



Field control of bacterial speck of tomato using some plant extracts

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Abstract

of the diseases of tomato, an important vegetable crop, that is known everywhere in every part of the world is Bacterial speck (*Pseudomonas syringae* pv. *tomato*). Chemical control has not been very effective and unsafe for the environment hence, the need for alternative methods of controlling the disease. This study accessed the effect of plant extracts on the frequency and ruthlessness of bacterial speck of tomato on two tomato cultivars (Roma VF and Gboko). Experimental construction and design was in form of a Randomized Complete Block Design (RCBD) having three (3) replicates. Plant extracts from *Moringa oleifera*, *Azadirachta indica*, *Chromolaena odorata* at 12.5% w/v and 25% w/v concentrations were applied. Data were subjected to Analysis of Variance (ANOVA) and means separated by the Duncan's Multiple Range Test ($p \leq 0.05$) using SAS 9.1 for windows. Results showed that *Chromolaena odorata* at 12.5% w/v greatly reduced the bacterial speck of tomato on Gboko variety while Roman VF responded greatly to treatments with *Moringaoleifera* at 12.5% w/v and 25% w/v concentrations, reducing.

Keywords: Disease, incidence, severity, *Moringa olivera*, *Azadirachta indica*, *Chromolaenaodorata*.

Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the *Solanaceae* family and is one of the most important vegetables in Africa and the world¹. It is grown in many parts of Nigeria as wet and dry season crops^{2,3}. The crop is prominent in the diet of Nigerians and also serves as a steady cash crop for many farmers¹. Recorded decline in yield of tomato produced in Nigeria has been reported ranging from 7,000kg/ha in 2003 to 6,419kg/ha in 2008, during the past decade¹.

However, the production potential of tomato in Nigeria has been constrained by pest and diseases, especially fungal and bacterial pathogens^{1,4}. Among the various diseases of tomato in Nigeria, bacterial speck incited by *Pseudomonas syringae* pv. *tomato* has been reported to be an economically important and devastating diseases reducing yield of tomato up to 75% in field, where high relative humidity and temperature ranging from 20 to 25°C are prevalent⁵.

Lesions created on the fruits of tomato by bacterial speck often reduce their market value and can also be serious enough to affect the rating and worth of processed products derived from them⁵. According to evaluation statistics, bacterial speck is responsible for values ranging between 75% and 5% of plants infected at early stage and those that were infected later in the growth season respectively^{5,6}.

Infected tomato seed is a potential vital origin of the primary inoculum for bacterial speck disease^{5,7}. Ability of the host plant to resist the pathogens has been described as a more reliable management strategy⁴. Approaches such as field sanitation (evacuation of plant debris, weeding, etc.), crop rotational farming practices and planting of pathogen-free seeds and seedlings are also good and effective management strategies while the use of chemical sprays is also common but with various accompanied challenges^{4,5}. Other peasant farmers' friendly control methods of bacterial speck remain underexploited. Indeed, the discovery of resistance by some cultivars of tomato to bacterial speck is a good way forward in the management system, yet there is still the possibility of resistant strains of *P. syringae* pv. *tomato* to evolve⁸.

On the other hand, the continuous usage of chemicals of copper origin to control diseases may cause toxicity to plants and it could also become accumulated in the soil over time as well as posing health risk to human⁸. An environmentally friendly and safe strategy to manage tomato bacterial speck could be through the natural compounds of plants^{9,10}.

Natural plant extracts, also known as botanicals or botanical pesticides have long history in the control of plants, animals and human pathogens¹¹. They have innate potential of producing various compounds with essential antimicrobial and antibiotic properties^{12,13}. However, the advent of synthetic pesticides has led to significant reduction or total neglect in the use of

botanicals. Even though chemical pesticides could be very potent in most cases, they are often accompanied by various undesirable aftermath records such as environmental pollution, soil degradation, untargeted beneficial microbes poisoning and toxicity to mammals. Over the years, the plant extracts have exhibited antimicrobial activity against phytopathogenic fungi and bacteria and their use is safe for human health and the environment¹¹. The growing tragic side effects associated with the management of plant diseases by the use of commercial chemicals have created apprehension to humans, particularly as many of the targeted pathogens are developing resistance continually to these chemicals. For example, averagely two hundred (200) species of plant pathogens are described to have become recalcitrant to certain chemicals¹⁴. Records based on soil and water pollution as said earlier are not left out¹⁵. Conversely, employing plant extracts for the control and operative controlling of plant diseases has fascinated momentous consideration in united pest management practices, particularly because plant metabolites have proven to impact no adverse effects to the ecosystem^{16,17}. Besides, bearing in mind the fact that the biopesticides are cheap, affordable and offer safer control of diseases by peasant farmers, majority of who are into tomato cultivation, coupled with the menace concomitant with the usage of chemicals to human environment, there is imperative obligation to look for such hopeful better replacements through plant products for the operational managing of the disease under consideration. Therefore, this study was conducted to assess the effect of plant extracts on the incidence and severity of bacterial speck disease on tomato.

