

# Compatibility of Mancozeb 75 WP with some plant extracts in the integrated management of *Cercospora* leaf spot disease of okra (*Abelmoschus esculentus* (L.) Moench)

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## ABSTRACT

The use of synthetic mancozeb fungicide has not been effective in the control of okra leaf spot (*Pseudocercospora abelmoschi*) disease amidst critical issues of environmental concerns. Therefore, this study evaluated the compatibility of mancozeb 75 WP with some botanicals in the integrated management of the disease. Laboratory assay was a 3×4×2 factorial laid out in a completely randomized design with three replications, while the field experiment was a randomized complete block design with 14 treatments. Four extract concentrations, 15, 30, 45 and 50% w/v and 0.5 g/l Mancozeb 75 WP were evaluated. The application of *Syzygium aromaticum* extract at 50% w/v concentration had the highest mycelial growth reduction of 71.89% of the pathogen. The soil textural class was sandy loam and treatments effect on okra growth indices did not differ significantly ( $p>0.05$ ). The highest pod yield of 15.48 t/ha<sup>-1</sup> was obtained in treatment inoculated with *P. abelmoschi*. but treated with a combination of *S. aromaticum* and Mancozeb. The same treatment had the lowest disease incidence of 4.01%, while combined application of *C. papaya*, *S. aromaticum* and Mancozeb recorded significantly ( $p<0.05$ ) lower disease severity among inoculated plants. Therefore, this combination is recommended as foliar spray on okra in place of Mancozeb.

**Keywords:** Botanicals; leaf spot disease; Laboratory assay; Mancozeb; Pathogen; Yield  
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## Introduction

Okra (*Abelmoschus esculentus* (L.) Moench), is an annual vegetable crop that is generally cultivated for its tender and succulent fruits. Nigeria is the third-largest producer of okra in the world and the crop is also about the third most consumed fruit vegetable in the country after tomato and pepper (Bello & Aminu, 2017). The immature fruits or pods contain digestible fibre and are used in making stews

and soups (Daniela *et al.*, 2012). The unripe fruits lack cholesterol, low in calories and are sources of important minerals like potassium, calcium, vitamins A and C (Reddy *et al.*, 2013; Das *et al.*, 2013). The mucilage from the fruit can be used as a medicinal preparation for plasma replacement or expansion of blood volume circulation (Maksudul *et al.*, 2015).

Fungal diseases constitute a major limiting factor to the growth and yield of okra.

These include *Cercospora* leaf spot, damping-off, powdery mildew, leaf blight and fruit rot (Farrang, 2011). Leaf spot is an important disease of okra. It limits the photosynthetic potential of the leaves, thereby reducing the overall yield performance of the crop. It is prevalent in West Africa and areas where okra is cultivated in the tropics, especially during the rainy season with high relative humidity. Symptoms of the disease on infected okra usually begin with the appearance of small brown irregular spots surrounded by pink colouration (Kumar *et al.*, 2010). Usually, symptoms first appear on the lower, older leaves while lesion appears on the younger leaves, which become necrotic at an advanced stage of development.

Breeding for resistance is a viable strategy in the prevention of okra leaf spot, but mutation could sometimes cause resistance breakdown in the plant. The disease is mainly controlled by the use of synthetic Mancozeb fungicide, especially in Nigeria. Mancozeb is a broad-spectrum protectant fungicide for the control of a wide range of diseases in crops. However, besides the deleterious effect of this chemical on man and the environment, it gives only short-term disease control and must be applied before fungal spores germinate. Therefore, multiple applications at regular intervals are required for effective control of plant diseases (Dania & Gbadamosi, 2019). The use of natural plant extracts as biopesticides in the control of plant diseases is cost-effective and harmless to biodiversity (Estrada-Cano *et al.*, 2017).

