Comparative Effect of Nigerian Indigenous Plants In The Control of Anthracnose Disease of Mango Fruits

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Abstract— Production of anthracnose-free mango fruits in Southwestern Nigeria rely heavily on the use of synthetic pesticides. But the hazardous effects of these pesticides on both human and the environment especially among illiterate farmers in the region offsets their benefits. This study, investigated the effect of aqueous and alcohol extract of *Anonna squamosa, Azadirachta indica* and *Vernonia amygdalina* in the control of fruit anthracnose disease of mango. The result of the investigation revealed that there was a significant reduction in the development and severity of anthracnose lesions on mango fruits treated after harvest with aqueous and alcohol extracts of the three indigenous plants. One-way ANOVA with Duncan Multiple Range Test conducted revealed that, the least disease index of 0.27 was recorded in both 10% (aqueous extract) and 30% (alcoholic extract) concentrations of *Anona squamosa* treatments and this was superior to 0.33 disease index recorded in benomyl treated fruits. Based on the result of this investigation, the three tested indigenous plants have the potential of good candidates for the replacement of synthetic pesticides in the control of fruit anthracnose disease of mango.

Keywords— Anthracnose disease, control materials, comparative effect, indigenous plants.

1 INTRODUCTION

ANTHRACNOSE caused by Colletotrichum gloeosporioides is presently recognized as the most common and most important field and postharvest disease of mango widely distributed in all mango-growing regions of the world [23], [6] [24]. It is the major disease limiting mango fruit production in all countries where mangoes are grown, especially where high humidity prevails during the cropping season and usually more serious in years when rain and heavy dews are frequent, from the onset of flowering until fruits are about half their sizes [12] [11]. The postharvest phase is the most damaging and economically significant phase of the disease worldwide, affecting directly and rendering marketable fruits unattractive and worthless [2], [3], [7]. There is presently no mango cultivar completely resistant to anthracnose [26] and therefore, to have anthracnose free mangoes, the trees must be protected with fungicides [9], [22]. Chemical control is not only very expensive; it is largely unavailable in Nigeria. In addition, the increasing attention given to the hazardous effects of chemicals and the development of resistance by pathogens worldwide [16], [17], [20], [14] does not make chemical control of anthracnose an attractive option anymore. This present research was initiated to investigate the fungicidal potentials of some naturally occurring indigenous plants capable of controlling fruit anthracnose of mango as an alternative to chemical control.

2 MATERIALS AND METHODS

2.1 Study areas

The study was conducted in Ibadan and Ogbomosho in the humid forest zone of Southern Nigeria during mango fruiting seasons in 2008, 2009 and 2010. Ibadan; a lowland rain forest zone situated at latitude 7° 23'N and longitude 3° 55'E, is 200 mm above sea level with annual rainfall of 1200 mm and mean daily temperature of between 24°Celsius (minimum) and 34°Celsius (maximum) lying between the humid forest and derived savannah agro-ecologies of Nigeria. Ogbomosho in Oyo state representing derived savannah vegetation lying between latitudes 8° 07'N and 8° 12'N; longitudes 4° 04'E and 4° 15'E and situated at about 600 millimeter above sea level with annual mean temperature of 26.2°Celsius and monthly temperature of 28°Celsius. Annual rainfall of this area is about 1247 millimeter with relative humidity ranging between 75 and 95 per cent.

2.2 Fungal pathogen

Pure cultures of Colletotrichum gloeosporioides were obtained from symptomatic mango parts. Portions of peeled epicarp and flesh of infected mango fruits were removed at the point of progression of the disease symptom. The removed portions were cut into small pieces; soaked in 70 per cent ethanol solution for 3 minutes, later, soaked into 1 per cent Sodium hypochlorite (NaOCI) for another 3 minutes, and then rinsed in two changes of sterile distill water. The parts were, dropped on sterile paper towels on laboratory bench, allowed to dry before plating them onto Potato Dextrose Agar (PDA) and incubated for 5 days at temperature range of 28 to 30°Celsius. Isolations were carried out in 3 replicates. Isolated colonies were, sub-cultured into fresh plates until pure cultures were obtained. Pure cultures obtained were identified by visual examinations and viewing under stereo and compound electronic microscopes. They were then identified based on conidia and colony morphology as described by [18] and [10]. Plating was carried out under a Lamina flow chamber in the Nigeria Agricultural Quarantine Service Plant Pathological laboratory, Moor Plantation, Ibadan.

