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Full length Research Paper

Comparative efficacy of Benomyl and plant Extracts in the management of Guava anthracnose (*Colletotrichum gloeosporioides* Penz) in Ado Ekiti, South Western Nigeria

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Abstract: Guava anthracnose caused by *Colletotrichum gloesporioides* is responsible for reduction in yield or productivity of guava with serious economic implication and hence the need for the control. Based on the above, laboratory studies were conducted to evaluate the effect of hot water leaf extracts of Drum stick tree (Moringa oleifera), Bright eyes leaf (Catharanthus roseus) and Cashew leaf (Anacardium occidentale) at three (15, 30 and 45%) concentrations and benomyl based synthetic fungicide on the mycelia growth of *Colletotrichum gloeosporioides* and in checking the spread of the disease on the field. 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. *M. oleifera* at 45% concentration was the most effective in reducing the growth of the fungus and incidence of the disease on the field. The effects of the 45% M. oleifera leaf extract compared favourably with the benomyl synthetic fungicide. The growth rate of *C. gloesporioides* treated with 45% M. oleifera and benomyl were respectively 2.88 and 2.76 mmday-[1]. Similarly, disease incidence in plots treated with benomyl (20.2%) compared favourably with that of *M. oleifera* (22.6%) when extracts were applied two days before inoculation at the highest concentration (45%) 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 guava anthracnose.

Keywords: Guava, anthracnose, plant extracts, fungicide.

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INTRODUCTION

Guava (*Psidium guajava*) a member of the family Myrtaceae, Class Magnoliopsida and Order mytales is widely cultivated in both tropical and sub-tropical regions of the world as a source of food (Akande *et al*., 2018). Guava is believed to have originated from Southern Mexico through central America, they were distributed by the spanlards Portugese to other parts of the world (Jamieson *et al*., 2021). Guava helps to improve skin colour, lower blood sugar level, relieves painful symptoms associated with menstruation, helps to improve digestion and boost libido and sperm production thereby increasing fertility. It also contains vitamin C, B6, D and a high amount of iron and magnesium needed for body growth (Naseer *et al*., 2018). World production of guava in 2020 was extimated at 54.73 MMT with India being the largest producer accounting for about 24.75 MMT which represents 45.22% of the world production. This is closely followed by Indonesia (3.62 MMT) [FAOSTAT, 2020]. Nigeria is the twelveth largest producer of guava accounting for about 0.87568 MMT which represents about 1.63% of world production (Andayanie *et. al.,* 2021). Guava production in Nigeria is mainly in the savanna and rainforest ecological zones. Most of the guava produced are consumed locally with little or no export hence the need to increase production.

Guava is affected by many fungal diseases such as fruit canker (*Pestalotia Psidi*), fruit spot (*Cephaleuros Virescens*), stem canker (*Botryoidiplodia theobromae*) all of which reduce photosynthesis, plant vigour, growth and abortion of fruits. The overall effect of which reduces yield or productivity with serious economic implications (Poonam *et. al.,* 2017). Guava is propagated by seed and since most of the pathogens are seed borne, they are likely to spread thus increasing production cycle from year to year. This contributes to the build-up of inoculum in the soil (Ahmadu *et. al.,* 2020).

Guava 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 (Binyamin, 2016) . Symptoms of the disease are small, slightly sunken dark or black necrotic lesions on immature fruits, spot which often enlarge up to 1-2cm in diameter, the central portion becomes dark black due to presence of acervuli. Infected area unripe fruit is usually corky and hardy which often develops cracks (Ansari *et. al.,* 2020).

The management of the disease 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 benomyl (Falade, 2017). 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 wih the farming practices of the farmers for pest and disease control (Ghost *et. al.,* 2002). Such methods can be applied solely or in combination with other methods.

Antifungal effects of leaf extracts of Drum stick tree (*Moringa oleifera*), (Ahmadu *et al*., 2020), Cashew leaf (*Anacardium occidentales*) (Tafinta *et al*., 2020) and *Catharathus roseus* (Zahari *et. al.,* 2018) are well known but their toxicity to *C. gloesporioides* and its use in the management of guava 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, benomyl on the mycelia growth and conidiation of the fungus in vitro and efficacy in the control of anthracnose disease on the infected guava plants in the field.

MATERIALS AND METHODS

Collection of Plant Leaves and Source of Fungicide

The leaves of *Moringa oleifera, Anacardium occidentales* and *Catharathus roseus* 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®, MixerGrinder), packaged into sealable nylon and refrigerated at 4°C until they were required for bioassay. The synthetic fungicide benomyl,® containing 65% Copper (1) Oxide in 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 (45 30 and 15) with 100 ml of distilled water in 500 ml flasks and kept in hot water bathshaker 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 45, 30 and 15% 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 benomyl® 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 guava plants showing symptoms of anthracnose were collected from the guava 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 guava plants and re-isolated to comply with Koch's postulate.

Evaluation of Growth

One centimeter agar disk of the pure culture was transferred unto the prepared plant extract- or Benomyl® -modified PDA media. After 24 hours, the colony diameter along premarked 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 thespread-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.