*Carica papaya* leaves and seeds are known to contain proteolytic enzymes such as papain, and other secondary metabolites including alkaloids, flavonoids and organic acids which confer anti-bacterial properties on the plant (Adebiyi & Adaikan, 2005; Doughari

*et al.*, 2007). Aqueous extracts from different papaya tissues have exhibited bioactivity against *Colletotrichum gloeosporioides* causing anthracnose disease of many crops (Bautista-Ban̄os *et al.*, 2002). The fungicidal effect of *Chromolaena odorata* (Siam weed) extract has been reported in the control of *Colletotrichum coccodes*, a rot-causing fungus of tomato fruits (Ngegbe *et al.*, 2018). Clove oil obtained from *Syzygium aromaticum* has useful antimicrobial effects on different types of plant pathogen such as fungi, bacteria and nematodes (El-Samawanty *et al.*, 2013; Mittal *et al.*, 2014; Manganayi *et al.*, 2015). Although the application of mancozeb and each of these botanicals singly could reduce disease incidence, combined application in varying doses within the context of integrated disease management is expected to yield a better outcome. Therefore, this study evaluated the efficacy of combining mancozeb and different plant extracts for the integrated management of okra leaf spot disease.

## Materials and Methods

### *Experimental site and sample collection*

The research was conducted in the Plant Pathology laboratory and crop garden of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. The University is located between latitude 7°23'N and longitude 3°54'E. The monthly rainfall at the experimental site varied between 137 and 192 mm with a mean temperature of 27.8°C and 86.61% relative humidity. The Seeds of a susceptible okra cultivar NHLe 47-4 were obtained from the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. Fresh leaves of *Chromolaena odorata* and *Carica papaya* were harvested from the Teaching and Research Farm, University of Ibadan, while

dried seeds of *Syzygium aromaticum* and Mancozeb 75% WP fungicide were purchased from the local market. A pathogenic isolate of *Pseudocercospora abelmoschi* was obtained from the mycological herbarium of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

#### *Preparation of aqueous plant extracts*

Fresh leaves of *Chromolaena odorata*, *Carica papaya* and seeds of *Syzygium aromaticum* were washed under running tap water and air-dried at 28-30°C for 12 days under a laminar flow hood chamber. Four weights 15, 30, 45 and 50 g of each plant material were pulverized into a smooth powder using a high-speed blender Model KSB 5017SR USA in 100 ml sterile distilled water, and then filtered through a Whatman No. 4 filter paper separately into a 250 ml Erlenmeyer flask to produce 15, 30, 45 and 50% w/v concentrations using cold water as the extraction medium. A synthetic fungicide, Mancozeb 75WP, which served as a positive check was formulated using the manufacturer's instruction at 0.5 g/L and applied as an amendment in potato dextrose agar (PDA). A hundred microlitre of Mancozeb was injected into each Petri dish using a 10 ml syringe, mixed with 10 ml PDA and allowed for 30 minutes to gel. The pathogen was inoculated at the centre and mycelial growth was measured for five consecutive days.

#### *Mycelial inhibition assays*

A 3×4×2 factorial design in a completely randomized design was used with three replicates to determine the best extract concentration that would inhibit the mycelial growth and sporulation of the test fungus and which was subsequently selected for the field trial. The constituents were three factors,

*C. odorata*, *C. papaya* and *S. aromaticum* evaluated at four levels (15%, 30%, 45% and 50% w/v), two controls, Mancozeb which was a positive check and negative control consisting of pathogen without extract amendment. *Pseudocercospora* isolate was maintained on potato dextrose agar (PDA) growth medium stored at room temperature (28-30°C) for four days before use in the antifungal bioassay according to Dania and Omidiora (2019). An aliquot of 100µl of each extract concentration was dispensed at the centre of the Petri dish in triplicates and 10 ml of sterilised PDA added. The mixture was stirred gently to ensure proper mixing. A four-day-old mycelium of the actively growing pathogen was removed using a sterilised 2-cm cork borer inoculated at the centre of the extract-amended PDA in Petri dishes and incubated at 28-30°C for seven days. Radial mycelial growth of treatments was measured at 24-hours intervals for five consecutive days post-inoculation using a ruler across two perpendicular lines drawn at the backside of the Petri dish and expressed as the percentage of radial growth of the control (Bautista-Baños *et al.*, 2002).