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2.3 Evaluation of botanicals as fruit anthracnose of mango control materials

The efficacy of plants' extract as control materials for the control of mango fruit anthracnose was evaluated on the linear mycilial growth and spore germination of *Collectotrichum gloeosporioides* and their effects on anthracnose disease index, decay and acceptability of mango fruits treated with them.

2.3.1 Plant materials

Plant selection was based on the fungicidal background previously reported in literature. Three indigenous plants (*Annona squamosa, Azadirachta indica* and *Vernonia amygadilina*) were used in the study.

2.3.2 Aqueous plant extract

Following [1] method, 100 grams each of *Azadirachta indica, Annona squamosa* and *Vernonia amygdalina* leaves; sun-dried for 2 days, were separately ground in a blender, added to 1000 milliliters of distilled water (i.e., 1:10w/v). The solution was allowed to stand under cold conditions for 24 hours and then filtered through a 45-micron mesh two-cheesecloth. The resultant filtrate was regarded as crude extract. Concentrations of 10, 20, 30, 40 per cent and the crude extract were used for the experiments. To prepare 10 per cent concentration level, 1 litre of crude extract was diluted into 9 litres of sterile distilled water.

2.3.3 Alcohol plant extract

Following the method described by Khan *et al.* (2004), fresh leaves of the selected indigenous plants were collected and airdried. The dried leaves were ground in a grinder to a powder state, subjected to cold extraction with 95 per cent alcohol for 8 days, and then filtered through two layers of cheesecloth.

2.3.4 Effect of botanicals on mycelial growth of anthracnose fungus

The effect of the plant extracts on the linear mycelial growth was evaluated using hole-plate diffusion method of [8]. Petri dishes containing 15 milliliters of potato dextrose agar each were inoculated with 5-millimeter disc of fungal pathogen at the center surrounded by 3 wells of 1 centimeter each in diameter at a distance of 1 centimeter from the fungal pathogen. Each well was, added with 100-micron liter of the aqueous or ethanol extracted plant extracts. Three plates were used for each plant extract as replicates and sterile water was used for control. Inoculated plates were incubated at 25 to 30°Celsius until mycelial growth of fungal pathogen covered the surface of the agar medium in control treatment. The antifungal activity of each plant extract was measured by measuring the mycelial growth of the pathogen on PDA with measuring rule and the percentage of linear growth reduction (LGR) of fungal pathogen in relation to the control was, calculated using the following formula:

 $LGR(\%) = n - x/n \times 100$ Where;

n =Growth in control

x = Growth in treatment.

2.3.5 Effect of botanicals on anthracnose fungi spore germination

On the effect of plant extracts on spore germination, slide technique method of [1] was used. Each plant extract was added to dried clean slides as films and then 0.1 millileter of spore suspension of fungal pathogen was sprayed over the films with hand sprayer while a control was prepared as a film of distilled water. Three slides were, used as replicates for each plant extract. Each slide was, placed on glass rod in Petri dish under moistened conditions and incubated for 24 hours at 25°C. Using 4 microscopic fields ($x = 10 \times 40$) for each replicate, spore germination (SG) percentages were calculated using the following formula;

SG (%) = $x/N \times 100$ Where; x = Spore germination number N = Total spore number

