Bactericidal Capacity Of Some Local Herbs On *Ralstonia solanacearum.* A Bacterium Wilt Pathogen Of Tomato (*Lycopersicon esculentum*).

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Abstract: The antibacterial effects of aqueous leaf extracts of five plants: *Azadirachta indica*, *Jatropha curcas*, *Vernonia amygdalina*, *Moringa oleifera, Bauhinia monandra* were evaluated for their antimicrobial effects on *R. solanacearum.* Fresh leaves of the test plant were collected, air dried and pulverized. Hundred grams of powdered leaves of each test plant was mixed with 200ml of distilled cold water at room temperature and left over night. These were filtered and the filtrate served as extracts. The antibacterial activities of the test plants were determined using agar diffusion method. *Bauhinia monandra* had the highest inhibition at 5%-20% on *R. solanaceraum* by 2.27cm and 2.84cm respectively, followed by inhibitory effects of cold water leaf extracts of *A. indica* which ranged from 2.41-2.50cm. *A. indica* extracts at 5%-20% concentration reduced the growth of *R. solanacearum* by 2.41-2.50cm. The effects of cold water leaf extracts of *Moringa oleifera* ranged from 0.92-2.13cm. The growth of *R. solanacearum* (0.93cm) was reduced by 10% with the least antibacterial effect at 5% by 2.13cm. The effects of cold water leaf extracts of *V. amygdalina* ranged from 1.08-1.72 cm, while *J. curcas* had the lowest inhibition at 5%-20% on *R. solanacearum* by 0.81-1.17cm and at 5%-20% on *R. solanacearum.* All the effects of varied concentrations of aqueous leaf extracts on *R. solanacearum* were significantly different from the standard (streptomycin) and control. Similarly, the test plants can play significant roles in containing phytobacterial infection on agricultural produce.
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Introduction

Ralstonia solanacearum is a soil borne, anaerobic, non-sporing, gram negative, free living, motile with a polar flagellar tuft and vascular phytopathogenic bacterium (Brlansky *et al.,* 1983). In the absence of host plants, *R. solanacearum* lives as a saprophyte in water or in the soil. Land infected with *R. solanacearum* is useless for planting susceptible crops for several years as it survives in cool weather entering a state of being viable but not culturable. *R. solanacearum* is widely distributed in the tropical and subtropical climates affecting vegetative and reproductive stages of host plants causing bacterial wilt of tomato through wounds by colonizing the xylem, thereby preventing water movement into upper portion of the plant tissue by plugging (Pradhanang *et al*., 2005).

The effect of bacterium wilt has been reported to be the most damaging (Taiwo *et al*., 2007). Though, chemicals are used in controlling bacterial wilt of tomatoes (Lesse, 1995). However, chemical treatments are short lived and less effective but biological control creates a long lasting effect thereby facilitating sustainable agriculture. In addition, high pathogen inconsistency and survival rate in diverse environments as well as extremely wide host range renders *R. solanacearum* difficult to control with chemicals (Nagouska *et al.,* 2007). Research efforts are required to reduce this problem and to understand the genetic mechanisms of resistance and for continuous studies of plant to develop new extracts (Cos *et al*., 2006).

Antimicrobial activities of crude extracts from different plants have been investigated against *R. solanacearum,* these reduced disease at different levels and increased the yield of tomato plant (Guo *et al.,* 2004).

Studies have been carried out to evaluate the effects of plant extracts against bacterial infections (Ung *et al*., 2010). Plant growth promoting bacterial strains and *Trichoderma* species were reported to be promising bio-control agents on *R. solanacearum*. Aqueous leaf extracts of some plants that possessed medicinal properties were inhibitive on *R. solanacearum.*

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 of $7^0$30N and longitude $3^0$45E. The latitude 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 and identification

Fresh leaves sample of *V. amydalina, J. curcas, A. indica, M. oleifera, B. monandra* were collected around the forest vegetation of Ekiti State University, Ado Ekiti. The plants were identified at the herbarium unit of the Department of Plant Science, Ekiti State University, Ado Ekiti. The plants were air dried at room temperature of about 37^{0}C for three weeks. The leaves were pulverized and the powered samples were packed into a clean storing bottle in the laboratory for use.

Preparation of Media

Twenty eight grams (28g) of powdered nutrient agar was weighed on Melter weighing balance into a clean 100ml beaker. This was dissolved by boiling in a water bath after dissolving to get homogenized; the media was autoclaved at 121^{0}C for 15 minutes.

Extraction of plant extracts

The plants extracts were obtained by weighing 100g of each plant sample into 200ml of distilled water and this was left overnight at room temperature. This was filtered with muslin cloth and stored in a bottle at 4^{0}C (refrigerating time).

