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MJ Falade
Department of Crop Production, Horticulture and Landscape Design, Ekiti State University, Ado-Ekiti, Nigeria

Correspondence
MJ Falade
Department of Crop Production, Horticulture and Landscape Design, Ekiti State University, Ado-Ekiti, Nigeria

Comparative efficacy of copper (I) oxide matalaxyl fungicide and plant extracts in the management of mango anthracnose caused by *colletotrichum gloeosporioides* (Penz) in Nigeria

MJ Falade

Abstract
Mango anthracnose caused by *Colletotrichum gloeosporioides* is responsible for about 60% yield loss of mango in Nigeria. Based on the above, laboratory studies were conducted to evaluate the effect of hot water leaf extracts of African egg plants (*Solanum macrocarpon*), Jimson weed (*Datura stramonium*) and African scent leaf (*Ocimum gratissimum*) at 20, 40 and 60% concentrations and copper (1) oxide metalaxyl based synthetic fungicide on the growth of *Colletotrichum gloeosporioides*. The extracts were applied in-situ to determine their efficacy of control. The result of the studies shows statistically significant (P<0.05) variation in *C. gloesporioides* growth depending on the extracts and concentrations. *S. macrocarpon* at 60% concentration was the most effective in reducing the growth of the fungus and incidence of the disease on the field. The effects of the 60% *S. macrocarpon* leaf extract compared favourably with the copper (1) oxide synthetic fungicide. The growth rate of *C. gloesporioides* treated with 60% *S. macrocarpon* and Copper 1 oxide Metalaxyl were respectively 2.91 and 2.81mmday-¹. Similarly, diseases incidence in plots treated with copper (1) metalaxyl (13.4%) compared favourably with that of *S. macrocarpon* (14.2%) when extracts was applied two days before inoculation at the highest concentration (60%) of the leaf extracts. The study concluded that the active compound in the leaf extracts can be developed into synthetic fungicide for the management of Mango anthracnose.

Keywords: Mango, anthracnose, plant extracts and fungicide.

Introduction
Mango (*Mangifera indica)* a member of the family Anacardiaceae, Class Magnoliopsida and Order Sapindale is widely cultivated in both tropical and sub-tropical regions of the world as a source of food (Fizza *et al.*, 2020) [7]. It is important in boosting human immune system and contains nutrients that support the eye and skin. Mango is a good source of fibre and vitamin C which help to fight against chronic diseases especially diabetes (Iram and Ahmad 2013) [11]. The bark of the mango trees is employed for making furniture, ceiling board, windows and agriculture implements. It also contains about16-20% tannin which is employed for the production of tannin hides (Krishna and Singh, 2007). World production of mango in 2019 was estimated at 55.85 million metric tons (MMT) (FAOSTAT 2019) [5] with India as the largest producer accounting for about 20/MMT while in Africa, Mali is the leading producer (Oluwole *et al.*, 2018). Nigeria is the tenth largest producer of mango in the world accounting for about 0.8 MMT. Most of the mango produced in Nigeria are consumed locally with little or no export hence, the need to increase production (Onyia *et al*., 2019) [18]. Mango is attacked by different fungal diseases like *Rhinocladium corticum* (Black banded disease), *Meliola mangiferae* (Black mildew), *Aspergillus niger* (Black mold) all of which impair growth, plant vigour, photosynthesis and abortion of fruits and flowers (Felipe, 2000) [6]. The overall effect of which reduces yield or productivity with serious economic implications. Mango is propagated by seed and since most of the pathogens are seed borne, theyare likely to spread thus increasing production cycle from year to year (Nasir *et al.*, 2018) [14]. This contributes to the buildup of inoculum in the soil.
Mango anthracnose is caused by the fungus *Colletotrichum gloeosporioides*, the disease is capable of infecting a wide variety of decidious trees, shrubs and oarks. The disease affects all parts of the plants (Nasir *et al.*, 2018) [14]. Symptoms are visible on the leaves, twigs, petioles, flowers cluster and fruits (Onyeani and Amusa 2015) [17].

On leaves, small angular brown to black spots are noticeable and enlarge to form extensive dead area. The lesion may drop out of leaf during dry weather; lesion size and distribution depend on severity. The fungus usually grows at a temperature of 75-85^{0}F and moisture is required for its germination (Igbari *et al.,* 2019).