2.3.6 Comparative effect of botanicals and different control materials on mango fruit anthracnose

One hundred and fifty (150) green matured mango fruits were randomly collected from the sampling areas. The fruits were mixed together and later subdivided into 10 parts of 15 fruits each with each fruit representing a replicate. Each part containing 15 numbered fruits was treated separately with botanicals (water or ethanol extraction), hot water and fungicide (benomyl). One part was treated with sterile distilled water to serve as control. For botanicals, the fruits were soaked in aqueous plant extract for 30 minutes and air-dried. For heat treatment, the fruits were soaked in hot water at 55°Celsius for 5 minutes as described by [25]. Benomyl 50 per cent w/w at 100 grams per 100 liter of water was sprayed over the fruits and completely covered with sterilized paper towels while in the case of control treatment; the fruits were thoroughly washed and soaked in sterile distilled water for 30 minutes and air dried. The treated fruits for each treatment were arranged in separate boxes laid out in a completely randomized design layout and allowed to ripen for 16 days under humid condition. The fruits were each examined for anthracnose lesions every other day beginning from the sixth day after treatment when ripening has commenced until the sixteenth day. Fruit anthracnose was assessed using the standards for the assessment of fruit anthracnose of mango proposed by [4]. Scale 1 to 5 was used, where scale 1 represents no fruit lesions, 2 represents 1 to 3 lesions, 3 represents 4 to 6 lesions, 4 represents 7 to 15 lesions and 5 where more than 30 per cent of fruit surface is covered with lesions. Disease index (number of infected fruits), and anthracnose rating (percentage of disease ratings on the average) was then obtained by the following formula:

DI(%) = x/N $DR(\%) = \sum (a+b)/N \times 100$ Where:

 $\sum (a+b) =$ Sum of disease ratings in fruits assessed

- \overline{N} = Total number of fruits assessed
- x = Number of infected fruits

2.4 Statistical analysis

Data collected were, subjected to One-way Analysis of Variance (One-way ANOVA) using Statistical Package for Social Sciences (SPSS) 14.0 version. Post Hoc tests were, conducted using Duncan Multiple Range Test to separate means of treatments into homogeneous subsets at 5 per cent level of significance.

3 RESULTS

3.1 Effect of aqueous and alcohol plant extracts on mycelial growth and spore germination of mango fruit rot fungi

Table 1 shows that aqueous and alcohol extracts of both Azadirachta indica and Vernonia amygdalina significantly reduced mycelial growth and spore germination of Colletotrichum gloeosporioides. Alcohol extract of Vernonia amygdalina was superior to other extracts in mycelial growth reduction. It reduced radial growth of mycelia to 3.0 centimeters representing 62.5 per cent reduction and spore germination to 127.33 millimeters representing 66.11 per cent reduction when compared with the mycelial growth and spore germination in the control. Aqueous extract of Azadirachta indica also significantly reduced radial mycelial growth to 3.5 centimeters representing 56.25 per cent reduction and spore germination to 134.30 milliliters representing 64.25 per cent reduction. Benomyl was the most superior in the reduction of mycelial growth with a record of 0.00 centimeter growth representing 100 per cent growth reduction and spore germination of 27.67 milliliters representing 92.63 reductions.

Table 1: Effect of plant extracts on in-vitro mycelial growth and spore germination of *Colletotrichum gloeosporioides*.

Plant extract	Mycelia	Per cent	Spore	Per cent
(Treatment)	I growth	decrease	germinatio	decrease
	(cm2)	over	n	over
		control	(ml-1)	control
Control	8.0 ^a	-	375.67 ^a	-
Benomyl	0.0 ^e	-100	27.67 ^d	-92.63
A. squamosa*	8.1 ^ª	+1.25	382.60 ^a	+1.84
A. squamosa ^q	6.3 ^b	-21.25	298.33 [°]	-20.57
A. indica*	3.5 ^{cd}	-56.25	134.30 [°]	-64.25
A. indica ^q	4.2 ^{cd}	-47.50	158.67 ^b	-57.76
V. amygdalina*	4.5 ^c	-43.75	173.67 [°]	-53.77
V. amygdalina ^q	3.0 ^d	-62.50	127.33°	-66.11

*Aqueous extract

Means with same letter are not significantly different at 5 per cent probability by Duncan Multiple Range Test. Values with positive or negative signs represent percentage differences in performance of treatments below or above control. 3.2 Effect of botanicals on fruit anthracnose index and severity on various mango varieties days after treatment:

