Asian Journal of Agricultural and Horticultural Research

1(2): 1-7, 2018; Article no.AJAHR.40392

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

Chigoziri Ekhuemelo[1*], Richard Isa Bwala[2] and Daniel Terfa[1]

[1]Department of Crop and Environmental Protection, Federal University of Agriculture, P.M.B. 2373 Makurdi, Benue State, Nigeria.
[2]Department of Crop Protection, University of Maiduguri, P.M.B 1069, Borno State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author CE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RIB and DT managed the analyses of the study. Author DT managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAHR/2018/40392
Editor(s):
(1) Magdalena Valsikova, Professor, Department of Vegetables Production, Faculty of Horticulture and Landscape Engineering,Slovak University of Agriculture (SUA), Nitra, Slovakia.
Reviewers:
(1) Sobia Chohan, Bahauddin Zakariya University, Pakistan.
(2) Baran Aslan, Mehmet Akif Ersoy University, Türkiye.
(3) Zakaria A. M. Baka, Damietta University, Egypt.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/23866

Original Research Article

Received 11th January 2018
Accepted 20th March 2018
Published 28th March 2018

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. amydalina* and *A. indica* significantly ($P \leq 0.05$) reduced decay of *C. frutescens* by 76% and 86% respectively. Total

**Corresponding author: Email: chiekhuemelo@gmail.com;*

soluble solid (TSS) of pepper fruits treated with the root bark extract of *A. indica* was significantly (P≤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 an 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 assist in the prevention of cancer, stroke and other diseases when consumed in diets [2]. Pepper is essential as food, medicinal and industrial crop [3]. Extract from the hot pepper is also used as a botanical pesticide for the control of insect pests and diseases of economic crops in organic farming systems. Peppers are used in pickles, for flavouring sauces and in canned products. They are also used for confectionery products like bread, meat pie, and burger.

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

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

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

2. MATERIALS AND METHODS

2.1 Collection of Infected Pepper Fruits

Infected pepper fruits with symptoms of anthracnose were purchased locally from North bank 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 (3-5 mm^2) were cut from the edge of the infected lesions to contain both diseased and healthy looking tissues [12]. The tissue pieces were sterilised for 1 minute in 10% Sodium hypochlorite solution after which they were rinsed in three changes of SDW and blotted dry with sterile filter papers. Potato Dextrose Agar (PDA) was prepared by adding 39 g in 1litre 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 0.2 g/L. The media was then poured into 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 subculturing into 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 was inoculated with the inoculum utilising the agar plug method.

2.4 Preparation of Plant Extracts

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

2.5 Inoculation of Pepper Fruits

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

2.6 Source of Pepper

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

2.7 Experimental Design and Treatments

The experiment was a completely randomized design consisting of one pepper variety *Capsicum frutescens* and three plant extracts (root bark extract of *A. indica*, stem bark extract of *A. indica* and the root bark extract of *V. amygdalina*), a positive (uninoculated) and a negative control (fruits inoculated with *C. capsici* alone) replicated three times.

2.8 Data Collection and Analysis

Various fruit quality parameters such as total soluble solids and titratable acidity were conducted at the Food Chemistry laboratory of the Department of Food Science and Technology, University of Agriculture, Makurdi, Nigeria. Data was recorded on lesion diameter of *C. capsici* on pepper fruits 7 days after inoculation. The severity of pepper anthracnose on pepper fruits was evaluated using a scale of 0 - 5 [13]:

1=0 to 1% of the fruit area is affected by anthracnose
2= 1 to 5% of the fruit area is affected by anthracnose
3 = 6 to 9% of fruit area is affected by anthracnose
4 = 10 to 49% of fruit area is affected by anthracnose
5 = 50% to 100% of fruit area is affected by anthracnose

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

$$\text{TTA} = \text{ml base x normality x} \frac{0.09}{1\text{ml}} \text{of sample x100} \quad (1)$$

The percentage decay was calculated using the formula:

$$\%\ \text{decay} = \frac{\text{A}}{\text{T}} \text{x100} \quad (2)$$

Where A= number of decayed fruits per treatment
T = Total number of fruits per treatment

Percentage lesion reduction was calculated using the formula below adopted from [14]:

$$\%\ \text{lesion reduction} = \text{DC} - \text{DT/DC x 100} \quad (3)$$

Where DC = Lesion diameter of control fruits
DT = Lesion diameter of treated fruit.

