

Effect of plant extracts on the postharvest quality and management of pepper anthracnose incited by *Colletotrichum capsici* (Synd) Butler and Bisby on pepper (*Capsicum frutescens* L.) fruits.

ABSTRACT

The experiment was conducted using the stem and root bark extract of *Azadirachta indica* and the root bark extract of *Vernonia amygdalina* at 10% concentration. The pepper fruits were dipped in each extract for five minutes and air dried after which 1mm agar plugs of *Colletotrichum capsici* were used to inoculate the fruits. The root, stem bark extract of *A. indica* and the root bark extract of *V. amygdalina* significantly ($P \leq 0.05$) reduced the lesion diameter of *C. capsici* by 94%, 68% and 20% respectively. Pepper fruits treated with the root bark extract of *A. indica* had a severity of 2.05 while pepper fruits treated with the root bark extract of *V. amygdalina* and *A. indica* both had a severity value of 2.33. *Capsicum frutescens* fruits inoculated with *C. capsici* alone had the highest severity value of 4.47. Treating pepper fruits with the root bark extract of *V. amygdalina* and *A. indica* significantly ($P < 0.05$) reduced decay of *C. frutescens* by 76 % and 86 % respectively. Total soluble solid (TSS) of pepper fruits treated with the root bark extract of *A. indica* was significantly ($P \leq 0.05$) lower (7.50°brix) compared with the TSS value of the control (9.20°brix). The root and stem bark extract of *A. indica* can be utilized as alternative to synthetic chemicals in the treatment of pepper fruits to reduce pepper anthracnose and increase pepper fruit quality.

Keywords: *Colletotrichum capsici*, Pepper, extract, *Azadirachta indica*, *Vernonia amygdalina*.

1. INTRODUCTION

Pepper (*Capsicum* spp) is a vegetable crop belonging to the family Solanaceae and the genus *Capsicum* [1]. Peppers contain phytochemicals which assists in the prevention of cancer, stroke and other diseases when consumed in diets [2]. Pepper is important as a food, medicinal and industrial crop [3]. Extract from hot pepper is also used as botanical pesticide for the control of insect pests and diseases of economic crops in organic farming systems. Peppers are used in pickles, for flavoring sauces and in canned products. They are also used for confectionary products like bread, meat pie, and burger.

1 Nigeria is known to be one of the major producers of pepper in the world accounting for
2 about 50% of the African production [4]. In Nigeria, pepper is known as the third most
3 important vegetable after onions and tomatoes [5]. Pepper was ranked seventh in North
4 Central Nigeria and listed among the commodities with returns greater than US\$1 billion
5 over a period of 17year [6]. Anthracnose caused by *Colletotrichum* spp is a destructive
6 disease which poses a big threat to pepper production. The disease affects the quality and
7 nutritive value of fruits [7]. Pepper fruits infected by anthracnose have unpleasant colour and
8 taste and are not generally accepted in the local and international market leading to economic
9 losses [8].

10 Although earlier management practices relied mainly on the use of chemical fungicides, the
11 production of chemical residues in the fruit and the contamination of the environment by the
12 fungicides have necessitated the search for alternative control measures. Several workers
13 have reported the management of pepper anthracnose disease in-vitro and on different fruits
14 using plant extracts [7, 9, and 10]. Fruit quality management can result in increased business
15 and farm profitability [11].

16 Although there is a growing interest in the use of medicinal plants to manage pepper diseases,
17 there is dearth of information on the management of anthracnose disease on pepper fruits and
18 the corresponding effect on the quality of such fruits. This study was therefore carried out to
19 determine the effect of plant extracts on the management of pepper fruit anthracnose and to
20 determine the effect of the plant extracts on the fruit quality.

21 **2. MATERIALS AND METHODS**

22 **2.1 Collection of infected pepper fruits**

23 Infected pepper fruits with symptoms of anthracnose were purchased locally from North bank
24 market in Makurdi Local Government Area of Benue State. Collected samples were placed in

sampling paper bags and brought to the pathology laboratory of the University of Agriculture, Makurdi for examination for fungal isolation and subsequent identification.