3.2.1 Effect botanicals on disease index

Effect of treatment of fruits with aqueous and alcoholic plant extract shows that anthracnose disease index increased as number of days of treatment increased. Comparatively, disease indexes after 6 days of treatment were lowest while after 16 days of treatment were highest (Table 2). Ten percent concentration of Annona squamosa was the most effective in reducing anthracnose disease index throughout the study period recording 0.27 disease indexes while the highest index of 1.00 was recorded in the control treatment. Benomyl had no disease index the first 10 ten days of treatment but recorded more index than Annona squamosa at the end of the study period. Hot water treatment and 30 per cent concentration of both Azadirachta indica and Vernonia amygdalina aqueous extract reduced anthracnose index to appreciable levels for up to 10 days after treatment. The trend was similar in alcoholic extract treatments where 10 per cent concentration of Annona squamosa reduced disease index to 0.27 and was also superior to benomyl at the end of the study period (Table 3)

3.2.2 Effect of botanicals on disease severity

Mangoes treated with benomyl had the least anthracnose severity of 28 per cent (Table 4) followed by 30.67 per cent severity recorded in both 30 per cent concentrations of *Annona squamosa* aqueous extract and *Vernonia amygdalina* alcoholic extract (Table 5). The highest severity of 86.67 per cent (10.67% above control treatment) was recorded in fruits treated with 40 per cent aqueous extract concentration of *Azadirachta indica*. Crude extract of *Vernonia amygdalina* also recorded higher disease severity of 82.67 per cent about 6.67 per cent increase in severity when compared with the control treatment.

^q Alcohol extract

Table 2: Effect of botanicals on fruit anthracnose index days after treatment with aqueous plant extract

Table 4: Effect of botanicals on fruit anthracnose severity days after treatment with aqueous plant extract

Plant extract	Disease index				Treatments	Disease severity (%)			6)
(Treatments)	Day 6	Day	Day	Day		Day 6	Day	Day	Day
		10	14	16		-	10	14	16
Control)	0.33 ^d	0.73 ^b	1.00 ^a	1.00 ^a	Control)	26.67 [†]	48.00 [†]	68.00 ^d	76.00 ^c
Benomyl	0.00 ⁱ	0.00 ⁱ	0.33 ⁱ	0.33 ^h	Benomyl	20.00 ⁱ	20.00 ^p	26.67 ^p	28.00°
Hot water	0.00 ⁱ	0.20 ^h	0.53 ^g	0.60 ^g	Hot water	20.00	25.33 ⁿ	33.33 ^m	44.00 ¹
Annona squamosa – Crude	0.27 ^e	0.53 ^d	0.60 ^f	0.60 ^g	Annona squamosa – Crude	33.33 ^b	49.33 ^e	57.33 ^g	58.67 ⁹
40% concentration	0.20 ^f	0.47 ^e	0.60 ^f	0.60 ^g	40% concentration	29.33 ^d	43.33 ⁱ	56.00 ^h	57.33 ^h
30% concentration	0.13 ^g	0.27 ^g	0.27 ^j	0.27 ⁱ	30% concentration	28.00 ^f	30.67 ^k	30.67 ⁿ	30.67 ⁿ
20% concentration	0.53 ^a	0.80 ^a	0.80 ^c	0.80 ^d	20% concentration	38.67 ^ª	65.33ª	69.33°	70.67 ^d
10% concentration	0.47 ^b	0.73 ^b	0.87 ^b	0.87 ^c	10% concentration	33.33 ^b	60.00 ^b	64.00 ^e	66.67 ^e
Azadirachta indica – Crude	0.07 ^h	0.33 ^f	0.60 ^f	0.93 ^b	Azadirachta indica – Crude	21.33 ⁿ	28.00 ¹	45.33 ^ĸ	70.67 ^d
40% concentration	0.33 ^d	0.73 ^b	1.00 ^a	1.00 ^a	40% concentration	30.67 [°]	46.67 ^g	72.00 ^b	86.67 ^b
30% concentration	0.00 ⁱ	0.20 ^h	0.47 ^h	0.73 ^e	30% concentration	20.00 ⁱ	24.00°	29.33°	30.67 ⁿ
20% concentration	0.20 ^f	0.53 ^d	0.67 ^e	0.67 ^f	20% concentration	26.67 ^f	48.00 ^f	57.33 ^g	58.67 ⁹
10% concentration	0.40 ^c	0.73 ^b	0.73 ^d	0.73 ^e	10% concentration	38.67 ^a	54.67 [°]	54.67 ⁱ	54.67j
<i>Vernonia amygdalina</i> – Crude	0.13 ^g	0.80 ^a	1.00 ^a	1.00 ^a	<i>Vernonia amygdalina</i> – Crude	22.67 ^g	52.00 ^d	77.33 ^a	82.67 ^a
40% concentration	0.07 ^h	0.33 ^f	0.53 ^g	0.60 ^g	40% concentration	21.33 ^h	28.00 ¹	37.33 ¹	41.33 ^m
30% concentration	0.00 ⁱ	0.20 ^h	0.53 ^g	0.80 ^d	30% concentration	20.00 ⁱ	26.67 ^m	37.33 [']	56.00 ⁱ
20% concentration	0.20 ^f	0.53 ^d	0.60 ^f	0.60 ^g	20% concentration	29.33 ^d	38.67 ^j	49.33 ^j	49.33 ^k
10% concentration	0.20 ^f	0.47 ^e	0.80 ^c	0.80 ^d	10% concentration	30.67 ^c	45.33 ^h	61.33 ^f	61.33 ^f

