

## An Evaluation Of Phytotoxic Activities Of Some Botanicals Against *Xanthomonas citri* (Hasse), A Canker Bacterial Pathogen Of Citrus

Ijato, J.Y

Department Of Plant Science, Faculty Of Science, Ekiti State University, Ado-Ekiti, P.M.B 5363, Ekiti State, Nigeria

E-mail: [considerureternity@gmail.com](mailto:considerureternity@gmail.com), GSM: 08067335124

**Abstract:** *Xanthomonas citri* is a gram-negative bacterium that causes citrus canker, reducing the external quality of citrus fruits. *Vernonia amygdalina* Del, *Jatropha curcas* L, *Azadirachta indica* .Juss, *Moringa oleifera* Lam, and *Bauhinia monandra* Kurz were evaluated for their antibacterial effects on the growth of *X. citri*. Hundred grams of powdered leaves of each was mixed with 200ml of distilled cold water 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 1.27-2.60cm. It was most and least inhibitive at 5% and 20% on *X. citri* by 1.27cm and 2.60cm respectively, followed by *J. curcas* which exhibited the highest and lowest inhibition at 5% and 20% on *X. citri* by 0.93 and 1.39cm respectively. *V. amygdalina* mostly reduced the growth of *X. citri* at 5% and 20% by 0.82cm and 2.20cm, followed by *M. oleifera* which was most antimicrobial on *X. citri* at 5% by 0.25cm while 20% of *M. oleifera* reduced the growth of *X. citri* by 2.74cm. *B. monandra* was most and least inhibitive at 5% and 20% on *X. citri* by 0.63cm and 1.77cm respectively. The results showed that, the higher the concentration of different aqueous leaf extracts, the higher the inhibitory capacity on *X. citri*.

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**Keywords:** Evaluation; Botanical; *Xanthomonas citri* (Hasse); Canker Bacterial Pathogen; Citrus

### Introduction

*Xanthomonas citri* pv. *citri* is a plant pathogen belonging to the family *Xanthomonadaceae*, it is a gram-negative, obligate aerobic, rod-shaped bacterium with polar flagella attached onto the membrane (Tondo *et al.*, 2011a). *X. citri* is a bacterium pathogen that causes serious canker disease on *Citrus aurantifolia*, *Citrus sinensis* and *Citrus limonia* and some citrus relatives. It reduces the external qualities of citrus fruits. Severe infection on young leaves and fruits cause extensive leaf abscission and fruit drop (Verniere *et al.*, 2003). The bacterium has a genome length of about five mega base pairs (Sahi *et al.*, 2007).

Transmission of *X. citri* occurs in plant tissues through natural openings (stomata) and mechanical injuries (wounds). *X. citri* produces colourless polysaccharides slime on media containing glucose and colonies of the species are mucoid, domed and glucose yeast-chalk agar. *X. citri* is one of the most virulent citrus diseases. The infection is characterized by occurrence of conspicuously raised necrotic lesions that develop on leaves, twigs and fruits.

Several infections can cause a range of symptoms from defoliation, blemished fruit, premature fruit drop and twig dieback to general tree decline (Francis *et al.*, 2009). Common control of the disease requires integrated cultural practices and chemical

sprays. One major limitation of using chemical control agents is that phytopathogenic bacteria frequently develop resistance to these compounds (Sigeo, 1993). Recently, much interest has been developed in the antibacterial effects of medicinal plants for phytodisease control.

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. In addition, they possess enormous therapeutic qualities. Although, citrus is an important fruit crop, yet its present status is threatened by a number of problems, including low production caused by diseases. Some plant extracts were reported as being effective inhibitors of phytopathogenic bacterial growth and *X. citri* was also suppressed by plant extracts (Leksomboon *et al.*, 2009). In recent years, the importance of vitamins as nutrients and as disease control agent has been emphasized (Pavet *et al.*, 2005). Various synthetic and biological compounds are capable of enrolling a large variety of plant diseases without displaying a direct antibiotic effect (Jakab *et al.*, 2001). Copper ammonium carbonate is a contact poison and used to achieve adequate control on susceptible citrus hosts such as grape fruit sweet orange (Graham *et al.*, 2004). The objective of this study is to evaluate the phytotoxic activities of some indigenous plants on *X. citri* that causes citrus canker disease on citrus fruits.

## Materials And Methods

### Location and experimental site

Laboratory studies were conducted at Advanced pathology Laboratory of the Institute of Agricultural Research and Training (IAR & T), Obafemi Awolowo University, Ibadan IAR & T is located to the North of Ibadan at latitude 7° 3N and longitude 3°45E. The altitude is 210m above sea level.