2.2 Isolation of the fungal organisms

Several small sections (3-5mm²) were cut from the edge of the infected lesions to contain both diseased and healthy looking tissues [14]. The tissue pieces were sterilized for 1 minute in 10% Sodium hypochlorite solution after which they were rinsed in three changes of SDW and blotted dry on sterile filter papers. Potato Dextrose Agar (PDA) was prepared by adding 39g in 1 litre of Sterile Distilled Water (SDW) in a conical flask. The flask was autoclaved at 121°C for 15 minutes. After autoclaving, the media was allowed to cool to about 40°C and Streptomycin Sulphate was added at the rate of 0.2g/L. The media was then poured in 9cm Petri dishes and allowed to solidify. The tissue pieces were plated on PDA (four pieces per plate). The plates were then incubated on the laboratory bench at ambient conditions of light and temperature (30± 2°C) for 7 days. Pure culture was obtained by sub culturing unto fresh PDA plates.

Microscopic examination was done by examining the colony characteristics. A sterile needle was used in taking a little portion of the hyphae containing spores on the sterile glass slide stained with lactophenol cotton blue and examined under the microscope for fungal structures. The morphology and cultural characteristics observed were compared with reference manual [8].

2.3 Source of inoculum

Seven days old pure culture of *C.capsici* isolated from pepper fruit with symptoms of anthracnose were used. The fruit were inoculated with the inoculum using the agar plug method.

1 **2.4 Preparation of plant extracts**

2 The stem bark, root bark extract of *Azadirachta indica* (A. Juss) and the root bark extract of
 3 *Vernonia amygdalina* (Del.) were collected around the Makurdi metropolis in October 2015.
 4 The samples were washed and air-dried for three weeks then ground separately. Ten percent
 5 weight by volume (w/v) of the plant extracts were prepared by soaking one hundred grams of
 6 each sample in 1litre of distilled water in separate flasks and left to stand for 48 hours. The
 7 sample was filtered with cheese cloth and the filtrate used as extract.

8 **2.5 Inoculation of pepper fruits**

9 The pepper fruits were dipped in each extract for five minutes and air dried after
 10 which the pathogen *C. capsici* was inoculated using a 1mm agar plugs from the
 11 pure culture of *C. capsici*. Seven inoculated fruits were kept in a sterile
 12 perforated polythene bag (twenty perforation per bag) sprayed with sterile
 13 distilled water to provide humidity and replicated three times. After seven days
 14 of post-inoculation the diameter of the lesion growth were measured with a metre
 15 rule.

16 **2.6 Source of pepper**

17 Healthy pepper fruit cayenne (*C. frutescens*) locally referred to as ‘Sombo’ were
 18 obtained from a local market, washed in 10% sodium hypochlorite solution and
 19 rinsed with sterile distilled water.

20 **2.7 Experimental design and Treatments**

21 The experiment was a completely randomized design consisting of one pepper
 22 variety *Capsicum frutescens* and three plant extracts (root bark extract of *A.*
 23 *indica*, stem bark extract of *A. indica* and the root bark extract of *V. amygdalina*),

1 a positive (uninoculated) and a negative control (fruits inoculated with *C.*
2 *capsici* alone) replicated three times.

3 **2.8 Data collection and analysis**

4 Various fruit quality parameters such as total soluble solids and titratable acidity were
5 conducted at the Food Chemistry laboratory of the Department of Food Science
6 and Technology, University of Agriculture, Makurdi, Nigeria. Data was recorded
7 on lesion diameter of *C. capsici* on pepper fruits 7 days after inoculation. The
8 severity of pepper anthracnose on pepper fruits was evaluated using a scale of 0 - 5 [15]:

9 1=0 to 1% of the fruit area is affected by anthracnose

10 2= 1 to 5% of the fruit area is affected by anthracnose

11 3 = 6 to 9% of fruit area is affected by anthracnose

12 4 = 10 to 49% of fruit area is affected by anthracnose

13 5 = 50% to 100% of fruit area is affected by anthracnose

14 The pH value of the pepper juice was measured with a pH meter. The total soluble solid
15 (TSS) was measured using a refractometer (Bellingham Stanley limited) while
16 the titratable acidity (TTA) was determined using the formula below:

$$TTA = ml\ base \times normality \times 0.09 / 1ml\ of\ sample \times 100 \dots \dots \dots equation\ 1.$$

17 The percentage decay was calculated using the formula:

$$\% \ decay = \frac{A}{T} \times 100 \dots \dots \dots equation\ 2$$

18 Where A= number of decayed fruits per treatment

19 T = Total number of fruits per treatment

20 Percentage lesion reduction was calculated using the formula below adopted from [16]:

1 % lesion reduction = $DC - DT / DC \times 100$equation 3

2 Where DC = Lesion diameter of control fruits

3 DT = Lesion diameter of treated fruit.

4 **2.9 Statistical analysis**

5 The data were statistically analyzed using Genstat 10 statistical package and means were
6 separated by least significant difference (LSD) at 5% level of significance [17].