The management of the diseases above in Nigeria can be achieved by the use of resistant varieties where they exist, intercropping of susceptible and non-susceptible crops, removing and destroying infected plants, pruning dead leaves from trees, use of plant extracts and control using selective fungicides like Copper (I) metalaxyl and benomyl. However, due to increased awareness of the side effects of synthetic pesticides on the soil and beneficial microorganisms coupled with the need to produce crop with minimal residual pesticides, attention is now being focused on alternative methods that are safe, cheap, less costly and in addition is compatible with the farming practices of the farmers for pest and disease control (Falade, 2017). The antifungal effects of African eggplant (*Solanum macrocarpon*), (Jayshrea and Srivastima 2010, Sharma *et al.,* 2011) [13] scent leaf (*Ocimum gratissimum*) (Okoi *et al.,* 2013) [5] and Jimson weed (*Datura stramonium*) (Usha *et al.,* 2009, Falade, 2017) [23] are well known but their toxicity to *C. gloesporioides* and its use in the management of mango anthracnose have not been studied. Therefore, this study was carried out to compare the effects of the extracts of these plants and the synthetic fungicide, Copper (I) metalaxyl on the mycelia growth and conidiation of the fungus in vitro and efficacy in the control of anthracnose disease on the infected mango plants in the field.

Materials and Methods

Collection of Plant Leaves and Source of Fungicide

The leaves of *S. macrocarpon, O. gratissimum* and *D. stramonium* were collected from the Ekiti State University Teaching and Research Farm (Latitude 7 7212°N and longitude 5.2575°E) in the South western Nigeria. The leaves were air-dried at ambient temperature (28±2°C) for 4-6 weeks, powdered using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated at 4°C until they were required for bioassay. The synthetic fungicide Tandem,® containing 65% Copper (1) Oxide in 12% Metalaxyl as wettable powder (WP) was purchased from Agro-stores in Ado-Ekiti, Nigeria.

Preparation of Plant Extracts

Extracts were prepared by mixing equivalent grams of the prepared plant powder (60, 40 and 20) with 100 ml of distilled water in 500 ml flasks and kept in hot water bath-shaker at 70 °C for 2 hours. Thereafter, the extract was separated from the shaft by vacuum filtration and stored at 4 °C in McCartney bottles and used as the stock solutions from which 60, 40 and 20% concentrations were prepared (Collin and Michael, 2000).

Preparation of Modified Media

Standard Potato Dextrose Agar (PDA, E. Merck, Darmstadt Germany) was modified either with different concentrations of the plant extracts or Tandem® at the recommended rate (0.1g/l) and autoclaved. Thereafter, the agar was allowed to cool to 50 °C, amended with 30 µg/L streptomycin sulphate, poured into 9 cm sterile petridishes (Sterilin® Product, UK) inside a laminar flow cabinet and left for 20 minutes to solidify.

Isolation and identification of *C. gloeosporioides*

Infected mango plants showing symptoms of anthracnose were collected from the mango fields in the Teaching and Research Farms Ekiti State University, Ado Ekiti. The leaves were cut into approximately 1-2 cm sizes and surface sterilized with sterile distilled water containing 0.2% hypochlorite solution followed by two rinses in sterile distilled water in a laminar flow cabinet. Three leaf cuttings were placed on standard PDA media containing 30 µg/L streptomycin sulphate to suppress bacteria growth. The plates were sealed with parafilm and incubated at 28 °C for 5-6 days. Single spores of developing colonies were isolated and subcultured to obtain pure cultures. The samples from the single spore cultures were used for morphological identification on Malt Extract Agar (MA) at x400 magnification of a compound microscope with Zivkovic et al., (2010). The conidia suspension of *C. gloeosporioides* were sprayed on healthy mango plants and re-isolated to comply with Koch's postulate (Enikuomehin *et al.,* 2010).

Evaluation of Growth

One centimeter agar disk of the pure culture was transferred unto the prepared plant extract- or Tandem® -modified PDA media. After 24 hours, the colony diameter along pre-marked orthogonal axes at the bottom of the Petridishes was done and this continued until the surface of the plate was covered. The values of the colony diameter were averaged and the percentage inhibition of mycelia growth (PIMG) was calculated for each treatment relative to control.

Evaluation of Conidia Germination.

