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# Effect of water extract of *Xylopia aethiopica* seed and *Zanthoxylum zanthoxyloides* root on rot-inducing fungi of *Capsicum annuum* and *Capsicum frutescens*

**Adejumo, O.A[1], Claudius-Cole, A.O.[2] and Peluola, C.O.[3]**

[1]*Emmanuel Alayande College of Education, Oyo, Lanlate Campus, Lanlate, Nigeria.*

[2]*Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria.*

[3]*AAFC, Saskatoon Research Centre SK, Canada*

## ABSTRACT

ot-inducing fungi are responsible for deterioration of fruits and vegetables. This study was to identify rot-inducing fungi on *Capsicum annuum* and *Capsicum frutescens* fruits and assess percentage rot of *C. annuum* and *C. frutescens* inoculated with rot-inducing fungi incubated at 28°C ± 2°C for six days. Water extract of *Xylopia aethiopica* seed and *Zanthoxylum zanthoxyloides* root at 25,000 $mgkgl^{-1}$, 50,000 $mgkgl^{-1}$ and 75,000 $mgkgl^{-1}$ concentrations was used to coat the pepper fruits using soft brush before inoculation with the respective fungi isolated from each pepper species. *Aspergillus aculeatus* and *Aspergillus flavus* were isolated from rotted *C. annuum* while *Rhizopus stolonifer, Aspergillus flavus, Fusarium sp, Penicillium purpurogenum, Aspergillus aculeatus* and *Penicillium capsulatum* were isolated from rotted *C. frutescens.* Extract concentration at75, 000 $mgkgl^{-1}$was most effective on the rot-inducing fungi of *C. annuum* and *C. frutescens* fruits. Water extract of *X. aethiopica* seed and *Z. zanthoxyloides* seed showed potential of reducing pepper fruit rot in storage.

**Keywords:** *Capsicum annuum*, *Capsicum frutescens,* rot-inducing fungi, *Xylopia aethiopica* and *Zanthoxylum zanthoxyloides*

Corresponding address: chemadex2007@yahoo.com

## INTRODUCTION

*Capsicum annuum* and *Capsicum frutescens* are classified as fruit vegetables. The fruits are an excellent source of vitamins A and C (Bosland and Votava, 2000). *Capsicum* contains capsaicin (methyl vanillyl nonenamide), a lipophilic chemical that can produce a strong burning sensation in the mouth of the unaccustomed eater. Most mammals find this unpleasant, whereas birds are unaffected (Mason *et al.*, 1991; Norman *et al.*, 1992). Capsaicinoid present is an effective antidote to poison arrows, asthma, boils, gonorrhea, epithelioma, malaria etc (Beskstron-Sternberg *et al.*, 1994).

The availability of these vegetables is greatly reduced during off-season and the available ones in the market are of poor quality in terms of appearance and nutritive value. Microbial deterioration of *C. annuum* and *C. frutescens* fruits is favoured by high moisture and nutrients of harvested fruits. Deterioration starts occurring in the field and continues during transportation, storage and marketing (Aworh and Olorunda, 1988; Wade and Beuchat, 2003). Harvesting, packing and transportation facilitate entry of pathogen due to injuries caused during these activities (Mehrotra and Aggarwal, 2003). The losses contributed by rot-inducing fungi on *C. frutescens* and *C. annuum* fruits can be reduced by application of fungicides. The application of synthetic fungicides however poses the problem of resistance by the pathogen and harzard to man in terms of toxic residues remaining on the crop (Janiesiewicz and Korsten, 2002). Pesticides of plant origin are bio-degradable, cheap, readily available and environmentally safe compared to synthetic chemicals (Okigbo and Nneka, 2005).

*Xylopia aethiopica* (Ethiopian pepper/Guinea pepper) belongs to the family of Annonaceae. The main constituents are mono and sesquiterpenes hydrocarbons (Karioti *et al.*, 2004). *X. aethiopica* fruits contain alkaloids, flavonoids terpenoids, fixed oil and volatile aromatic oil. Key constituents are diterpenic and xylopic acids (Iwu, 1993; Shanmugam *et al.*, 2008).