7 **3. RESULTS**

8 Data

9 Data presented in Table 1 shows the effect of plant extracts on the lesion diameter, severity of
10 anthracnose and percentage decay of pepper fruits inoculated with *C. capsici*. The lesion
11 diameter of *C. capsici* was significantly ($P < 0.05$) reduced by all plant extracts tested
12 compared with the negative control (*C. capsici* alone). *Capsicum frutescens* fruits treated
13 with the root bark extract of *A. indica* significantly ($P < 0.05$) reduced the lesion diameter of
14 *C. capsici* by 94% (1.51cm) compared with the negative control (*C. capsici* alone) having a
15 lesion diameter of 2.45cm. Treating pepper fruits with the stem bark extract of *A. indica*
16 reduced the lesion diameter of *C. capsici* by 68% (1.67cm) while treating pepper fruits with
17 the root bark extract of *V. amygdalina* reduced lesion diameter of *C. capsici* by 20%
18 (1.97cm).

19 Treating pepper fruits with the aqueous extracts of *A. indica* and *V. amygdalina* significantly
20 ($P \leq 0.05$) reduced anthracnose severity on pepper fruits. Pepper fruits treated with the root
21 bark extract of *A. indica* had a severity value of 2.05 (5% of fruit area affected) while pepper
22 fruits treated with the root bark extract of *V. amygdalina* and pepper fruits treated with the
23 stem bark extract of *A. indica* had a severity value of 2.33 (9% of fruit area affected) which
24 was significantly ($P \leq 0.05$) reduced compared with the severity of pepper anthracnose on

1 untreated but inoculated pepper fruits with a severity value of 4.47 (49% of fruit area
2 affected).

3 Pepper fruits treated with the root bark extracts of *A. indica*, *V. amygdalina* and the stem
4 bark extract of *A. indica* significantly ($P \leq 0.05$) inhibited decay of pepper fruits by 86 %, 76
5 % and 62 % respectively. Untreated pepper fruits inoculated with *C. capsici* had the
6 highest percentage decay of 96.67%.

7 Table 1: Effect of plant extracts on the lesion diameter, severity of anthracnose
8 and percentage decay of pepper fruits inoculated with *C. capsici*

Treatment	Lesion diameter (cm)	Severity	Percentage decay	Per cent inhibition of decay
<i>C. capsici</i> alone	2.45	4.47	96.67	0.00
<i>A. indica</i> root bark	1.51	2.05	14.00	86.00
<i>A. indica</i> stem bark	1.67	2.28	38.00	62.00
<i>V. amygdalina</i> root bark	1.97	2.33	24.00	76.00
Control	0.00	1.00	10.00	-
Grand Mean	1.52	2.43	36.53	
LSD(0.05)	0.36	0.23	2.39	

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10

11 Data presented in Table 2 shows the effect of plant extracts on the quality parameters of
12 pepper fruits inoculated with *C. capsici*. Pepper fruits from each treatment had pH values
13 that ranged from 5.42 to 6.00. Untreated pepper fruits inoculated with *C. capsici* produced the
14 highest pH value (6.00) but this was not significantly different from the pH of pepper fruits
15 treated with the stem and root bark extract of *A. indica* with a pH value of 5.83 and 5.85
16 respectively. The lowest pH value was recorded from un-inoculated pepper fruits with a pH
17 of 5.42.

1 The total soluble solid (TSS) of pepper fruits treated with the stem bark extract of *A. indica*
 2 had TSS value of 6.50°brix while those treated with the root bark extract of *V. amygdalina*
 3 had TSS value of 6.37°brix and pepper fruits treated with the root bark extract of *A. indica*
 4 had TSS value of 7.50°brix and this was significantly ($P \leq 0.05$) lower than the TSS value for
 5 the un-inoculated pepper fruits (9.20°brix). Un-inoculated pepper fruits had significantly
 6 ($P \leq 0.05$) greater titratable acidity (TA) content of 2.31% than those of treated and inoculated
 7 pepper fruits. Pepper fruits treated with the stem bark of *A. indica* had TA content of 1.28%
 8 while pepper fruits treated with the root bark of *A. indica* had a TA content of 1.62% and
 9 pepper fruits treated with the root bark extract of *V. amygdalina* had TA of 1.37% and these
 10 were not significantly different at 5% level of significance. However, pepper fruits treated
 11 with *C. capsici* alone produced the lowest percentage titratable acidity of 0.90%.