#### *Determination of physical and chemical properties of the experimental soil*

Five core samples of unsterilised soil were collected from five different points at the arable crop garden in the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria and mixed thoroughly to form a composite sample and air-dried at 60°C for two days. The soil was not sterilised in this experiment to avoid vapourisation of some nutrient constituents that may have impacted negatively on the results obtained. The soil was sieved through a 2-mm filter mesh preparatory to further analysis.

Particle size distribution was determined by the hydrometer method (Pauwels *et al.*, 1992). Soil pH was assessed after mixing 1.0 g of the soil with 10 ml sterile water and allowed to stand for 12 hours using a Philips pH meter model PW9215 (Kacar, 1997). Organic carbon (C) content was measured using dichromate-oxidation according to the modified method of Walkley and Black (1934). Total nitrogen (N) content was measured using an elemental analyzer (EA 1125 CHNS-0- Zhang, China). Exchangeable potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na), were determined using an Absorption Atomic spectrophotometer UV-VIS 2001-India. The Bray-2 method was used to determine available phosphorus (Fomenky *et al.*, 2018). Total N was determined by Kjeldhal method (Pauwels *et al.* (1992).

#### *Effect of treatment combinations on growth and yield of okra*

Field experiment was conducted at the peak of the rainy season between May and August, 2019. The soil type is a well-drained, flat, sandy loam with a mean monthly temperature of 27.8°C (Dania & Gbadamosi, 2019). The experiment was laid out in a randomized complete block design with three replications and 14 treatments. It consisted of three blocks, each having 14 plots with a total of 42 plots. Each plot had a dimension of 3.2 × 1.4 m. An alley of 1.0 m and 0.5 m was left between the blocks and plots respectively. Plant spacing was 50 × 60 cm with six plants per unit plot and 84 plants per block. Four seeds of okra were sowed per hill and later thinned to two at one week after sowing. Plots were kept weed-free by manual weeding throughout the experiment. The treatments consisted of the following combinations:

T1= Okra plants inoculated with pathogen only (Control)

T2= Uninoculated okra plants

T3= *Carica papaya*

T4= *Chromolaena odorata*

T5= *Syzygium aromaticum*

T6= Mancozeb

T7= *C. papaya* + Mancozeb

T8 = *C. odorata* + Mancozeb

T9= *S. aromaticum* + Mancozeb

T10= *C. papaya* + *C. odorata* + Mancozeb

T11= *C. papaya* + *S. aromaticum* + Mancozeb

T12= *C. odorata* + *S. aromaticum* + Mancozeb

T13= *C. papaya* + *C. odorata* + *S. aromaticum*

T14= *C. papaya* + *C. odorata* + *S. aromaticum* + Mancozeb

Of the four extract concentrations that were evaluated in the *in vitro* experiment, 50% w/v concentration of each extract which had the best inhibitory potential *in vitro* was selected and used in the field trial. *Pseudocercospora* spore concentration was prepared using the serial dilution method. This was done using two Petri dishes containing pure cultures of *P. abelmoschi*. Nine 100 ml beakers were filled with sterile distilled water up to 90 ml gauge. An aliquot of 0.1 ml Tween 20 was dispensed to the culture and 50 ml of sterile distilled water was used to scoop out the spores and mycelia on each Petri dish into a beaker. The medium in both beakers was decanted through Whatman filter paper No. 4 to collect the spores and remove the mycelia. The medium from both Petri dishes was poured into a beaker to make 100 ml of the inoculum which then served as the stock solution. Ten millilitres were transferred from the stock solution to the 90 ml in the first beaker to make 10<sup>1</sup> concentration. The procedure was used to produce three concentrations 10<sup>4</sup>, 10<sup>6</sup> and 10<sup>8</sup> and eventually 10<sup>6</sup> was selected as the optimal concentration.