*Zanthoxylum zanthoxyloides* (Candle wood) belongs to family

Rutaceae. The species was formerly known under the generic name *Fagara* Linn. *Z. zanthoxyloides* found abundantly in the savannah and dry forest vegetables and found in the drier part of South-West Nigeria extending to Niger States (Adesina, 2005). Benzophenanthridine, chelerythrine and aporphine, berberine, and the phenolic canthine-6-one isolated from the powdered root of *Z. zanthoxyloides* have also been identified to possess antimicrobial effect (Odebiyi and Sofowora, 1973). The purpose of the study was to assess the effect of water extract of *X. aethiopica* seed and *Z. zanthoxyloides* root on rot-inducing fungi of *C. annuum* and *C. frutescens.*

## MATERIALS AND METHODS

### Sample collection

The infected samples of *C. annuum* and *C. frutescens* fruits were randomly selected from different parts of Bodija market, Oyo State, Nigeria. *C. annuum* and *C. frutescens* were characterized with bell and cayenne pod types (Bosland and Votava, 2000). The seed of *X. aethiopica* and root of *Z. zanthoxyloides* were obtained from Oyo State, Nigeria.

### Isolation of rot-inducing fungi

Infected fruits were surface sterilized by dipping in 0.5% of commercial bleach for one minute and rinsed in five changes of sterile distilled water. Small segments (1-2mm) of tissues from areas bordering the infected portion were cut and prepared Potato Dextrose Agar (PDA) for seven days. The purity of culture was based on visual and microscopic examination of conidia and colony colour.

### Preparation of seed of *X. aethiopica* and root of *Z. zanthoxyloides* extract

Fifty grammes of seed of *X. aethiopica* and root of *Z. zanthoxyloides* were weighed into heating bottle and five hundred millitre of distilled water was added to plant part then heated over waterbath at 100°C for one hour, allowed to cool and the extract was sieved through Watman No 1 filter paper placed in funnel (Olabiyi *et al.,* 1992). The filtrate obtained was taken to be the stock extract (100, 000mgkg$^{-1}$). Extract concentration of 25, 000mgkg$^{-1}$, 50, 000mgkg$^{-1}$ and 75, 000mgkg$^{-1}$ were prepared from stock concentration while the control at 0 mgkg$^{-1}$ concentration consist of sterile distilled water.

**Preparation of inoculum**

Identified fungi were carefully lifted by using sterile inoculating needle into Potato Dextrose Agar (PDA) plates and placed at centre of the plates. The plates were incubated at 28 ± 2°C for ten days. The spore suspension of fungi isolated was prepared by adding 10 ml of sterile distilled water to pure culture plates. The spores were dislodged using a wad of sterilized muslin. The concentration of spore suspension was adjusted by addition of water and counted to be $1\times10^6$ conidia/ ml with the aid haemocytometer.

**Effect of plant extracts concentrations on percentage rot of *C. annuum* and *C. frutescens***

Four clean healthy *C. annuum* and *C. frutescens* fruits each were wounded at the mesocarp regions respectively. The wounded region was coated extracts of *X. aethiopica* and *Z. zanthoxyloides* before coating with fungal spores using soft brush. The control consists of fruits from each crop coated with sterile distilled water. Each fruit was placed separately in transparent polythene bags (11.7 x 25cm) to avoid contamination.

The fruits treated with spore suspension and the untreated fruits were arranged in completely randomized design with four replicate per treatments at temperature 28 ± 2°C. The rot severity was assessed after six days.

$$\text{Percentage rot} = \frac{\left[\frac{Lv}{Tv} \times \frac{100}{1}\right] + \left[\frac{Lh}{Th} \times \frac{100}{1}\right]}{2}$$

Lv = vertical lesion length; Tv = total vertical length of fruit; Lh = horizontal lesion length; Th = total horizontal length of fruit.

**Data Analysis**

Data obtained was statistically analyzed using ANOVA and the means were separated using Students Newman Keuls test (SNK) at 5% level of probability.

