a dietary protein source complementing staple cereal and tuber crops. Cowpea also contains essential minerals and vitamins, including folic acid and vitamin B, crucial for preventing birth defects (Singh *et al.,* 2014).

The rich nutritional profile of cowpea makes it a vital crop in the developing world, particularly in Africa. Despite this significance, cowpea production faces challenges, with pests and diseases identified as major constraints in Nigeria. Diseases like brown blotch and anthracnose pose significant threats, leading to yield reduction (Mark and Channya*.,* 2016). Natural solutions, such as the use of *Moringa oleifera*, are being explored as biopesticides. *Moringa oleifera*, a medicinal plant, exhibits antimicrobial properties in its various parts, making it a potential alternative to chemical fungicides (Li Destri Nicosia *et al*., 2016). Phytochemical analysis reveals the presence of antioxidant compounds, making it effective against microorganisms, fungi, and bacteria (Yadav *et al.,* 2017).

The study aims to assess the phytochemical composition of *Moringa oleifera* leaf extract, evaluate its antifungal activity against *Botrytis cinerea* (*B. cinerea*), and examine ultrastructural changes in *B. cinerea* conidia and mycelia at the Minimum Inhibitory Concentration (MIC) of *M. oleifera* leaf extract.

MATERIALS AND METHODS

Collection of Disease Plant and Isolation of Pathogen from Infected Plants

Ten (10) plants out of the hundred plants examined on a cowpea farm in Gombe State were selected at random before taken for diagnosis in the laboratory. The samples collected were preserved into a sterilized cellophane bag before the isolation of the pathogen. The pathogens were isolated following the procedures described by Zakari *et al.* (2016). Using a scalpel, a section of unhealthy and healthy tissues was excised from the diseased roots of the plants. Samples were sterilized in 75 % ethanol for 40 seconds then washing in 1% hypochlorite for 3 minutes followed by soaking in 75% ethanol for 30 seconds to remove excess hypochlorite and finally rinsed in three changes of sterile distilled water before blot-drying on clean sterile filter paper to remove excess moisture. Samples were then transferred aseptically onto a petri dish of 2 % PDA supplemented with streptomycin sulphate at 50 mg/l to check bacterial growth. Petri plates were incubated at room temperature. Pure culture was obtained through sub culturing on fresh PDA plates and thereafter stored on PDA slant in McCartney bottle.

Identification of the Fungal Isolates

The fungal isolates were identified based on the following morphological characteristics; colony growth, presence or absence of aerial mycelium, colony colour, presence of wrinkles and furrows and pigment productions and compared with those in the identification keys of Watenabe *al*., (2010). The procedure involved corking portion of seven-day-old mycelium of the fungi and aseptically placing it on a clean slide, spread with inoculation needle and added a drop of lacto phenol cotton blue. Slip was then covered with cover slip, mounted on the light

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microscope and observed under x10, x40 and x100 objective lenses.

Preparation of the Spore Suspensions

Suspension of the pathogen spore was prepared following the procedures reported by Burgess (2008). Seven-day old culture of the fungal isolate was flooded with 25 ml of sterile distilled water. With the aid of a Carmel hair brush, the spores were carefully brushed off the sporophores and decanted into a sterile Petri dish. Fifteen millilitre of sterile water was added and filtered through 0.2×0.2 mm nylon mesh to get rid of mycelia fragments. This filtrate containing the spores which was adjusted to a concentration of 1×10^4 spores per ml through the addition of five millilitre of sterile distilled water.

Pathogenicity Tests

Pathogenicity test was conducted to ascertain that pathogen isolated from diseased cowpea plants and soils are responsible for the disease symptoms observed on the samples. This was done following the procedures described by Burgess *et al*., (2008). Cowpea plants that were used for the test were raised in polyethene pots containing 2 kg steam-sterilized sandy-loam soil. Plants were irrigated twice a week to evade water stress. Cowpea seedlings were sprayed with the pathogen suspension with hand sprayer two weeks after germination. Cowpea seedlings in the control pots were sprayed with distilled water only. Seedlings were retained in the humidity chamber for 24 hours and thereafter removed and taken to open pavelion for growth. Seedlings were assessed on their variation in seedlings emergence, plant height, number of leaves per stand, stem girth on weekly basis.

