Assessment Of The Antibacterial Activities Of Some Aqueous Plant Extracts Against *Erwinia carotovora.* subsp. A Soft Rot Bacterium Of Vegetables

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Abstract: *Erwinia carotovora* is a gram-negative bacterium that causes soft rot disease on variety of crops. *Jatropha curcas, Moringa oleifera, Vernonia amygdalina, Azardirachta indica* and *Bauhinia monandra* were evaluated for their antibacterial effects on the growth of *E. carotovora*. Hundred grams of powdered leaves of each was mixed with 200ml of distilled cold H_2O and left overnight. This was filtered and the filtrate served as extracts. The antibacterial activities of the test plant were determined using agar diffusion method. The effects of cold water leaf extracts of *A. indica* ranged from 0.55-2.85cm. It was most and least inhibitive at 5%and 20% on *E. carotovora* by 0.55cm and 2.85cm respectively, followed by *J. curcas* which had the highest and lowest inhibition at 5% and 20% on *E. carotovora* by 0.52cm and 1.68cm. *V. amygdalina* mostly reduced the growth of *E. carotovora* at 5% and 20% by 0.52cm and 0.73cm, followed by *M. oleifera* which was most antimicrobial on *E. carotovora* at 5% by 0.12cm while 20% of *M. oleifera* extracts highly reduced the growth of *E. carotovora* by 2.27cm. *B. monandra* was most and least inhibitive at 5% and 20% on *E. carotovora* by 0.20cm and 1.69cm respectively. The results showed that, the higher the concentration of different aqueous leaf extracts, the higher the inhibitory capacity on *E. carotovora*.

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Introduction

Erwinia carotovora subsp. a rod-shaped, facultative anaerobe, gram-negative bacterium of family enterobacteriaceae. *E. carotovora* is a catalase negative and oxidase positive. It can be found in the bodies of water introduced by aerosols, runoff into rivers and dumping of vegetables and also, in the guts of insects, transferable from insect to plant and vice versa. After rainfall upon diseased plants, an aerosol containing the bacteria is created. Fifty percent of the bacteria that become suspended in aerosols can survive for five to ten minutes and may travel for miles (Perombelon *et al*., 2009).

E. carotovora produces extracellular pectic enzymes that destroy the integrity of pectin. Also, it produces extracellular cellulase to degrade cellulose. Other enzymes thought to be important in pathogenesis of *E. carotovora* include arabanases, hemicellulases, protease and xylanases. The bacterium causes soft rot on variety of plant species like carrot, potatoes, cucumbers, onions, tomatoes, lettuce and ornamental plants like iris by creating an osmotically fragile cell through cell wall degrading enzymes (Bell *et al*., 2004).

High humidity and temperatures around 30^0C favour development of *E. carotovora* infection. Amadioha, (2004) reported that late maturity varieties of potato have slightly less weight loss than earlier maturity varieties, which varies from 0.1 to 16.4%. In the present study, efficacy of the five test plants extract for antibacterial activity against soft rot pathogen were tested and the plant extracts included: *A. indica, V. amygdalina, M. oleifera, J. curcas* and *B. monandra.* All the test plants are readily available across Nigeria. They are used by farmers and local indigenes for medicinal purposes. Therefore, the objective of this study is to evaluate the antibacterial effects of the leaf extracts of some plants on *E. carotovora*.

Materials And Method

Sterilization of Laboratory materials

Glass-wares used in these studies were washed in detergent, rinsed with water and allowed to dry. Erlenmeyer flasks, beakers and pipettes were wrapped in aluminum foil while Petri-dishes were placed in canisters and oven-sterilized at 160^oC for at least 3 hours. Inoculating needle, cork borers, and scalpels were sterilized before use after dipping in 70% ethanol. The inoculating chambers (laminar flow hood) and all other working surfaces were sterilized by swabbing with 70% ethanol. Sterilization of media and distilled water were done in Erlenmeyer flask plugged with non-absorbent cotton wool and autoclaved at 121^oc pressure for 15 minutes.