Healthy okra plants with fully developed first trifoliate leaves were inoculated with an inoculum concentration of  $1 \times 10^6$  conidia/ml of the pathogen at three weeks after sowing according to Dania and Gbadamosi (2019). The treatment combinations were applied as foliar spray at two weeks after inoculation before the appearance of visible symptoms on the okra plants. The aqueous extracts and Mancozeb fungicide were mixed in a 1:1 equal volume ratio prior to application. Second spraying was done four weeks after the first when symptoms had emerged as curative therapy. Data were collected on growth parameters such as plant height, number of leaves, stem girth and yield indices including pod weight, pod length, seed weight, number of seeds per pod and yield per hectare following standard procedures. The field experiment was terminated after collection of all growth and yield data at 90 days after sowing. Fruit yield was determined according to the modified method of Biya (2018)

$$\text{Fruit yield/ha}^{-1} = \frac{\text{Length of row} \times \text{Area of hectare}}{\text{Fruit weight} \times \text{No. of rows per plot} \times \text{spacing}}$$

Fruit yield per hectare ( $\text{kg/ha}^{-1}$ ) was divided by a factor of 1000 to convert to tonnes per hectare ( $\text{t/ha}^{-1}$ ).

#### *Determination of incidence and severity*

Disease incidence was measured as the number of the crop population, which was counted as individual plants that were infected and expressed as a percentage of total plants per treatment (Brown & Kean, 2012). Disease severity was evaluated as the proportion of the area of the plant leaf that showed symptoms of the disease. The assessment was based on visual observation of the leaves of test plants for typical symptom development of brown necrotic lesions. Disease severity was rated on

a scale of 1-6 according to the modified method of Inglis *et al.* (1988):

1= No disease symptom, 2= 1-10% of leaflet area with lesion, 3= 11-25% leaflet area with lesion, 4= 26-50% leaflet area with lesion and limited chlorosis, 5= >50% of the leaflet area with lesion and sporadic necrosis and 6= Defoliation.

From the rating scale, disease index (DI) was calculated according to the method described by Nagesh and Mudge (2017):

$\text{DI} (\%) = 100 \cdot \frac{\sum [(f.v) / (n.x)]}{x}$ , where, f = number of plants with the same grade; v = observed grade; n = total number of plants evaluated and x = maximum grade on the scale.

Data were collected progressively on disease incidence and severity beginning at two weeks after inoculation (WAI) and continued at 4, 6, 8 and 10 WAI all through the growth stages. The final data recorded at 10WAI was selected and used in data analysis.

#### *Statistical analysis*

Data were subjected to one-way analysis of variance (ANOVA) using the Generalized Linear Model (GLM) procedure of the Statistical Analysis System (SAS) Version 9.4. Significant differences among means were separated using the Duncan's Multiple Range Test (DMRT) at 5% level of probability.

## **Results**

#### *Mycelial inhibition assay*

The application of the plant extracts significantly ( $p < 0.05$ ) reduced the mycelial growth of the test fungus with increasing extract concentration (Table 1). Growth inhibition of the pathogen in Petri dishes varied from 18.56-37.01% at 15% w/v concentration of *C. papaya*, *C. odorata* and *S. aromaticum*. Compared to all the test botanicals, *S. aromaticum* extract had a significant mycelial reduction of 71.89%

at 30% w/v. The extract completely inhibited pathogen growth as the concentration was increased to 45 and 50% w/v. *Carica papaya* extract inhibited *Pseudocercospora* growth by 74.78% and was more effective than *C. odorata* at 50% w/v concentration. Mancozeb

75 WP amended plates completely inhibited growth of the pathogen. Comparatively, there was zero inhibition in the control treatment without extract application.

TABLE 1  
*Inhibitory effect of botanicals on mycelial growth of Pseudocercospora abelmoschi*

<i>Treatment</i>	<i>Mycelial growth (cm)</i>	<i>Inhibition (%)</i>
Control	9.0a	0.0c
15% <i>Carica papaya</i>	7.33a	18.56bc
15% <i>Chromolaena odorata</i>	6.08a	32.44b
15% <i>Syzygium aromaticum</i>	5.71ab	37.01b
30% <i>Carica papaya</i>	5.01ab	44.33b
30% <i>Chromolaena odorata</i>	5.23ab	41.89b
30% <i>Syzygium aromaticum</i>	2.53b	71.89ab
45% <i>Carica papaya</i>	3.72ab	58.67ab
45% <i>Chromolaena odorata</i>	4.6ab	48.89ab
45% <i>Syzygium aromaticum</i>	0.0bc	100a
50% <i>Carica papaya</i>	2.27b	74.78ab
50% <i>Chromolaena odorata</i>	3.74b	58.45ab
50% <i>Syzygium aromaticum</i>	0.0bc	100a
Mancozeb	0.0bc	100a

Means with same letters along a column are not significantly different using DMRT test at  $P < 0.05$ .