### 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°C 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°C pressure for 15 minutes.

### Sample collection

Fresh leaves of *A. indica*, *V. amygdalina*, *M. oleifera*, *J. curcas* and *B. monandra* were collected at Ekiti State University, Ado-Ekiti premises. The plants were identified at the herbarium unit of the Department of Plant Science, Ekiti State University. These 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 Mettler balance into 1000ml of distilled water. This was placed inside a water bath and the agar was allowed to dissolve and homogenized by boiling. This was latter autoclaved at 121°C for 15minutes.

### Extraction of plant extracts

Hundred grams of powdered leaves of each test plant were mixed with 200ml of distilled cold H<sub>2</sub>O at room temperature and left overnight. Thereafter, this was filtered and the filtrate served as extract. Each extract 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 test plants

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 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 1ml of respective plant extracts. The plates were incubated at 37°C for 24hours. The antibacterial activities of the test plants were determined by measuring the diameter 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 24hours. The antibacterial activities were assessed by measuring the diameter of the zone of inhibition using meter rule.

## Results

### Growth inhibition of *X. citri* by some extracts

The inhibitory activities of the test plants against the growth of *X. citri* were presented in Table 1. All the plant extracts inhibited the growth of *X. citri* irrespective of the concentration. However, there were significant differences in the antibacterial activities of the plant extracts on *X. citri*. The strongest inhibitory activity of 2.74cm was exhibited by 20% concentration of *Moringa oleifera*. *Azadirachta indica* at the lowest concentration at 5% inhibited the growth of *Xanthomonas citri* (1.27cm) as compared with other plant extracts and negative control (0.00cm).

The result also showed that increased in the concentration of the plant extract lead to increase in the antibacterial activities of the extracts. *Jatropha curcas* was less effective. Streptomycin thoroughly inhibited the growth of *X. citri*.

## Discussion

Bacterial disease in *Citrus* crop possess a great challenge while the currently used bactericides are not as effective as plant because they lack sufficient residual activity to protect leaf and fruit surfaces for extended periods. So, developing effective alternative plant extract to control citrus canker is more crucial (Graham *et al.*, 2000). Citrus crop and protection have

been supported by the use of botanical in developing countries in some third world countries; *in-vitro* evaluation of plant extracts for antibacterial activity is the first step toward achieving the goal of developing eco-friendly plant protection strategies (Rinaldi *et al.*, 2000). There were significant differences in the levels of effectiveness exhibited among the plant extracts,

the results also showed that increase in the concentrations of the plant extracts lead to increase in the antibacterial activities of the extracts. The use of *M. oleifera* seeds for the treatment of hard water has been reported. Similarly, it has been proved that removal of hardness of *M. oleifera* increased with increasing dosage (Muyibi and Evison 1995).

**Table 1: Inhibitory effects of different plant extracts on *X. citri*.**

Plant extracts	5%	10%	15%	20%	LSD
<i>B. monandra</i>	0.63 <sup>d</sup>	1.20 <sup>c</sup>	1.74 <sup>d</sup>	1.77 <sup>c</sup>	0.07
<i>V. amygdalina</i>	0.82 <sup>c</sup>	1.29 <sup>c</sup>	1.41 <sup>c</sup>	2.20 <sup>c</sup>	0.43
<i>A. indica</i>	1.27 <sup>b</sup>	1.85 <sup>b</sup>	2.40 <sup>b</sup>	2.60 <sup>b</sup>	0.27
<i>J. curcas</i>	0.93 <sup>c</sup>	1.11 <sup>c</sup>	1.34 <sup>d</sup>	1.39 <sup>c</sup>	0.04
<i>Streptomycin</i>	4.00 <sup>a</sup>	4.00 <sup>a</sup>	4.00 <sup>a</sup>	4.00 <sup>a</sup>	
Control	0.00 <sup>f</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	
LSD	0.18	0.23	0.43	0.55	

Values followed by the same letter(s) within the column are not significantly different from each other ( $P \leq 0.05$ ).

#### Corresponding author:

Dr. Ijato, J.Y  
Department Of Plant Science, Faculty Of Science,  
Ekiti State University, Ado-Ekiti, P.M.B 5363, Ekiti  
State, Nigeria  
E-mail: [considerureternity@gmail.com](mailto:considerureternity@gmail.com)  
GSM: 08067335124

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