Isolation of surface contaminants on the test plants

After the extraction, 1ml of each plant extracts was dispensed into 9ml of sterile water using hypodermic syringe. This process was serially diluted. The final sample in the test tube was corked with sterile 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, and the bacterial inoculum was streaked on the medium. Wells were punched into the agar using 4mm cork borer and the holes were 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 with 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 holes were 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 with meter rule.

Result

Bacterial associated with dried leaves of the test plants used in inhibiting the growth of *R. solanacearum*.

The result showed that the higher the concentration of different aqueous leaf extracts, the higher the inhibitory capacity on *R. solanacearum.* The result in the Table 1 showed that *B. subtlis*, *B. cereus*, *Corynebacteria*, *E. coli* and *S. aureus* were cultured as surface contaminants from various plant leaves used in this study. The result showed that *B. subtlis* was associated with all the plants. *B. cereus* and *B. subtlis* were associated with *J. curcas*, while both *S. aureus* and *E. coli* were cultured from only *V. amygdalina* and *A. indica*.

Table 1: Surface contaminants associated with dried leaves of the test plants used in inhibiting the growth of *R. solanacearum*.

Plant extracts	*B. subtilis*	*B. cereus*	*Corynebacterium*	*E. coli*	*S. aureus*
V. amygdalina	+	+	-	+	+
J. curcas	+	+	+	-	-
B. monandra	+	-	+	-	-
A. indica	+	+	-	+	+
M. oleifera	-	-	+	-	+
Ba					

Present= + **Absent=-**

Effects of some extracts on the growth of *R. solanacearum*

The inhibitory activities of *J. curcas, V. amygdalina, M. oleifera, A. indica* and *B. monandra* on *R. solanacearum* are shown in Table 2. All the plant extracts inhibited *R. solanacerum* irrespective of the concentration. However, antibacterial activities of various concentrations test plants were significantly different. The strongest inhibitory activity was exhibited by 15% aqueous extract of *B. monanadra* (2.84%), 5% aqueous extract of *J. curcas* strongly inhibited the growth of *R. solanacearum* (0.81%) compared to other plant extracts and the control (0.00%). The results also showed that, increase in concentration of the plant extracts lead to increase in antibacterial activities of the test extracts.

Table 2: Effects of Different Plant Extracts on the Growth of *R. solanacearum*

Plants Extracts	5%	10%	15%	20%	LSD
A. indica	2.41[c]	1.79[c]	1.24[c]	2.50[c]	0.16
J. curcas	0.81[e]	0.92[e]	0.84[e]	1.17[e]	0.04
B. monandra	2.27[b]	2.67[b]	2.74[b]	2.84[b]	6.45
V. amygdalina	1.08[d]	1.24[d]	1.27[d]	1.72[d]	0.08
M. oleifera	2.13[b]	0.92[e]	1.52[e]	1.87c	0.15
Streptomycin	4.00[a]	4.00[a]	4.00[a]	4.00[a]	
Control	0.00	0.00	0.00	0.00	
LSD	0.25	0.24	0.21	2.24	

Values followed by the same letters are not significantly different at $p<0.005$ (Fisher's LSD)

Discussion

Plants are rich sources of natural products used for centuries to cure various diseases that represent major constraints to food production. Natural plant products derived from plant species have the capacity to control disease caused by viruses, bacteria and fungal pathogens. Therefore, the capacity of most of the medicinal plants in controlling various plant diseases may lie in their antioxidant effects (Akinmoladun *et al.,* 2007).

Plants are one of the main sources of natural products for new therapies, particularly in poor countries, as they cost less, affects a wide range of antibiotic resistant micro-organisms. Another benefit of plant products is that, they exhibit less adverse effects (Chethana *et al.,* 2012).

Plants produce antimicrobial agents through secondary metabolism to protect themselves from pathogenic attack; many plants possess antimicrobial activity (Macdonald, 2008). All the extracts obtained from the plants showed antagonistic effects on *R. solanacearum*. The antibacterial activity of *R solanacearum* with plant extracts have been earlier reported (Lopez *et al.,* 2005; Larkin *et al*., 2007). Extracts of these plants are naturally available to play key roles in crop production, they enhance plant growth. The proliferation of undesirable microorganisms is ecologically realistic. Therefore, a plant diseases management strategy through production of bio-pesticides enhances commercial crop production (Gottlieb *et al;* 2002). Chethana *et al*., (2012) reported that most of the botanicals used in this study have antibacterial effects on most of the plant pathogenic bacteria. Therefore, in recent years medicinal plants are found to be effective reservoirs for bioactive molecules and can provide valuable sources for the discovery of natural pesticides, and the extracts were intensively analyzed with an aim of isolating the bioactive compounds (Akhtar *et al,* 1997*).*

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