EFFECT OF PLANT EXTRACTS AND FUNGICIDE ON DISEASE INCIDENCE

The field experiment was conducted at Ekiti State University Teaching and Research Farm, Ado-Ekiti (7.712^{o}N, 5.2523^{o}E). The first trial was done in May 2000 and repeated during the same period in 2021. Total area of the farm was 1200m^{2}. Guava variety (Apple) was planted at a spacing of 3mx 3m separated by boarder row of 1m, there were 100 stands of guava sown at two seeds/hole. The experiment was laid in a Randomized Complete Block Design (RCBD) with three replicates. Germination was observed at five weeks after planting. The plants were later thinned to one plant per stand. Five weeks after germination, three concentrations (15, 30, 45%) of leaf extracts (*Moringa oleifera*, *Catharanthus roseus* and *Anacardium occidentale*) were sprayed on the 10 weeks old guava in the first subplot and inoculated with the conidia suspension after 48hours. Benomyl a conventional synthetic fungicide was applied at the rate of 0.1g/litre while the control plot was sprayed with distilled water. Plants in the second subplot were inoculated with the spore suspension and later sprayed either with the plant extracts or benomyl after 48hours. In the third plot, the guava was sprayed with the spore suspension followed by immediate application of benomyl or plant extract. In all the experiments, conidia suspension containing 10^{4} Conidia ml^{-1} was used. Thereafter, plants were tagged in each plot and assessed for diseases incidence.

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 10 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 (45% w/v), the growth rate induced by *Moringa oleifera* leaves was 2.88 while that of *Anacardium occidentales* and *Catharathus roseus* were 3.22- and 3.30-mm day-^{1} respectively.

Table 1: Effect of three concentrations of hot water leaf extracts of three plants on growth rate *C. gloeosporioides* in Ado-Ekiti.

	Extracts Concentrations % (w/v)/ Growth rate (mmday^{-1})		
Plants extracts	15	30	45
M. Oleifera	3.65^{c} (15.70) *	3.40^{c} (21.48)	2.88^{d} (33.6)
A. occidentale	3.67 ^{c} (15.44)	3.54^{c} (18.43)	3.22^{c} (25.64)
C. roseus	3.68^{b} (15.21)	3.55^{b} (18.20)	(3.30) ^{b} 24.0
Benomyl	2.76^{d}	2.76^{d}	2.76^{d}
Control	4.34^{a}	4.34^{a}	4.34^{a}

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

Table 2 shows the effects of the plant extracts on conidia germination *C. gloesporiodes*. There was 68-100% germination of conidia irrespective of the plant extracts or concentrations. At the highest concentration, conidia germination was low but as the concentrations reduced, conidia germination was high

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

	Conidia Germination (%)		
Conc. Extracts	*M. Oleifera*	*A. occidentale*	*C. roseus*
0	100	100	100
15	82 (18) *	84(16)	86 (24)
30	75 (25)	76 (24)	77 (27)
45	68 (32)	70 (30)	72 (28)

Values in parentheses are % reduction in Conidia germination
Means with the same letter in each column are not significantly different (P≤0.05) (Turkeys. 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 extracts of *M. Oleifera* at 45% Concentration two days before innoculation compared favourably with the benomyl synthetic fungicide

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Table 3: Sequence of application of inoculum, benomyl and plant extracts on disease incidence in guava anthracnose disease in Ado-Ekiti, Nigeria.

Plant extracts	Conc.	2DBI(%)	2DAI(%)	CAE1(%)
M. oleifera	45	22.6^{d}	26.5^{c}	28.8^{c}
	30	26.2^{c}	28.3^{c}	30.2^{c}
	15	30.4^{b}	32.7^{b}	34.6^{b}
A. occidentales	45	25.4^{c}	27.3^{c}	29.5^{c}
	30	27.2^{c}	29.4^{c}	31.3^{c}
	15	31.3^{b}	33.5^{b}	34.8^{b}
C. roseus	45	25.8 ^{c}	27.9^{c}	29.6^{c}
	30	27.6^{c}	29.9^{c}	32.9^{c}
	15	31.7^{b}	33.9^{b}	35.0^{b}
Benomyl	0.1g/l	20.2^{d}	20.1^{d}	20.3^{d}
Control		74.2^{a}	76.4^{a}	77.8^{a}

DBI = Days before Inoculation
DAI = Days after Inoculation
CAE = Concurrent application extracts followed by Inoculation
Means with same letter are not significantly different with Turkeys (HSD).

Table 4 shows the comparative efficacy of benomyl synthetic fungicide and the three plant extracts on the incidence of guava anthracnose disease. Incidence of the disease was concentration dependent and higher where lower concentrations of the plant extracts were used on pooled mean basis. Disease incidence with the application of benomyl synthetic fungicide (19.8) was not significantly different from those of the three plant extracts at the highest concentration used in the study

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Table 4: Comparative Effect of Benomyl fungicide and three plant extracts on incidence of Guava anthracnose disease in Ado-Ekiti, Nigeria.