Sample collection

Fresh leaves of *V. amygdalina* Del, *J. curcas* L, *A. indica* A. Juss, *M. oleifera* Lam *and B. monandra* Kurz were collected around the school premises at Ekiti State University, Ado-Ekiti. The plants were identified at the herbarium unit of the Department of Plant Science, Ekiti State University. The plants were air dried at room temperature of about 37°C for two weeks. The plants were ground into fine powder using an electric blender and stored in polythene bags until needed.

Preparation of Media

Twenty-eight grams of powdered prepared nutrient agar (NA) was weighed on analytical Metller balance into 1000ml of distilled water. It was placed inside a water bath and allowed the agar to dissolve by boiling to get homogenized; the media was then sterilized in an autoclave at 121°c for 15minutes.

Extraction of plant extracts

Hundred grams of powdered leaves of each test plants were mixed with 200ml of distilled cold water at room temperature and left overnight. Thereafter, this was filtered and the filtrate served as extract. Each extracts was stored in a sterile bottle at 4°C (Refrigerating time).

Isolation of surface contaminants on the test plants

After the extraction, 1ml of each of the plant extracts was taken, using a syringe and dispensed into 9ml of sterile water. This process was serially diluted. The final sample in the test tube was corked with cotton wool to avoid contamination.

Determination of antibacterial activity

The antibacterial activities of the leaf extracts of the test plants were determined using pour plates method. The molten nutrient agar was dispensed into a sterile Petri-dish. This was allowed to cool down to 45°C, the bacterial inoculum was streaked on the medium. Wells were punched into the agar using 4mm cork borer and the hole was filled with 1ml of respective plant extracts. The plates were incubated at 37°C for 24 hours. The antibacterial activities of the test plants were determined by measuring the diametre of the zone of inhibition using meter rule.

Preparation of standard antibacterial agent (streptomycin)

The molten nutrient agar was dispensed into a sterile Petri-dish and this was allowed to cool down to 45°C, the bacterial inoculum was streaked on the medium. Wells were punched into the agar using 4mm cork borer and the hole was filled with a drop of streptomycin. The plates were incubated at 37°C for 24 hours. The antibacterial activities were assessed by measuring the diameter of the zone of inhibition using metre rule.

Results

Bactericidal effects of some plant extracts against pathogenic *E. carotovora*

The inhibitory activites of five different extracts namely: *J. curcas, V. amygdalina, M. oleifera, A. indica* and *B. monandra* against the bacteria growth of *E. carotovora* were presented in Table 2. All the plant extracts inhibited the growth of *E. carotovora* irrespective of the concentration. The result also showed that increase in the concentration of the plants extracts lead to increase in the antibacterial activities of the extract. *V. amygdalina* showed less effectiveness.

Table 1: Inhibitory Effects Of Different Plant Extracts On *E. Carotovora*

Plant Extracts	5%	10%	15%	20%	LSD
M. oleifera	0.12^{d}	1.08^{c}	1.31^{d}	2.27^{c}	0.21
J. curcas	0.52^{c}	0.92^{c}	1.07^{d}	1.68^{d}	0.07
A. indica	0.55^{b}	1.59^{b}	1.03^{d}	2.85^{b}	0.08
B. monandra	0.20^{d}	0.22^{e}	1.67^{b}	1.69^{d}	0.14
V. amygdalina	0.53^{c}	0.66^{d}	0.72^{e}	0.73^{e}	0.07
Streptomycin	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	
Control 0.00^{e}	0.00^{f}	0.00^{f}	0.00^{f}		
LSD 0.17	0.19	0.22	0.32		

Values followed by the same letters are not significantly different at P≤ 0.05 at (fisher's LSD)

Discussion And Conclusion

Bacterial disease of agricultural crop poses a great challenge while the currently used chemicals are less effective and of a great environmental concern. *In vitro* evaluation of plants for antimicrobial properties is a step toward achieving the goal of developing eco-friendly food production strategies. Inhibitory effects were obtained against *E. carotovora* from screened aqueous extracts of five plants in this study has been reported (Afolayan, *et al*; 2006). Significant differences in the levels of effectiveness exhibited among various concentrations of plant extracts may be

as a result of toxicity of different extracts which may be due to their solubility in water.

The finding of this present investigation is an important step towards developing plant based bactericides which are eco-friendly for the management of plant diseases.

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