Collection of Plant Materials for Control of *C. capsici*

A fresh and healthy roots, leaves, and stembarks of actively growing *Moringa oleifera,* were collected at Gwandum, Shongom local government area of Gombe state. The plant materials were washed with distilled water and air dried under shed at room temperature until all the water molecules evaporated. After drying, the plant materials were cut into small pieces and ground well using mechanical blender into fine powder and transferred into airtight containers with proper labelling for future use.

Preparation of Plant Extracts and Solvent Extraction

The extraction of the active metabolites that are toxic to pathogens from the test plant of

Moringa oleifera, was conducted following the procedures describe by Zakari *et al.* (2016). The grinded powder of the root, stemback and leaves of *Moringa oleifera* were sieved separately to fine powder and dissolved in ethanol in the ratio of 10:1 (v/w). The mixtures was then stirred using a sterile glass rod and vigorously shaken for 10 min and allowed to stand for 24 hours before filtering; filtrate was allowed to sediment and dried under in water bath at 55^o C. About 10 g of the filtrate was dissolved in 100 ml of sterile distilled water to make 100 % concentration of the extract. The Aqueous preparation of the test , leaves, and stembark extracts was used for the control test.

***In-vitro*- Control Experiment**

Poisoned food technique was adopted for this study. Seven-day-old fungal culture was punched aseptically with a sterile of 5 mm diameter. The fungal discs were inoculated on the gelled agar plate. The agar plates were impregnated with the various concentrations of plant materials at a room temperature. The

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plates were then incubated at room temperature for the fungal growth. Growth diameters of the fungal colonies were recorded by measuring the two opposite circumference of the culture. Percentage inhibition of mycelial growth was evaluated by using the formula given below;

$$\text{Inhibition percentage (\%)} = \frac{\text{DC} - \text{DT}}{\text{DC}} \text{x } 100$$

Where;
DC - Average Diameter of fungal spore in control.
DT - Average diameter of fungal spore with treatment

Experimental Design and Statistical Analysis

For the *in-vitro* control trial, completely randomized design (CRD) with three replications was used. Treatments consisted of Leaves, Stem, and Root extract of *M. oleifera,* at three concentration levels (2.00 ml, 2.50 ml and 3.00 ml per 10 ml of PDA). The *in-vivo* experiment was conducted in Randomized Completely Block Design with three replications. Treatments consisted of leaves Stemback, Root extract of test at three concentration levels (2.00 ml, 5.00 ml and 7.00 ml). Data obtained were analyzed statistically using analysis of variance (ANOVA) with Genstart. Version 14 Statistical Analysis System (SAS) (PC/Window) April 2023. Means were separated at ($p<0.05$) by Least Significance Difference (LSD).

RESULTS

Result on the effect of different concentration levels of Leaves, roots and Stemback extract of *Moringa oleifera,* on the mycelia growth and inhibition zone of *Colletotrichum capsici* on the three days after inoculation were significantly different with the control at $P \leq 0.05$, as shown in (Tables 1). The stemback extracts of *Moringa oleifera* at 2.00 ml, 2.50 ml and 3.00 ml reveals the least mycelia growth of *Colletotrichum capsici* (1.37 cm) on a PDA, with the highest inhibition zones of (2.03 cm) when extract at various concentration levels ware applied. Thus a lower control was seen when 2.00 ml of *M. oleifera* (2.70 cm) was recorded which gave inhibition zone of 0.70 cm. while the distilled water control has the highest zone of mycelia growth of 3.40 cm. The mycelia growth is in conformity with the inhibition which has zero (0.00 cm) zones of inhibition.

Table 1: Effect of Different Concentrations of Plant Parts Extract of *Moringa oleifera* on *C. capsici* on the Three Days after Inoculations

				Mycelia growth inhibition		
Conc / Plant Parts	**Root**	**Leaves**	**Stembarks**	**Root**	**Leaves**	**Stembarks**
0.00	3.40	3.40	3.40	0.00	0.00	0.00
2.00	2.70	2.03	1.37	0.70	1.37	2.03
2.50	2.37	1.97	1.40	1.03	1.23	2.03
3.00	2.03	1.90	1.37	1.37	1.60	2.03
P-value	0.05			0.05		
L.S.D	1.71			1.71		

Result of analysis of variance on the sixth day for the effect of different concentration levels of plants parts extract of *Moringa oleifera* on the mycelia growth and inhibition zone of

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Colletotrichum capsici were significantly different with the control at P≤0.05 as shown in Table 2. Stem extracts of *M. oleifera;* showed the least mycelia growth at various concentrations compare to other plant parts. Thus giving the highest zones of inhibition (2.72, cm 2.83. cm 2.90 cm) followed by the root extract and subsequently the leaves extracts while the distilled water control has the highest zone of mycelia growth at 4.37cm with inhibition zones of 0.00 cm.