#### *Determination of physical and chemical properties of the experimental soil*

The experimental soil comprised 7.2% silt, 81.31% sand and 11.5% clay, belonging to the sandy loam textural class (Table 2). It had a slightly acidic pH of 5.39

and contained average proportions of the major macronutrients, N, P and K, with an organic matter content of 6.05%. Moderate amounts of other micronutrients were also present in the soil.

TABLE 2  
Physical and chemical properties of soil  
used in the experiment

Soil parameter	Value
Silt (%)	7.22
Sand (%)	81.31
Clay (%)	11.50
Textural class	Sandy loam
pH	5.39
Organic matter (%)	6.05
Organic carbon (%)	8.64
Bulk density (kg/m <sup>3</sup> )	2.88
Available Phosphorus (mg/kg)	10.33
Available Potassium (Cmol/kg)	0.72
Total Nitrogen (Cmol/kg)	2.50
Copper (mg/kg)	2.63
Sodium (Cmol/kg)	0.52
Iron (mg/kg)	93.04
Manganese (Cmol/kg)	102.77
Magnesium (Cmol/kg)	1.41
Calcium (Cmol/kg)	2.68
CEC (Cmol/kg)	4.08

Effect of treatment combination on growth and  
yield of okra

Plant height varied between 24.71 and 40.34 cm at the vegetative stage and increased progressively through the reproductive to maturity stage (Table 3). There was no significant difference ( $p>0.05$ ) among the treatments in plant height, number of leaves and stem girth. Control plants that were inoculated with the pathogen but without treatment did not differ significantly ( $p>0.05$ ) in seed and pod yield characteristics (Table 4). However, inoculated plants that were treated with a combination of *S. aromaticum* and Mancozeb fungicide produced the highest yield of 15.48 t/ha<sup>-1</sup>, which was significantly higher than single-dose application of each extract and in the control plants.

Table 3  
Effect of treatments on growth performance of okra

Treatment	Plant height (cm)			Number of leaves			Stem girth (cm)		
	Veg	Rep	Maturity	Veg	Rep	Maturity	Veg	Rep	Maturity
Control	24.71ab	53.2ab	73.11ab	3.82a	5.88a	6.12a	0.77a	3.13a	4.05a
Uninoculated okra plants	27.3ab	45.22b	56.62bc	4.55a	6.44a	8.32a	1.24a	2.57a	3.97a
<i>C. papaya</i>	25.85ab	50.08ab	71.93ab	4.72a	5.88a	7.14a	2.15a	4.07a	5.03a
<i>C. odorata</i>	31.77ab	57.20a	80.61a	5.01a	7.43a	9.05a	1.2a	2.33a	4.31a
<i>S. aromaticum</i>	36.23a	47.88b	63.55b	3.51a	5.05a	8.77a	1.33a	3.48a	4.22a
Mancozeb	27.41ab	45.44b	60.81b	4.08a	6.54a	9.13a	1.72a	3.95a	5.01a
<i>C. papaya</i> + Mancozeb	40.34a	51.67ab	79.22a	3.95a	4.93a	6.89a	1.65a	3.02a	4.73a
<i>C. odorata</i> + Mancozeb	37.21a	48.81b	66.41b	4.17a	5.77a	7.44a	0.97a	2.43a	3.85a
<i>S. aromaticum</i> + Mancozeb	26.40ab	43.50b	58.12bc	4.22a	5.33a	8.51a	0.85a	2.33a	4.77a
<i>C. papaya</i> + <i>C. odorata</i> + Mancozeb	39.22a	56.71a	81.15a	3.66a	6.75a	8.76a	1.74a	3.52a	4.62a
<i>C. papaya</i> + <i>S. aromaticum</i> + Mancozeb	38.12a	55.84a	77.33a	3.26a	6.03a	8.31a	1.83a	3.14a	4.73a
<i>C. odorata</i> + <i>S. aromaticum</i> + Mancozeb	36.11a	46.60b	73.87ab	4.01a	5.60a	7.80a	0.82a	3.21a	4.11a
<i>C. papaya</i> + <i>C. odorata</i> + <i>S. aromaticum</i>	39.40a	52.44ab	66.21b	4.31a	6.45a	8.13a	1.34a	3.65a	4.39a
<i>C. papaya</i> + <i>C. odorata</i> + <i>S. aromaticum</i> + Mancozeb	32.77ab	45.32b	74.04ab	4.35a	5.51a	9.06a	1.55a	2.98a	5.0a