Means in a column with same letter are not significantly different at 5 per cent probability by Duncan Multiple Range test. Values are means of 15 replicates.

Table 3: Effect of botanicals on fruit anthracnose index days after treatment with alcohol plant extract

Means in a column with same letter are not significantly different at 5 per cent probability by Duncan Multiple Range test. Values are means of 15 replicates.

Table 5: Effect of botanicals on fruit anthracnose severity days after treatment with alcohol plant extract

Treatments	Disease index				Treatments	Disease severity (%)			
	Day	Day	Day	Day	-	Day 6	Day	Day	Day
	6	10	14	16			10	14	16
Control)	0.33 ^a	0.73 ^ª	1.00 ^ª	1.00 ^a	Control)	26.67 ^a	48.00 ^a	68.00 ^a	76.00 ^a
Benomyl	0.00 ^d	0.00	0.33 ⁱ	0.33 ⁱ	Benomyl	20.00 ^e	20.00 ^m	26.67 ^p	28.00 [°]
Hot water	0.00 ^d	0.20 ⁱ	0.53 ^g	0.60 ^f	Hot water	20.00 ^e	25.33 ^k	33.33 [′]	44.00 ^h
Annona squamosa – Crude	0.00 ^d	0.47 ^e	0.73 ^d	0.73 ^d	Annona squamosa – Crude	20.00 ^e	37.33 [°]	50.67 ^d	50.67 ^d
40% concentration	0.20 ^b	0.67 ^b	0.73 ^d	0.73 ^d	40% concentration	24.00 ^b	42.67 ^b	48.00 ^e	48.00 ^e
30% concentration	0.00 ^d	0.47 ^e	0.60 ^f	0.60 ^f	30% concentration	20.00 ^e	32.00 ^g	44.00 ^g	44.00 ^h
20% concentration	0.00 ^d	0.47 ^e	0.67 ^e	0.67 ^e	20% concentration	20.00 ^e	33.33 ^f	42.67 ^h	42.67 ⁱ
10% concentration	0.07 ^c	0.13 ^j	0.27 ^j	0.27 ^j	10% concentration	21.33 ^d	26.67 ^j	32.00 ^m	32.00 ^m
Azadirachta indica – Crude	0.00 ^d	0.60 ^c	0.67 ^e	0.67 ^e	Azadirachta indica – Crude	20.00 ^e	40.00 ^c	45.33 ^f	45.33 ⁹
40% concentration	0.00 ^d	0.27 ^h	0.60 ^f	0.60 ^f	40% concentration	20.00 ^e	29.33 ⁿ	38.67 [,]	38.67 [,]
30% concentration	0.07 ^c	0.20 ⁱ	0.33 ⁱ	0.40 ^h	30% concentration	21.33 ^d	25.33 ^k	30.67 ⁿ	33.33 ¹
20% concentration	0.00 ^d	0.33 ^g	0.53 ^g	0.67 ^e	20% concentration	20.00 ^e	29.33 ^h	41.33 ⁱ	46.67 ^f
10% concentration	0.07 ^c	0.47 ^e	0.67 ^e	0.93 ^b	10% concentration	21.33 ^d	29.33 ^h	41.33 ⁱ	54.67 ^c
<i>Vernonia amygdalina</i> – Crude	0.00 ^d	0.33 ⁹	0.40 ^h	0.40 ^h	<i>Vernonia amygdalina</i> – Crude	20.00 ^e	28.00 ⁱ	30.67 ⁿ	32.00 ^m
40% concentration	0.07 ^c	0.40 ^f	0.40 ^h	0.40 ^h	40% concentration	22.67 ^c	32.00 ^g	34.67 ^k	34.67 ^k
30% concentration	0.00 ^d	0.07 ^k	0.40 ^h	0.47 ^g	30% concentration	20.00 ^e	21.33 ¹	28.00°	30.67 ⁿ
20% concentration	0.07 ^c	0.67 ^b	0.87 ^b	0.93 ^b	20% concentration	21.33 ^d	38.67 ^d	66.67 ^b	76.00 ^ª
10% concentration	0.00 ^d	0.53 ^d	0.80 ^c	0.87 ^c	10% concentration	20.00 ^e	37.33 ^e	60.00 ^c	70.67 ^b