Six different fungi were isolated from rotted *C. annuum* and *C. frutescens* fruits. *Aspergillus aculeatus* and *Aspergillus flavus* were isolated from rotted *C. annuum* while *Rhizopus stolonifer, Aspergillus flavus, Fusarium sp, Penicillium purpurogenum, Aspergillus aculeatus* and *Penicillium capsulatum* were isolated from rotted *C. frutescens.*

Table 1: Effect of *X. aethiopica* and *Z. zanthoxyloides* on percentage rot of *C. annuum* artificially inoculated with *Aspergillus flavus* and *A. aculeatus* incubated at 28 ± 2°C for six days

| Fungi + botanicals | 25,000mgkgl$^{-1}$ | 50,000 mgkg1$^{-1}$ | 75,000mgkgl$^{-1}$ |
|---|---|---|---|
| Control | 12.50$^{b}$ | 12.28$^{c}$ | 12.67$^{c}$ |
| *A. flavus* + *X. aethiopica* | 48.98$^{a}$ | 24.87$^{c}$ | 19.96$^{bc}$ |
| *A. flavus* + *Z. zanthoxyloides* | 56.11$^{a}$ | 42.13$^{b}$ | 26.27$^{abc}$ |
| *A. aculeatus* + *X. aethiopica* | 49.11$^{a}$ | 44.09$^{b}$ | 31.35$^{ab}$ |
| *A. aculeatus* + *Z zanthoxyloides* | 66.27$^{a}$ | 59.28$^{a}$ | 39.17$^{a}$ |

Means with the same letter(s) in a column are not significantly different at $p \leq 0.05$ using SNK.

The percentage rot of *C. annuum* inoculated with *Aspergillus flavus* and *A. aculeatus* treated with *X. aethiopica* and *Z. zanthoxyloides* for six days ranged from 12.28% to 66.27% as shown in table 1. The control fruits treated with sterile distilled also showed rot ranging from 12.28% to 12.67%. At the end of the storage period, fruit rot by *A. flavus* treated with *X. aethiopica* at 50,000 mgkg$^{-1}$ was not significantly different (P≤0.05) from control. *A. flavus* treated *X. aethiopica* and *Z. zanthoxyloides* were not significantly different (P≤0.05) from control at 75,000 mgkg$^{-1}$.

In table 2, the percentage rot of *C. frutescens* fruits inoculated with *Aspergillus flavus, Fusarium sp, Penicillum purpurogenum, Penicillum capsulatum, Aspergillus aculeatus* and *Rhizopus stolonifer* ranged from 17.41% to 100%. At 25,000 mgkg$^{-1}$ and 50,000 mgkg$^{-1}$, there is significant difference (P≤ 0.05) in percentage rot of control compared with *A. flavus* treated *X. aethiopica* and *Z. zanthoxyloides, Fusarium sp* treated *X. aethiopica* and *Z. zanthoxyloides, A. aculeatus* treated *X. aethiopica* and *Z. zanthoxyloides, P. purpurogenum* treated *X. aethiopica* and *Z. zanthoxyloides, P. capsulatum* treated *X. aethiopica* and *Z. zanthoxyloides* and *R. stolonifer* treated *X. aethiopica* and *Z. zanthoxyloides.* At 75,000 mgkg$^{-1}$, the control is significantly different (P≤ 0.05) from *A. aculeatus* treated *X. aethiopica* and *Z. zanthoxyloides P. capsulatum* treated *Z. zanthoxyloides* and *R. stolonifer* treated *X. aethiopica* and *Z. zanthoxyloides.*

