

Full Length Research Paper

Evaluation of some botanicals against bacterial wilt of tomatoes

*Sangoyomi, T. E.,¹ Owoseni, A. A.² Adebayo O. S.³ and Omilani, O. A.¹

¹Department of Crop Production, Soil and Environmental Management, Faculty of Agriculture, Bowen University, Iwo, Nigeria.

²Department of Biological Sciences, Faculty of Science and Science Education, Bowen University, Iwo, Nigeria.

³National Institute for Horticultural Research and Training (NIHORT) P.M.B 5432 Ibadan, Nigeria

Accepted 12 October, 2011

Tomato production is affected majorly by pests and diseases; one of the major diseases being Bacterial wilt caused by *Ralstonia solanacearum*. Healthy tomato plants (variety *Beske*) were subjected to infection with the bacterium to determine its pathogenicity. Studies were also carried out to determine the anti-microbial effects of water extracts of *Zingiber officinale*, *Azadirachta indica*, *Cymbopogon citratus*, *Ocimum gratissimum*, *Allium sativum*, *Venonia amygdalina*, *Jatropha curcas*, *Senna obtusifolia*, *Senna occidentalis* and *Senna sophera* at varying concentrations on *Ralstonia solanacearum*. The crude extracts at various concentrations (0.5%, 1% and 10%) did not have any inhibitory effects on Bacterium. The use of other types of solvents may be necessary as the active ingredients of the plant materials may not be soluble in water. Sensitivity to antibiotics was tested on the bacterium. Seven of the fourteen antibiotics used showed zones of inhibition while the other seven did not have any effects. Ciprofloxacin, Ofloxacin, Pefloxacin, Drovid, Cotrimozazole, Norfloxacin and Clindomycin resulted in varying zoning diameters (2.5-13mm) while Tetracyclin, Amoxycylin, Gentamycin, Nitrofurantoin, Ceftriazone, Augmentin, and Erythromycin did not have effects on the bacterium. The effective antibiotics may be incorporated into the development of an integrated management approach for the control of bacterial wilt of tomatoes.

Keywords: *Ralstonia solanacearum*, Tomato, Crude extracts Antibiotics.

INTRODUCTION

Tomato is one of the most popular vegetables in the world. The global production area was estimated at 4, 397,873 ha with world production estimated at one hundred and thirty million tonnes in 2008 (FAO, 2008). Tomato is a very versatile plant and it could either be grown for fresh market tomatoes or for processing tomatoes in which mechanical processes are involved. The world consumption of tomato has increased significantly over the past 25 years (FAO 2000). Despite the importance of tomato in many developing and

developed countries, this crop has often been neglected in the making of several agricultural policies. This coupled with several constraints like poor soils, use of unimproved local varieties, land tenure system and damage by pests and diseases, has caused the crop to be produced below its full potential. The yield of tomato in West Africa particularly Nigeria is not encouraging, especially when compared to developed countries. For instance, Nigeria production was estimated at 170 tonnes in 2008 while the U.S.A estimate for the same year was put at 1, 257 tonnes (FAO, 2008). Yield per hectare in Nigeria was estimated at 1/7th of that of the U.S.A (FAO, 2008). Many factors have been reported to be responsible for this with diseases and pests representing major constraints to production. Several authors have

*Corresponding author: t.sangoyomi@yahoo.com; Phone +234 803 4510 695

reported the contributions of many types of diseases affecting tomato production; however the effect of bacterium wilt has been reported to be the most damaging (Taiwo *et al* 2007). Bacterial wilt affects hundreds of plant species such as potato, tobacco and egg plant all over the world and this makes it among the most important plant diseases worldwide. It affects the plants at their vegetative as well as their reproductive stages (Buddenhagen and Kelman (1964); Hayward (1991) and Koenig *et al.*, (1999). The use of chemicals has not been effective in the control of bacterial wilt of tomatoes because the pathogen causing the disease *Ralstonia solanacearum* is a soil borne pathogen and is systemic in its action. The use of botanicals or non chemical methods however, has a long history in the control of diseases (Secoy and Smith (1983); Khanna *et al.*, (1991); Calvet *et al.*, (2001) Pradhanang *et al.*, (2003) and Taiwo *et al.*, (2007). Crude extracts from ten different plants were therefore investigated for their antimicrobial activities against *Ralstonia solanacearum* as well as carry out antibiotics sensitivity tests of various antibiotics.

MATERIALS AND METHODS.

Source and preparation of culture medium

Bacterium culture of *Ralstonia solanacearum* was collected from National Institute for Horticultural Research and Training (NIHORT). Throughout the investigation, the bacterium *Ralstonia solanacearum* was grown on Triphenyl Tetrazolium Chloride (TTC) agar prior to inoculation and maintained on nutrient agar for long term storage of cultures.

Pathogenicity of *Ralstonia solanacearum* on Tomato plant variety Beske.