Means in a column with same letter are not significantly different at 5 per cent probability by Duncan Multiple Range test. Values are means of 15 replicates.

Means in a column with same letter are not significantly different at 5 per cent probability by Duncan Multiple Range test. Values are means of 15 replicates.

4. DISCUSSIONS

Several reports have shown that production of anthracnose free mango fruits rely heavily on the use of fungicides. Nevertheless, the use of fungicides has reduced drastically due to development of resistance by fungal pathogens and public perception that fungicides have harmful effect on human health and the environment. This study tested the efficacy of botanicals as control materials capable of replacing synthetic fungicides. The results showed that, there were significant differences among all the treatments. Control treatment was slightly superior to some botanical treatments. This result tend to be in agreement with the reports of [16], [20], [14] on the decreased efficiencies of fungicides due to development of resistant by fungi pathogens worldwide. Anthracnose index was lesser in fruits treated with some concentrations levels of Annona squamosa than it was in those treated with benomy which is an indication that the extract has the potential to suppress anthracnose disease infection. Hot water treatment was only able to inhibit anthracnose infection for ten days after treatment and was inferior to some concentration levels of plant extracts in the inhibition of anthracnose infection at the end of the study period. This result confirms the observations of [21] and [5] who found hot water treatment effective in reducing anthracnose only up to the tenth day after treatment. Similarly, this result tends to support [27] who recommended that hot water treated mango fruits should be marketed as quickly as possible to avoid spoilage. Ikisan [13] and [19] reported that no control material could totally control anthracnose disease. In this present study, neither benomyl nor any other control material completely inhibited fruit anthracnose manifestation. Some concentration levels of plant extracts were inferior to the control treatment both in anthracnose index and severity.

5 CONCLUSIONS

There were significant differences between benomyl and other control materials tested in this study. Benomyl was consistent and was more effective than other materials in the overall evaluation. Most plant extracts tested in the study, rather than reduce anthracnose index and severity enhanced the growth of the fungus. This is evident in their inability to reduce fruit anthracnose index and severity below what were obtained in the control treatments. This suggests that synthetic fungicide use remain the surest means of controlling anthracnose on mango fruits. However, the limited successes recorded in the control of the disease in this study, using the available control materials, emphasizes the need and importance of further search and development of effective and safe alternative control strategies.

6 ACKNOWLEGDEMENTS

We sincerely give God all the glory and are grateful to all the people who contributed to make this study a success.

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