Veg= Vegetative growth stage, Rep= Reproductive growth stage

Means with same letters along a column are not significantly different using DMRT test at  $P<0.05$ .

Each extract was at 50% w/v concentration and Mancozeb 75 WP at 0.5 g/l.

TABLE 4  
Effect of treatments on yield of okra inoculated with *Pseudocercospora abelmoschi*

Treatment	Pod wt (g)	Pod length (cm)	Pod width (cm)	100 seed wt (g)	Seeds		No. of pods/Plant	Yield (t/ha <sup>1</sup> )
					pod	per		
Control	11.72a	10.75ab	7.21a	50.86a	26.32ab	6.74a	6.87b	
Uninoculated okra plants	14.81a	12.37a	5.06a	54.76a	31.86ab	8.15a	10.33ab	
<i>Carica papaya</i>	16.08a	13.78a	4.83a	51.45a	40.73a	6.17a	8.21ab	
<i>C. odorata</i>	10.24a	15.72a	8.17a	44.51ab	29.33ab	9.88a	9.23ab	
<i>S. aromaticum</i>	12.34a	10.83ab	6.33a	40.80ab	36.11a	6.77a	7.55b	
Mancozeb	15.22a	15.73a	5.64a	46.77ab	26.32ab	5.39a	11.65ab	
<i>Carica papaya</i> + Mancozeb	16.73a	8.78ab	7.65a	49.43a	31.71ab	4.33a	8.46b	
<i>Chromolaena odorata</i> + Mancozeb	17.23a	14.77a	9.41a	48.33a	36.88a	8.93a	11.17ab	
<i>Syzygium aromaticum</i> + Mancozeb	12.13a	14.66a	8.55a	45.50ab	26.77ab	6.07a	15.48a	
<i>C. papaya</i> + <i>C. odorata</i> + Mancozeb	14.88a	11.69ab	6.09a	52.21a	35.44a	7.77a	12.45a	
<i>C. papaya</i> + <i>S. aromaticum</i> + Mancozeb	13.41a	15.08a	8.40a	56.04a	33.10ab	8.62a	10.0ab	
<i>C. odorata</i> + <i>S. aromaticum</i> + Mancozeb	15.07a	14.01a	6.07a	48.20a	37.20a	6.04a	10.22ab	
<i>C. papaya</i> + <i>C. odorata</i> + <i>S. aromaticum</i>	16.31a	15.44a	5.81a	51.62a	34.03ab	6.06a	13.08a	
<i>C. papaya</i> + <i>C. odorata</i> + <i>S. aromaticum</i> + Mancozeb	15.41a	15.03a	7.32a	46.03ab	41.22a	6.02a	13.77a	

Means with same letters along a column are not significantly different using DMRT test at  $P < 0.05$ . Each extract was 50% w/v concentration and Mancozeb 75WP at 0.5 g/l.

#### Determination of incidence and severity

Plants inoculated with the pathogen and treated with a combination of 50% w/v *S. aromaticum* and 0.5 g/l Mancozeb 75WP fungicide had the lowest incidence of 4.01% at maturity, while plants treated with sole extracts had higher incidences that varied between 11.31 and 15.24%. (Table 5). The treatments differed significantly ( $p < 0.05$ ) in their inhibitory ability of leaf spot disease with control plants having the highest incidence of 24.77%. Similarly,

disease severity was significantly ( $p < 0.05$ ) lower in plants treated with extract and Mancozeb combination at the various stages of growth, including the positive check relative to control. Disease severity reduction among the treatments was significantly ( $p < 0.05$ ) higher than control plants that were inoculated with the pathogen without the application of extracts. Percent disease index (PDI) varied between 5.0 and 24.28% and control plants had significantly higher PDI than other treatments.