Healthy seedlings of tomatoes were raised in the greenhouse using completely randomized design for four (4) weeks and then transplanted into 3litre pots at the rate of two (2) plants per pot and inoculated with 30 ml of pure liquid culture of *R. solanacearum* one week after transplanting. Colonies were washed from the agar surface with sterile distilled water, centrifuged and calibrated to final population of approximately 10^7 CFUml⁻¹. The tomato plants were inoculated using two different methods. In one set of experiments, plants were inoculated by pouring 30ml of *R. solanacearum* culture containing 10^7 CFU ml⁻¹ into the pots in ring form around the root zone of the plants. In the other set of pots, inoculation was done by pouring *R. solanacearum* culture around the root zone immediately after pricking the plants

roots (2 points) just below the soil surface. Percentage survival was calculated by using the formula:

$$\% \text{ Survival} = \frac{\text{Number of survival}}{\text{Total number of plants}} \times 100\%$$

Effects of plant extracts on Bacterial growth.

Healthy leaves of *Azadirachta indica*, *Cymbopogon citratus*, *Ocimum gratissimum*, *Venonia amygdalina*, *Jatropha curcas*, *Senna obtusifolia*, *Senna occidentalis* and *Senna sophera*, bulbs of *Allium sativum* and rhizomes of *Zingiber officinale* were used for the preparation of plant extracts and used immediately. Three different concentrations of water extracts were prepared by blending 10g, 1g and 0.5g of each plant part in 100ml sterile distilled water to produce extract concentrations of 10%, 1% and 0.5% respectively. The extracts were sieved through four (4) layers of cheese cloth. One ml of bacteria was introduced into sterile Petri-dishes using a sterile syringe and the Petri-dishes were filled with 20ml of Agar at 45°C and allowed to cool and gel. After this, wells were made using sterile 3mm cork borer using three wells per plate and the wells were filled with the plant extracts and placed in an incubator at 37°C for 24 hours using completely randomized design with three replicates for each of the different plant extracts used. After this period, the Petri-dishes were then assessed for anti-microbial activities (Owoseni and Ajayi 2010).

Antibiotic sensitivity tests

The pathogen *Ralstonia solanacearum* was tested using Antibiotic test kit manufactured by FONDOZ laboratories and MAXICARE medical laboratories Nigeria. This was done by first introducing 1ml of the bacterium into sterile Petri-dishes with molten agar. The agar was allowed to gel and the antibiotic test kit were placed on the agar surface and incubated at 37°C for 24 hours (Owoseni *et al* 2010). The antibiotics Petri-dishes were replicated twice. The zoning diameter was taken as the mean zone along the two directions on perpendicular lines using a ruler on the reverse side of the plates. Data obtained were analyzed using descriptive analysis such as frequency count and simple averages.

RESULTS

Pathogenicity of *R. solanacearum* on tomato

Ralstonia solanacearum was found to be pathogenic on

Table 1. Pathogenicity of *R. solanacearum* (10^7 CFU ml⁻¹) on tomato (variety *Beske*) 2 weeks after inoculation.

Treatment	Percentage (%) survival
Inoculation around root zone	50
Inoculation by pin-prick	30
Control	100

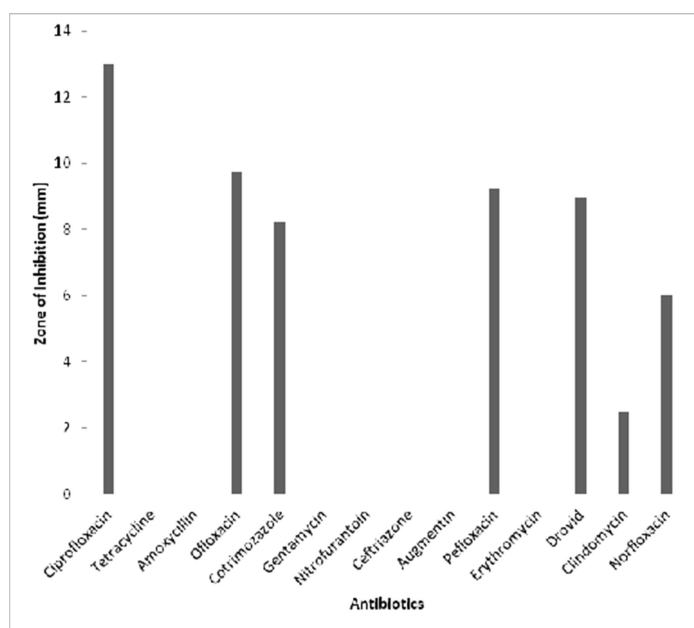


Figure 1. Antibiotic Sensitivity of *Ralstonia solanacearum*

tomato variety tested 14 days after inoculation causing wilting of the plants (Table 1). Percentage survival was lowest in plants inoculated by pin-prick (30%). The bacterium resulted in wilting of the plants i.e. loss in turgidity of both the leaves and the stem.

Effects of plant extracts on Bacterial growth.