2.9 Statistical Analysis

The data were statistically analysed using Genstat 10 statistical package and means were separated by a least significant difference (LSD) at 5% level of significance [15].

3. RESULTS

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

Treating pepper fruits with the aqueous extracts of *A. indica* and *V. amygdalina* significantly ($P \leq 0.05$) reduced anthracnose severity on pepper fruits. Pepper fruits treated with the root bark extract of *A. indica* had a severity value of 2.05 (5% of fruit area affected) while pepper fruits treated with the root bark extract of *V. amygdalina* and pepper fruits treated with the stem bark extract of *A. indica* had a severity value of 2.33 (9% of fruit area affected) which was significantly ($P \leq 0.05$) reduced compared with the severity of pepper anthracnose on severity value of 4.47 (49% of fruit area affected).

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

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

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

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

Treatment	Lesion diameter (cm)	Severity	Percentage decay	Per cent inhibition of decay
C. capsici alone	2.45	4.47	96.67	0.00
A. indica root bark	1.51	2.05	14.00	86.00
A. indica stem bark	1.67	2.28	38.00	62.00
V. amygdalina 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	

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

Treatment	pH	Total soluble solute (^{o}brix)	Titrable acidity (%)
C. capsici alone	6.00	7.20	0.90
A. indica root bark	5.85	7.50	1.62
A. indica stem bark	5.83	6.50	1.28
V. amygdalina 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

4. DISCUSSION

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

The reduction of lesion diameter of *C. capsici* by the root bark and bark extract of neem in this study agrees with the findings of [9] who reported reduction of colony growth of *C. capsici* in-vitro and the work of [14] who recommended the use of *Azadirachta indica* as a bio protective agent on tomato fruits. Fruit decay is the major cause of the termination of commercial life span of fruits, which can be the result of various postharvest disease and other physiological disorders. The reduction of lesion growth of *C. capsici* in this study by the root bark extract of *V. amygdalina* is collaborated by the report of [10] in which the aqueous extract of *Vernonia amygdalina* showed a potential in prolonging the shelf life of mango fruits thereby increasing the marketability of the fruits above 42%. The observed range of the TSS contents of all treated pepper fruits agrees with the report of [16] who reported TSS range of 6.1 to 7.9°Brix in pepper grown hydroponically in the greenhouse. The decrease in TSS content of treated pepper fruits may be due to the use of the solids present in the pepper fruit for respiration by the fruit cells. The presence of the pathogen *C. capsici* may have influenced the production of total soluble solids hence the increase of the TSS for pepper fruits inoculated with *C. capsici* alone. Also, this increase in TSS content could be attributed to moisture loss by the fruits and conversion of organic acids to sugars. [10] observed that the increased rate of respiration, synthesis of carbohydrates to sugars and other metabolites resulted in higher TSS in mango fruits.

The increment in TA value for the un-inoculated fruits might be due to the presence of pectin methylesterase enzyme activity; while the reduction in TA of treated fruits could be due to high respiration rate and reduction in organic acids [17].

The present study shows that pepper fruits treated with various plant extracts had reduced decay compared with the untreated pepper fruits. This agrees with the report of [10] in which plant extracts were reported to check the growth of microbes that are responsible for rotting thereby reducing metabolic rate of the fruits. This is also collaborated by the report of [9] who reported strong fungitoxicity of the root and stem bark extracts of *A. indica*. and *V. amygdalina* against *C. capsici* in- vitro. In a similar study, [18] reported that neem leaf extract completely inhibited the production of aflatoxin in cottonseed, thus showing differential effects of active ingredients on pathogens. [9] Attributed the fungitoxicity of the plant extracts to the presence of tannins, glycosides, alkaloids, saponin and flavonoid. [19] however related the potency of *A. indica* to the compound Azadirachtin. Also [20] attributed the fungicidal and bactericidal properties of neem leaf extract during in - vivo and in vitro trials to the presence of several antimicrobial active ingredients in neem leaves such as desactylimbin, quercetin and sitosterol.

In this study, the fruits with a high rate of disease incidence and severity had higher pH. [10] had reported that the rate of respiration could raise pH of infected banana fruits over time. However, [21] attributed these changes in pH to the organic acids present in the fruit tissue.