		Incidence of Disease %		
Plant extract	Conc %	2020	2021	Pooled mean
M. Oleifera	45	20.40^{d}	22.60^{d}	21.50^{d}
	30	26.54^{c}	28.40^{c}	27.47^{c}
	15	32.42^{b}	34.50^{b}	33.46^{b}
A. occidentalis	45	21.72^{d}	22.32^{d}	22.02^{d}
	30	27.40^{c}	29.54^{c}	28.47^{c}
	15	31.74^{b}	33.92^{b}	32.83^{b}
C. roseus	45	21.90^{d}	23.95^{d}	22.93^{d}
	30	28.52^{c}	30.65^{c}	29.54^{c}
	15	32.64^{b}	34.86^{b}	33.75^{b}
Benomyl		19.40^{d}	20.20^{d}	19.8^{d}
Control		72.40	76.80^{a}	74.60^{a}

Means with same letter are not significantly different with Turkeys (HSD).

DISCUSSION

Anthracnose caused by *Colletotrichum gloeosporioides* is known to affect the nutritional and market value of guava in Nigeria with over 40% of the fruits severely infected. The non-infected fruit has been reported to have higher carbohydrate, crude fibre, ash, fat, protein, calcium, Iron and phosphorus than the infected ones and hence the need for the control (Amusa *et al.,*2006). In the study, the leaf of *M. oleifera, A. occidentales and C. roseus* were chosen for the control of guava anthracnose based on previous reports that indicated its antifungal and antimicrobial properties, which is effective against phytopathogens (Bera and Anita, 1983, Andayanie *et al*., 2021, Zahari *et al*., 2018 and Ahmad *et al*., 2021). In addition to this, the leaf is relatively available in the environment.

The method of extraction of the bioactive ingredients in the study involves the use of hot water to extract the active components from the morphological part of the plant that was used (leaf). The result of which shows that the active ingredients were present in sufficient amount to inhibit growth and conidial germination and also check the spread of the disease on the field. This method of extraction is cheap and can easily be recommended to peasant farmers for diseases control (Ghost *et al*., 2002).

In this study, the extracts from the leaf of the three plants were mixed with PDA in different concentrations and tested against *C. gloeosporiodes* causing anthracnose disease of guava, the result of which shows that the growth of the fungus was inhibited or impaired. The rate of inhibition was concentration dependent, higher inhibition of growth occurred at relatively higher concentrations of the plant extracts. *M. oleifera* at 45% concentration was the most effective and closely followed by *A. occidentales and C. roseus*. The inhibition was probably due to availability of antifungal phytochemical in the medium. Resvey *et al*., (2014) evaluated the antifungal activity of *M. oleifera* oil and seed extract against seven pathogenic fungi, the result of which shows that the extracts reduced the growth rate of the fungus.

Similarly, Zahari *et al.,* (2018) evaluated the antifungal compound isolated from *C. roseus* to control the mycelia growth of root rot disease of rubber caused by the pathogen *Armillaria mellea*, the study shows that the antifungal compound inhibits mycelia germination of the fungus which is in agreement with the present study.

In this study, all the three extracts at the tested concentration inhibited conidial germination of *C. gloeosporiodes* by 18-32% irrespective of plant extracts or concentration. Ahmadu *et al*., 2020 evaluated the antifungal activity of moringa leaf and seed against *Botrytis cinerea* causing grey mold disease of tomato, the study shows that the extracts inhibit conidia germination of the fungus which is in agreement with the present study.

In this study, field trials were conducted to evaluate disease incidence where the three extracts and benomyl were applied before inoculation, shortly after inoculation and inoculation of the plant before application of the extracts and benomyl in the possible shortest time. Disease incidence was significantly lower when extracts

and benomyl were applied two days before inoculation of the guava plant, the trend common to all the extracts and benomyl compared to application of the extracts and benomyl two days after inoculation or inoculation followed by application of extracts and benomyl.

This result suggests that extracts and benomyl were more effective when applied as a preventive rather than curative means. The result of the present study agrees with the work of Amadioha and Obi, (1999) who reported the control of rice blast caused by the fungus *Pyricularia oryzae* with extracts of *Azadirachta indica* and carbendazim fungicide, the study shows that both the fungicide and extracts were more effective on the field when applied before inoculation.

In this study, disease incidence was lower at higher concentrations of the three plant extracts on pooled mean basis and was not significantly different from that of benomyl fungicide. However, at lower concentrations of the extracts, significant differences were observed among the three plant extracts and benomyl such that incidence of disease in benomyl treated plots were lower than that of the extracts treated plots. Falade (2021) controlled downy mildew disease of cucumber caused by *Pseudoperenospora cubensis* using extracts of *Datura stramonium*, *Ricinus communis* and *Sida acuta* alongside with benomyl synthetic fungicide, the study shows that three plant extracts at the highest concentration (60%) compared favorably with the use of benomyl fungicide which is in agreement with this study.

CONCLUSION

This research provided information that guava anthracnose can be controlled with the use of plant extracts in the laboratory as well as on the field. The three leaf extracts used in the study are readily available in homesteads and the method of extraction is simple, this can easily be adapted by peasant farmers for the control of guava anthracnose disease, thereby replacing the conventional fungicide that are costly alongside the attendant side effects.

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