TABLE 5  
Effect of treatments on incidence and severity of leaf spot disease of okra

Treatment	Disease incidence (%)			Disease severity			
	Vegetative	Reproductive	Maturity	Vegetative	Reproductive	Maturity	DI (%)
Control	17.85a	23.46a	24.77a	4.30a	4.50a	4.50a	24.28a
Uninoculated okra plants	0.0b	0.0b	0.0c	1.0b	1.0b	1.0b	0.0d
<i>Carica papaya</i>	5.28ab	7.24ab	15.24ab	2.22ab	3.10ab	3.40ab	18.33ab
<i>Chromolaena odorata</i>	2.8ab	4.22ab	13.87ab	2.81ab	2.8ab	3.02ab	17.26ab
<i>Syzygium aromaticum</i>	3.77ab	5.31ab	11.31ab	2.31ab	2.55ab	2.85ab	12.43b
Mancozeb	0.74ab	2.8ab	8.10b	1.43ab	2.77ab	2.94ab	13.08b
<i>Carica papaya</i> + Mancozeb	1.83ab	2.51ab	7.67b	1.66ab	1.95bc	2.07b	10.24b
<i>Chromolaena odorata</i> + Mancozeb	2.56ab	3.09ab	9.22b	1.20ab	2.07bc	2.15b	9.07bc
<i>Syzygium aromaticum</i> + Mancozeb	0.41ab	2.74ab	4.01bc	1.20ab	1.88bc	1.94b	5.03c
<i>C. papaya</i> + <i>C. odorata</i> + Mancozeb	1.80ab	3.40ab	9.70b	1.23ab	1.78bc	3.40ab	18.33ab
<i>C. papaya</i> + <i>S. aromaticum</i> + Mancozeb	1.22ab	2.04ab	7.35b	1.13ab	1.77bc	1.88b	2.86cd
<i>C. odorata</i> + <i>S. aromaticum</i> + Mancozeb	0.81ab	1.23ab	7.13b	1.1ab	1.55c	2.0b	8.77bc
<i>C. papaya</i> + <i>C. odorata</i> + <i>S. aromaticum</i>	0.55ab	1.08ab	6.77ab	1.23ab	1.37c	1.90b	8.04bc
<i>C. papaya</i> + <i>C. odorata</i> + <i>S. aromaticum</i> + Mancozeb	0.63ab	1.30ab	6.84ab	1.28ab	1.42c	1.94b	8.23bc

Means with same letters along a column are not significantly different using DMRT test at  $P < 0.05$ .

1= No disease symptom, 2= 1-10% of leaflet area with lesion, 3= 11-25% leaflet area with lesion, 4= 26-50% leaflet area with lesion and limited chlorosis, 5= >50% of the leaflet area with lesion and sporadic necrosis and 6= Defoliation.

Each extract was at 50% w/v concentration and Mancozeb 75WP at 0.5 g/l.

## Discussion

Physical and chemical properties of the experimental soil showed that it belonged to the sandy loam textural class, contained moderate quantity of the major macronutrients, N, P, K and organic matter. Soil is a complex matter and comprises minerals, organic matter, water, and air. These fractions greatly influence their texture, structure, and porosity, which subsequently affect air and water movement in the soil layers, and thus the soil's ability to function. Therefore, soil physical and chemical properties have a great influence on soil quality and crop growth and yield performance (Watanabe et al., 2015). The textural category of soil is decided by the relative amount of various particle sizes in soil, that is, whether it is sandy loam, clay or loam. The sandy loam used in this experiment was well-drained,

aerated and contained significant amount of organic matter which may have enhanced the growth of the okra plants, hence the application of manure was unnecessary.