12 Table 2: Effect of plant extracts on some quality parameters of pepper fruits inoculated with
 13 *Colletotrichum capsici*

Treatment	pH	Total Soluble Solute (°Brix)	Titration Acidity (%)
<i>C. capsici</i> alone	6.00	7.20	0.90
<i>A. indica</i> root bark	5.85	7.50	1.62
<i>A. indica</i> stem bark	5.83	6.50	1.28
<i>V. amygdalina</i> root bark	5.54	6.37	1.37
Control(Un-inoculated)	5.42	9.20	2.31
Grand mean	5.73	7.35	1.51
LSD(0.05)	0.24	0.20	0.35

1 **4. DISCUSSION**

2 The effectiveness of plant extracts from different plant species in reducing decay of fruits and
3 maintaining the quality of pepper fruits as found in the present study can be corroborated by
4 previous findings. [10] reported the use of plant products for the management of postharvest
5 plant diseases, maintenance of fruit quality, prevention of environmental pollution and
6 accumulation of toxic substances in the produce.

7 The reduction of lesion diameter of *C. capsici* by the root bark and bark extract of neem in
8 this study agrees with the findings of [9] who reported reduction of colony growth of *C.*
9 *capsici* in-vitro and the work of [16] who recommended the use of *Azadirachta indica* as a
10 bio protective agent on tomato fruits. Fruit decay is the major cause of the termination of
11 commercial life span of fruits, which can be the result of various postharvest disease and
12 other physiological disorders. The reduction of lesion growth of *C. capsici* in this study by
13 the root bark extract of *V. amygdalina* is collaborated by the report of [10] in which the
14 aqueous extract of *Vernonia amygdalina* showed a potential in prolonging the shelf life of
15 mango fruits thereby increasing the marketability of the fruits above 42%. The observed
16 range of the TSS contents of all treated pepper fruits agrees with the report of [18] who
17 reported TSS range of 6.1 to 7.9 °Brix in pepper grown hydroponically in the greenhouse.

18 The decrease in TSS content of treated pepper fruits may be due to the use of the solids
19 present in the pepper fruit for respiration by the fruit cells. The presence of the pathogen *C.*
20 *capsici* may have influenced the production of total soluble solids hence the increase of the
21 TSS for pepper fruits inoculated with *C. capsici* alone. Also, this increase in TSS content
22 could be attributed to moisture loss by the fruits and conversion of organic acids to sugars.
23 [10] observed that the increased rate of respiration, synthesis of carbohydrates to sugars and
24 other metabolites resulted in higher TSS in mango fruits.

1 The increment in TA value for the un-inoculated fruits might be due to the presence of pectin
2 methylesterase enzyme activity; while the reduction in TA of treated fruits could be due to
3 high respiration rate and reduction in organic acids [13].

4 The present study shows that pepper fruits treated with various plant extracts had reduced
5 decay compared with the untreated pepper fruits. This agrees with the report of [10] in which
6 plant extracts were reported to check the growth of microbes that are responsible for rotting
7 thereby reducing metabolic rate of the fruits. This is also collaborated by the report of [9]
8 who reported strong fungitoxicity of the root and stem bark extracts of *A. indica*. and *V.*
9 *amygdalina* against *C. capsici in- vitro*. In a similar study, [19] reported that neem leaf
10 extract completely inhibited the production of aflatoxin in cotton seed, thus showing
11 differential effects of active ingredients on pathogens. [9] attributed the fungitoxicity of the
12 plant extracts to the presence of tannins, glycosides, alkaloids, saponin and flavonoid. [20]
13 however related the potency of *A. indica* to the compound Azadirachtin. Also [21] attributed
14 the fungicidal and bactericidal properties of neem leaf extract during *in - vivo* and *in vitro*
15 trials to the presence of several antimicrobial active ingredients in neem leaves such as
16 desactylimbin, quercetin and sitosterol.

17 In this study, the fruits with high rate of disease incidence and severity had higher pH. [10]
18 had reported that the rate of respiration could raise pH of infected banana fruits over time.
19 However, [22] attributed these changes in pH to the organic acids present in the fruit tissue.

20 **CONCLUSION AND RECOMMENDATION**

21 The result of this work shows that treating pepper fruits with the root bark and stem bark
22 extracts of neem reduced anthracnose on pepper fruits. The root bark and stem bark extract of
23 neem can be used in the treatment of pepper fruits in order to reduce pepper anthracnose.

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