Table 2: Effect of *X. aethiopica* and *Z. zanthoxyloides* extract on percentage rot of *C. frutescens* fruits artificially inoculated with *Aspergillus flavus, Fusarium sp, Penicillum purpurogenum, Penicillum capsulatum, Aspergillus aculeatus* and *Rhizopus stolonifer* incubated at 28°C± 2°C for six days

| Fungi + botanicals | 25,000mgkgl$^{-1}$ | 50,000 mgkgl$^{-1}$ | 75,000mgkgl$^{-1}$ |
|---|---|---|---|
| Control | 12.92$^{d}$ | 12.99$^{e}$ | 12.83$^{d}$ |
| *A. flavus + X. aethiopica* | 62.45$^{bc}$ | 55.11$^{cd}$ | 31.00$^{cd}$ |
| *A. flavus + Z. zanthoxyloides* | 55.01$^{bc}$ | 39.07$^{d}$ | 25.31$^{cd}$ |
| *Fusarium sp +X. aethiopica* | 45.74$^{c}$ | 39.88$^{d}$ | 19.00$^{cd}$ |
| *Fusarium sp + Z. zanthoxyloides* | 56.44$^{bc}$ | 46.65$^{cd}$ | 24.57$^{cd}$ |
| *A. aculeatus + X. aethiopica* | 68.39$^{abc}$ | 43.60$^{d}$ | 36.16$^{c}$ |
| *A. aculeatus + Z. zanthoxyloides* | 88.41$^{ab}$ | 63.98$^{bc}$ | 62.90$^{b}$ |
| *P. purpurogenum + X. aethiopica* | 87.86$^{ab}$ | 59.39$^{cd}$ | 19.51$^{cd}$ |
| *P. purpurogenum+ Z. zanthoxy-loides* | 62.59$^{bc}$ | 51.76$^{cd}$ | 30.66$^{cd}$ |
| *P. capsulatum + X. aethiopica* | 73.07$^{abc}$ | 46.91$^{cd}$ | 17.41$^{cd}$ |
| [illegible] | [illegible] | [illegible] | [illegible] |

| | | | |
|---|---|---|---|
| *P. capsulatum* + *Z. zanthoxyloides* | $80.36^{abc}$ | $75.03^{b}$ | $54.35^{b}$ |
| *R. stolonifer* + *Z. zanthoxyloides* | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| *R. stolonifer* + *X. aethiopica* | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |

Means with the same letter(s) in a column are not significantly different at $p \leq 0.05$ using SNK.

## DISCUSSION

*Aspergillus aculeatus* and *Aspergillus flavus* induced rot on healthy *C. annuum* fruits while *Rhizopus stolonifer, Aspergillus flavus, Fusarium sp, Penicillium purpurogenum, Aspergillus aculeatus* and *Penicillium capsulatum* were capable of inducing rot on healthy fruits of *C. frutescens* fruit. *Penicillium sp* occurring on most fruits and vegetables during storage or transport caused post harvest losses (Agrios, 2005). *Aspergillus sp* are opportunistic pathogens without host specialization as proved in *A. flavus* (St. Leger *et al.*, 2000). Balogun *et al.* (2005) reported *Aspergillus flavus* being pathogenic on *C. annuum* and *C. frutescens* fruits. *R. stolonifer* was also reported to cause food spoilage and decay in fruits, particularly peaches, strawberries, raspberries and grapes (Northover and Zhou, 2002). Species of *Alternaria*, *Fusarium*, *Penicillium* could produce mycotoxins while grown on fruits (Stinson *et al*., 1980) even during refrigeration (Tournas and Stack, 2001).

The percentage rot of control was significantly different ($p \leq 0.05$) from percentage rot of *C. annuum* and *C. frutescens* fruits inoculated with rot-inducing fungi at 25, 000 $mgkgl^{-1}$ and 50, 000 $mgkgl^{-1}$ except *C. annuum* inoculated with *A. flavus* treated with *X. aethiopica.* Extract concentration at75, 000$mgkgl^{-1}$ was most effective on the rot-inducing fungi of *C. annuum* and *C. frutescens* fruits in storage. Okigbo and Nneka (2005) reported mycelia inhibition by *X. aethiopica.*

The use of chemicals has been regulated due to their carcinogenicity, tetratogenicity, and other residual effects. Alternatives to synthetic fungicides are now employed in order to comply with food safety standards. The advantage of water extraction of plant extracts over ethanol or acetone extraction include easy preparation and application, and cheaper cost for the benefit of peasant farmers. Water extract of *X. aethiopica* seed and *Z. zanthoxyloides* seed showed potential of reducing pepper fruit rot in storage.

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