5. CONCLUSION AND RECOMMENDATION

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Berke T. The Asian Vegetable Research and Development Center Pepper Project. Proceedings of the 16th International Pepper Conference, Tampico, Mexico. November 10-12, Tamaulipas, Mexico. 2002;1-16.
2. Ademoyegun OT, Fariyike TA, Aminu-Taiwo RB. Effects of poultry dropping on the biologically active compounds in *Capsicum anuum* L (var. Nsukka yellow). Agricultural and Biology Journal of North America. 2011;2(4):665-672.
3. Ashilenje DS. Learn how to grow pepper. Phoenix publishers Limited Nairobi, Kenya. 2013;37.
4. Idowu - Agida OO, Adetimirin VO, Nwanguma EI, Makinde AA. Effects of Seasonal variability on the Performance of Long Cayenne Pepper Collected from South-western Nigeria. Researcher. 2010; 2(10):85-92.
5. Agbogidi OM, Okonmah UL. Performance of sweet pepper (*Capsicum annuum*) as influenced by weeding frequency. International Journal of Advanced Biological Research. 2011;1(1):119-122.
6. Manyong VM, Ikpi A, Olayemi JK. Agriculture in Nigeria: Identifying opportunities for increased commercialization and Investment. A summary report by IITA, USAI and University of Ibadan. 2004;28.
7. Jadesha G, Haller H, Mondhe MK, Hubballi M, Prabakar K, Prakasam V. Application of plant extracts induced the changes in biochemical composition of Banan fruits. Annals of Biological Research. 2012;3(11):5133-5140.
8. Nayaka SC, Shankar ACU, Niranjana SR, Prakash HS, Mortensen CN. Anthracnose disease of chili pepper. Technical Bulletin published by Asian Seed Health Centre Karnataka, India and Danish Seed Health Centre for Developing Countries, Denmark. 2009;14.
9. Nduagu C, Ekefan EJ, Nwankiti AO. Effect of some crude plant extracts on growth of *Colletotrichum capsici* (Synd) & Bisby, causal agent of pepper anthracnose. Journal of Applied Biosciences. 2008;6(2): 184–190.
10. Alemu K, Ayalew A, Woldetsadik K. Antifungal activity of plant extracts and their applicability in extending the shelf life of Mango fruits. Journal of Food Science and Quality Management. 2014;33:47-54.
11. Amusa NA, Ashaye OA, Amadi J, Oladapo O. Guava fruit anthracnose and the effects on its nutritional and market values in Ibadan. Journal of Applied Sciences. 2006; 6(3):539–542.
12. Agrios GN. Plant Pathology. 5th ed. Academic Press, San Diego. 2005;922.
13. Corkidi G, Balderas-Ruiz KA, Taboada B, Serrano-Carreon, L.Galindo E. Assessing mango anthracnose using a new three-dimensional image-analysis technique to quantify lesions on fruit. Plant Pathology 2006;55(2):250-257.
14. Ijato JY, Adebiyi AO, Ijadunola JA. Antifungal effects of four tropical plant aqueous and ethanol extracts on post harvest rot of tomato (*Lycopersicum esculentum*) in Ado–Ekiti, Nigeria. New York Science Journal. 2011;4(1):64-68.
15. Obi IU. Statistical methods of detecting differences between treatment means and research methodology issues in laboratory and field experiments 2nd edition AP Express Publishers, Nsukka. 2002;117.
16. Fox AJ, Pozo-Infran DD, Lee JH, Sargent SA, Talcott ST. Ripening-induced chemical and antioxidant changes in bell peppers as affected by harvest maturity and postharvest ethylene exposure. Hortscience. 2005;40:732-736.
17. Tsegay D, Tesfaye B, Mohammed A, Yirga H, Bayleyegn A. Effects of harvesting stage and storage duration on postharvest quality and shelf life of sweet bell pepper (*Capsicum annuum* L.) varieties under passive refrigeration system. International Journal of Biotechnology Molecular Biology Resources. 2013;4(7):98-104.

18. National Research Council (NRC). Neem: A Tree for Solving Global Problems. National Academy Press, Washington. 1992;141.
19. Okwute SK. Plant derived pesticidal and antimicrobial agents for use in agriculture: A review of phytochemical and biological studies of some Nigerian plants. Journal of Agriculture Science and Technology. 1992; 2 (1):62-70.
20. Singh UP, Singh HB, Singh RB. The fungicidal effect of neem (*Azadirachta indica*) extracts on some soil borne pathogens. Mycologia. 1980;7:1077-1093.
21. Salisbury FB, Ross CW. Fisiologia vegetal. Grupo Editorial Iberoamericana S.A. de C. V. Mexico. 1994;441.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/23866