*Carica papaya* extract significantly ( $p < 0.05$ ) reduced radial mycelial growth of *Pseudocercospora* sp. at increasing concentrations in the *in vitro* assay. This result agrees with previous reports of Chiejina and Ukeh (2013) that fungitoxicity of plant extracts is concentration dependent. The variation in the efficacy of the extracts may be attributed to the inherent active ingredient constituents. The main secondary metabolites produced by plant extracts include phenols, flavonoids, alkaloids, saponins, tannins and steroids (Seseni et al., 2015) and their fungicidal properties against various plant pathogens have been reported (Dania & Thomas, 2019). *Carica papaya*

leaves contain alkaloids which are important elements in plant defence system against plant pathogens (Azarkan *et al.*, 2004). Also, latex constituent in papaya leaves contains chitinase enzymes, which have been shown to have strong antifungal activity against biochemical processes in fungi (Chen *et al.*, 2007; Nwinyi *et al.*, 2010). Although no analyses were done in this study to identify chitinase in the extract, these enzymes may have enhanced its antifungal activity against the test pathogen. Though *C. odorata* was the least effective among the test extracts, it however reduced the mycelial growth of the pathogen by 58.45%. Ikechi-Nwogu *et al.* (2018) had reported the efficacy of *C. odorata* extract in the management of post-harvest biodeterioration of onions.

The use of botanicals in the management of plant diseases has been reported as an economically sound approach (Farrang, 2011; El-Mohamedy *et al.*, 2013; Dania & Gbadamosi 2019). The various treatment combinations significantly reduced the incidence of okra leaf spot disease at maturity, while severity showed mild to moderate expression of visible symptoms relative to control. However, the lowest disease incidence of 4.01% was recorded in plants that were inoculated and treated with a combination of *S. aromaticum* and mancozeb. This result is consistent with the previous findings of Kumar *et al.* (2017) that reported the efficacy of combining Mancozeb fungicide with neem (*Azadiractha indica*) and garlic (*Allium sativum*) in the integrated management of *Rhizoctonia solani* causing sheath blight of rice. Estrada-Cano *et al.* (2017) reported the potential of clove oil obtained from *S. aromaticum* in reducing the incidence and severity of *Fusarium* wilt disease in tomato grown under greenhouse conditions. Similarly,

Seseni *et al.* (2015) showed the efficacy of the essential oil in inhibiting the prevalence of the damping-off disease among pine seedlings.

Plant height was not significantly influenced by the different treatment combinations that were evaluated in this study. This result is consistent with the findings of Dania and Omidiora (2019) who reported that the application of garlic extract as treatment for damping-off disease did not significantly influence growth parameters in tomato plants. However, it disagrees with report of Thabet and Khalifa (2018) that *S. aromaticum* extract treatment used in the control of *Rhizoctonia solani* strains in tomato enhanced plant height, root and shoot fresh weights of the crop. The disparity in the ability of certain plant extracts to increase plant growth may be due to elicitor's effect on physiological processes in plants such as ion uptake, cell division, enzymatic activation and protein synthesis (El-Mohamedy *et al.*, 2013).

Leaf spot disease is characterized by self-limiting necrotic lesions which reduces the leaf area available for photosynthetic and ultimate yield of infected plants. This was evident in the control which consisted of okra plants that were inoculated with the test pathogen and untreated having the lowest yield of 6.87 t/ha<sup>-1</sup>. Okra yield was significantly ( $p < 0.05$ ) higher among treatments that were inoculated and treated with a combination of extract sprays and the synthetic Mancozeb fungicide which served as a positive check. Inoculated plants that were sprayed and treated with a combination of 50% w/v of *S. aromaticum* extract and 0.5 g/l mancozeb WP produced the highest yield of 15.48 t/ha<sup>-1</sup>. This result agrees with the previous reports of Dania & Omidiora (2019) that the combination of treatments as an integrated approach has the

overall effect of reducing disease index and boosting obtainable yield among tomato plants.

### Conclusion

This study shows the efficacy of combining readily available and ecologically safe botanicals with synthetic mancozeb fungicide in the management of incidence and severity of leaf spot disease of okra. The combinations enhanced better control of the disease than the single application of mancozeb or single extract application, thus providing a credible and firm basis for an integrated approach in the management of the disease. The technology is quite simple and can easily be adopted by rural farmers who are the target beneficiary of agricultural research.

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