Sterile PDA in 9 cm Petri dishes were inoculated with 10 ml of *C. gloesporioides* conidia suspension measured with a micropipette and spread-plated using Drigalsky spatula, the lids were replaced and sealed with parafin. The incubation was carried out at ambient temperature (25±2°C) for 24 hours. Thereafter, sterile cover slip was placed on the spread-plated area and percentage germinated conidia was estimated for 100 conidia in the cover slip area under a compound microscope using x40 magnification. The conidium with germ tube length longer than it's diameter was considered as germinated. The Percentage Conidia Germination (PCG) was calculated as;

$$PCG = \frac{\text{No of germinated conidia}}{\text{Total counted conidia within field of view}} \text{ X } 100$$

Effect of plant extracts and fungicide on disease incidence

The field experiment was conducted at Ekiti State University Teaching and Research Farm Ado-Ekiti (7.7129°N, 5.2523°E). The first trial was conducted in May, 2019 and repeated during the same period in 2020. The size of each plot was 15 m x 15 m separated by a border row of 1 m. The total area of the farm was 900 m² and planting of Mango was done at spacing of 2 m x 2 m and there were 100 stands of mango. Sugar mango variety was sown at one seed per hole. The experiment was laid in a Randomized Complete Block Design (RCBD) with three replicates. Germination was observed at three weeks after planting and six weeks after plant establishment. Three concentrations (20, 40 and 60%) of leaf extracts (*S. macrocarpon, O.*

gratissimum and *D. stramonium)* were sprayed on the 9-week old mango. The plants in the first subplots were inoculated with the conidia suspension of *C. gloeosporioides* after 48 hours. Methaxyl, fungicide was applied at rate 0.1 g while the control plot was sprayed with distilled water. The plants in the second subplot were inoculated with the spore suspension and later sprayed either with the plant extracts or Copper (1) methaxyl fungicide after 48 hours. In the third subplot, the plants were sprayed with the spore suspension followed by immediate application of copper (I) methaxyl or the extracts. In all the treatment, conidia suspension containing 10^6 conidia was used.

Disease assessment

Assessment of the incidence of the disease was determined by using five randomly tagged plants per plot. The number of diseased leaves was counted and expressed as a percentage of tagged plant. The assessment commenced at 9 weeks after planting (WAP) and continued till 14 WAP.

Results

Table 1 shows the effects of hot water extracts of the three plants on growth of *C. gloesporiodes*. The growth rate differed significantly in relation to plant extracts and their concentrations. As the concentration of the extracts increased, the growth rate of *C. gloesporiodes* reduced in all the three plant extracts. At the highest concentration (60% w/v), the growth rate induced by *S. macrocarpon* leaves was 2.91 while those of *O. gratissimum* and *D. stramonium* were 3.18 and 3.30 mm day^1 respectively.

Table 1: Effect of three Concentration of hot water leaf extracts of three plants on growth rate of *C. Gloesporiodes* in Ado Ekiti, Nigeria

Concentrations			
Plant extracts	**20**	**40**	**60**
S. macrocarpon	3.60c (12,11$^{1\prime}$	3.33c (23.3)	2.91d(32.9)
O.at,0.1ssimurn	3.63c (16.4)	3.43b (21.0)	3.18c (26.7)
D. stramonium	3.89b (10.4)	3.40b (21.7)	3.30b (24.0)
Copper (1) Instalayx 1	2.81"	2.81d	2.81d
Contro14.34a	4.34a4.34a		

Values in parenthesis are GRI (Growth Rate Inhibition %)
Means with the same letter in each column are not significantly different (P<0.05) (Turkey' HSD)

Table 2 shows the effects of the plant extracts on conidia germination *C. gloesporiodes*. There was 65-100% germination of conidia irrespective of the plant extracts or concentrations. At the higher concentration, conidia germination was low but as the concentrations reduced, conidia germination was high

Table 2: Germination of Conidia of *C. gloesporiodes* after 12 hours incubation on modified PDA at three concentrations of the plant extracts.

Extracts			
Concentrations (w/v)	***S. macrocarpon***	***O. gratissimum***	***D. stramonium***
0	100^b	100^a	100^a
20	80^b (20)*	84^b (16)	86^b (24)
40	72^c (28)	75^c (25)	77^c (27)
60	65^c (35)	69^c (31)	70^c (30)

Values in parenthesis are % reduction in conidial germination
Means with the same letter in each column are not significantly different (P<0.05) (Turkey' HSD)

Table 3 shows the effects of the three plants extracts on the incidence of *C. gloesporioides*. The incidence of disease was concentration dependent, being least where the highest concentration of all the extracts was used. The incidence of the disease in all the treated plots was significantly (P≤0.05) lower than the control. Application of the three extracts at 60% Concentration compared favourably with the Copper I metaxyl.