Crude extracts from 10 plant species showed no antimicrobial properties at the different concentrations tested (0.5%, 1% and 10%) i.e. there were no zoning around the agar wells filled with the various plant extracts.

Antibiotic sensitivity tests

Antibiotics placed on the agar wells impregnated with *R. solanacearum* showed varying degrees of zoning. The

result of the antibiotics on the bacterium culture measured after 24 hours of incubation at 37°C is shown in Figure 1 and Plate 2. The antibiotics on the test kit were Ciprofloxacin, Tetracyclin, Amoxycylin, Ofloxacin, Cotrimoxazole, Gentamycin, Nitrofurantoin, Ceftriazone, Augmentin, Pefloxacin, Erythromycin, Droid, Clindomycin and Norfloxacin, the various antibiotics used resulted in various levels of zoning. Seven out of the fourteen antibiotics tested had zoning diameters of between 3 and 13mm while the remaining 7 antibiotics did not result in any zoning. Ciprofloxacin resulted in the highest result with a zoning diameter of 13mm followed by Ofloxacin (10mm) while Pefloxacin, Droid and Cotrimoxazole produced zoning diameters between 8 and 9mm. Norfloxacin and Clindomycin had very low zoning diameters of 6mm and 2.5mm respectively. Tetracyclin, Amoxycylin, Gentamycin, Nitrofurantoin, Ceftriazone, Augmentin, and Erythromycin did not have any effects on *R. solanacearum* (Plate 2).



Plate 1a. Antibiotic sensitivity test on *R. solanacearum* (Test kit manufactured by FONDISCS Laboratories)

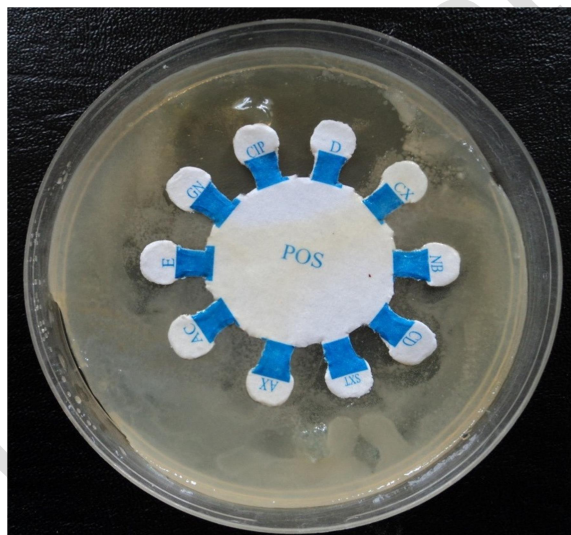


Plate 1b. Antibiotic sensitivity test on *R. Solanacearum* with another set of antibiotics (Test kit manufactured by MAXICARE Laboratories)

DISCUSSION

Tomato is an important vegetable crop as well as a notable cash crop in Nigeria. However, its production and availability is often limited by a number of factors, for example, yield per hectare in Nigeria is as low as 170 tonnes when compared to that of the U.S.A (1, 257

tonnes) (FAO, 2008). The tomato variety *Beske* used in this study is an important variety in the south western Nigeria where this research was carried out. The variety is more preferred to the sour, watery, local variety by consumers during the off season when the most preferred variety *Alausa* is scarce. Bacterial wilt is an important soil borne pathogen causing severe loss of

production. This agrees with the report of Ji *et al* (2007) that described the effect of the disease on tomato yield. Wounding will increase the pathogenicity of the bacterium on the test plant. Bacterial wilt of tomato caused by *R. solanacearum* is a systemic disease that cannot be effectively managed with foliar application of chemical pesticides. The use of plants in the control of diseases is as old as man and presents no potential toxicity. Anti-microbial effects of ten plant extracts were studied *in vitro* using crude extraction method. None of the ten plants screened showed effectiveness in their ability to control the test organism (*R. solanacearum*). Although, many of these plants have been shown to have anti-microbial properties against some other pathogens Sangoyomi *et al.*, (2010); Okigbo and Nneka (2005) and Ji *et al* (2007), it is therefore suggested that other forms of extraction should be exploited to review the anti-microbial properties of these same set of plant materials. The lack of efficacy of these plant materials used in their crude form may be due to the insolubility of their active ingredients in water. Ji *et al.*, (2007) has also reported the use of plant derived volatile compound thymol to suppress bacterial wilt of tomatoes as part of an integrated management package for the disease. The extracts investigated in this study are from plants that are locally available and environmentally friendly. A range of antibiotics were screened for their efficacy in the control of *R. solanacearum*, out of which seven (Ciprofloxacin, Ofloxacin, Pefloxacin, Drovid, Cotrimozazole, Norfloxacin and Clindomycin were found to be effective in controlling the disease. The incorporation of these effective antibiotics into integrated management approach will ensure a better management of the disease. Since the water extracts had no effects, other solvents are being used to extract active ingredients from these plants and their efficacies are being tested on the bacterium.

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