Table 2: Effect of different concentrations of plant parts extract of *Moringa oleifera* on *C.capsici* on the six days after inoculations

	Mycelia growth inhibition					
Conc / Plant Parts	**Root**	**Leaves**	**stembark**	**Root**	**Leaves**	**stembark**
0.00	4.37	4.37	4.37	0.00	0.00	0.00
2.00	2.93	3.03	1.60	1.43	140	2.72
2.50	2.77	2.97	1.53	1.60	1.03	2.83
3.00	2.50	3.00	1.47	1.87	1.07	2.90
P-value	0.05			0.05		
L.S.D	2.40			1.56		

The ninth day of observation on the effect of different concentration levels of plant extract of *Moringa oleifera* on the mycelia growth and inhibition zone of *Colletotrichum capsici* were significantly different with the control at P≤0.05 (Table 3). The best control treatments were seen in 3.00 ml of leaves and stem extracts of *Moringa oleifera,* with least mycelia growth of 1.63 cm. Followed by stemback at concentrations of 2.50 and 2.00 which yielded mycelia growth of 1.67 and 1.70 while their inhibition zones are 3.23 and 3.20 respectively. The distilled water control has the highest mycelia growth of 4.37cm, and the inhibition zones of the extracts also concur with the mycelia growth recording zero inhibition zone.

Table 3: Effect of different concentrations of plant parts extract of *Moringa oleifera* on *C.capsici* on the nine days after inoculations

	Mycelia Growth inhibition					
Conc / Plant Parts	**Root**	**Leaves**	**stembark**	**Root**	**Leaves**	**r**
0.00	4.90	4.90	4.90	0.00	0.00	0.00
2.00	3.03	1.87	1.70	1.87	140	3.20
2.50	2.97	2.15	1.67	1.93	1.89	3.23
3.00	2.30	1.63	1.63	1.97	3.27	3.27
P-value	0.05			0.05		
L.S.D	2.40			1.56		

DISCUSSION

Three crude plants extract from the roots, stem and leaves of *Moringa oleifera,* were evaluated against *Colletotrichum capsici* species under laboratory condition at three different concentrations of 2.00ml/10ml, 2.5ml/10ml, and 3.00ml/10ml of PDA and then compared with distilled water as control. They were evaluated against the fungi using poisoned food technique. The fungal growth was recorded at 3, 6, and 9 days after inoculation and the data were recorded. The entire treatments were found to be superior over control. (p ≤0.05). Zakari *et al*. (2016) tested and found Moringa extracts effective in the reduction of radial growth of *Colletotrichum capsiciI* under laboratory condition in Nigeria. In Zakari *et al*. (2016),

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the pathogen was isolated from pepper while in this experiment on cowpea seeds.

The antifungal properties in root, leaves and stembark extracts of *Moringa oleifera* that was found effective in controlling the mycelia growth of *Colletotrichum capsici* may be due to chemical substances present in the plant extracts. Alhakmani *et al*. (2013) found that chemicals present in plants were effective in suppressing most fungal strains growth. Arora, and Onsare (2014) reported that oil that contain cittral, triterpene, phytosteroid, sulfide, sallysteine, allyppropl have antimicrobial effect of which some of this chemical are also present in *Moringa oleifera* extracts**.**

Apart from the aforementioned chemicals, *M. oleifera* was reported by Enyiukwu (2014) to have secondary metabolites such as Tannins, Alkaloids, Flavonoids, Saponnins, Steroids, Triterponoids, Phenolic compounds and Terpenoids, these compounds are generally considered to be responsible for most of the pharmacological properties of *Moringa oleifera*. Some of these compounds may be responsible for the antifungal property of *Moringa oleifera* which has been effective against *Colletotrichum capsici* in this study.

CONCLUSION

The overall result concludes that the extracts used in this research are of potent antifungal activity. Thus, should be explored further for fungal control activities and other uses as this is important in combating the recent observed emergence of drug resistance organisms decreases the colony growth of *Colletotrichum capsici.* In this study the extract has some potential to be effective antifungal agent against brown blotch of cowpea.

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