Table 3: Effects of three plant extracts at three concentrations on the incidence of *C. gloesporioides*in Ado Ekiti, Nigeria

Plant Extracts	Conc	2IA%)	2DAI (%)	CAEI (%)
S. macrocarpon	20	22.3b	25.6b	29.4b
	40	17.4c	22.4c	27.0c
	60	14.2d	15.2d	15.0d
^{0}. 83=183Zira	20	22.6b	27.3b	30.7b
	40	17.8c	26.4c	30.2c
AMON&	60	14.8d	15.3d	15.4d
	20	22.8b	27.4b	30Ab
	40	18.0c	24.9c	29.6c
	60	14.9d	15.4d	14.6d
Control	100a		100a	100a
Copper (1)gmtwal	13.4d		m,4,	gx,

DBI = Days Before Inoculation, DAI = Days After Inoculation
CAE = Concurrent Application Extract followed by inoculation
Means with the same letter in each column are not significantly different (P<0.05) (Turkey' HSD)

Discussion

Anthracnose disease undermines mango production in Nigeria. The pathogen survives in soil for long period of time making crop rotation ineffective. Thus successful identification of plants with bioactive components is an important requirements for its control. The three plants used in the study; *S. macrocarpon*, *O.gratissimum*and *D. stramonium* are readily available in homestead with bioactive constituents endogenously synthesized and present in varied concentrations in the plant tissue (Gideon and Anita 2013, Ghost *et al*.,2002) [9, 8]. In the study, the leaves of the three plants materials were collected, air-dried and powdered to increase the surface area between samples and the extraction solvent. This is because air-dried material are less fragile and do not tend to deteriorate, an advantage which it has over fresh leave sample (Falade, 2020). Hot water was used for the extraction of the bioactive components of the plants because it is less costly and can be recommended for small and medium enterprises, apart from this, it has the potential of preserving the chemical constituents of the plants (Vongsak *et al.,* 2013, Enyiukwu and Awurum, 2013) [24, 2]. In the study, hot water leaf extracts of the three plants reduced mycelia growth of *C. gloesporioides* on PDA and the rate of inhibition was concentration-dependent. The higher inhibition of growth occurred at relatively higher concentration of the plant extracts, this was probably due to increased availability of antifungal phytochemicals in the medium. Jawdah *et al*., (2021) [12] evaluated the antifungal activities of nine plants extracts against seven pathogenic fungus, the study showed that all the nine extracts had moderate to high inhibition of mycelia growth which is in agreement with the current study.

In this study, all the three extracts at the tested concentrations inhibited conidia germination of *C. gloesporiodes* by 20-35% irrespective of plant extracts or concentration. Shaidul *et al.,* (2002) [21] evaluated the antifungal activities of extracts of *Vincarosea*, *Azadirachta*

indica and smoke of rice against four pathogenic fungi and reported that the plant extracts of the plant inhibited spore germination which is in agreement with the current study. Falade 2017 reported the activities of six plant extracts in the control of mycelia growth and conidia germination of *C. lindemuthianum* causing anthracnose disease of cowpea and found that all the extracts had no effect on conidia germination which contradict the present study. The mechanism of some plants extracts and some fungicides causing inhibition of mycelia growth without significant deterence to germination is not fully understood. The susceptibility of phytopathogenic fungi to botanicals are influenced by a number of factors, which include the part of the plant that was used, chemical constituent of the plant, fungus strain, mode of extraction bioactive components among other factors can be responsible for the results obtained in the earlier study.

In this study, field trials were conducted to evaluate disease incidence where the three extracts and copper I metalaxyl fungicide were applied before inoculation, shortly after inoculation and inoculation of the plant before application of the extracts and fungicide. Disease incidence was significantly lower when extracts and fungicide were applied two days before inoculation of the mango plants. The trend common to all the extracts and fungicide when compared to application of extracts and fungicide two days after inoculation or inoculation followed by application of extracts and fungicide within the shortest possible time of 20-30 minutes. This result suggests that extracts and fungicide were more effective when applied as a preventive rather than curative means. This finding agree with the work of Amadioha who controlled rice blast caused by the fungus *Pyricuilaria oryzae* with extracts of *Azadirachta indica* and Carbendazin fungicide, the studies show that both the fungicides and extracts were effective on the field when applied before inoculation.

Conclusion

This research provided information that the three plant extracts have anti-microbial properties and hence the potential for the inhibition of *C. gloeosporiodes* causing anthracnose disease of mango. The research will contribute to the development of a disease control strategy that can be used by small holder farmers and scientist.

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