Materials and methods

Experimental site: The experiment was carried out on DelPHE-5Project Research field, Federal University of Agriculture Abeokuta (FUNAAB), Nigeria during the late planting season (15th of August) in 2017.

Source of seeds, treatments and experimental design: Seeds of two tomato cultivars (Roma VF and Gboko) were obtained from tomato germplasm collection Centre in Tissue Culture Laboratory, Department of Crop Protection, FUNAAB. Treatments consisted of extracts from *Moringa oleifera* (12.50% and 25% w/v), *Azadirachta indica* (12.50% and 25% w/v) and *Chromolaena odorata* (12.50% and 25% w/v). Streptomycin (0.3% w/v) served as positive control while sterile distilled water served as negative control. The experiment was laid out in a randomized complete block design (RCBD), with three replications. Dimension of each plot was 3meter x 2meter, consisted of 30 plants per plot with inter and intra row spacing of 60cm by 50 cm respectively. Pathway of 1meter was left in-between plots. Seedlings were raised inside screen-house in nursery trays containing sandy-loam steam-sterilized soil.

Preparation and application of plant extracts: The fresh mature leaves of *M. oleifera*, *A. indica* and *C. odorata* obtained from the University premises were taken to the laboratory,

washed with running tap water and chopped into pieces with sterilized knife. Separately, each type of air-dried leaf samples were weighed (125 and 250g) and blended in 1 litre of sterile distilled water using electric blender and left overnight. The paste was filtered through a clean cheese cloth to give 12.50 and 25% w/v stock filtrate. Plots that were sprayed with streptomycin (0.3% w/v) served as positive control while plots sprayed with sterile distilled water served as negative control. Three spraying regime were observed at two weeks after transplanting, flowering and fruiting stages.

Transplanting and field management: Tomato seedlings were transplanted into the open field four weeks after sowing in the nursery. Tomato plants were staked and tied over time. Weeding was regularly observed. Pests were controlled with foliar application of cyperforce® 10% E.C (cypermethrin) at 2 weeks intervals.

Isolation of *Pseudomonas syringae* pv. tomato: Infected tomato leaf samples were collected from experimental field, washed with clean tap water, surface sterilized with 1% sodium hypochlorite solution for 2mins, rinsed in three change of sterile distilled water and air dried at room temperature. Tissue segments of about 2mm² were cut from advancing lesion margin of leaf samples. The tissue segments were inoculated in Petri plates contained nutrient agar and incubated for 72 hours at 25-28°C. Presumptive colonies of the pathogen were purified by sub-culturing. The pathogen was identified as *P. syringae* pv. *tomato* through biochemical tests, pathogenicity test on “Beske” tomato cultivar seedlings and hypersensitivity on tobacco.

Data collection and analysis: Data were collected from plant height (cm), number of leaves, number of flowers, number of fruits harvested and weight of fruits harvested (kg). Disease incidence was calculated as percentage of plants showing symptom of bacterial speck in relation to the total number of plant in a unit. Disease severity was assessed using 1-5 point rating scale described by James¹⁸ viz: 1 = no lesions, 2 = 1–10 lesions/plant, 3=11–20 lesions/plant, 4=21–40 lesions/ plant, 5 = more than 40 lesions/plant. Data were subjected to Analysis of Variance (ANOVA) and means separated by the Duncan’s Multiple Range Test ($p \leq 0.05$) using SAS 9.1 for windows.

Results and discussion

Agronomic parameters of the two tomato cultivars used in this study, as influenced by plant extracts, are shown in Table-1. Majorly, plant height (cm) and number of leaves/plant were not significantly affected ($p \geq 0.05$) by plant extracts, sterile distilled water (SDW) and streptomycin in both varieties. However, at 2 weeks after transplanting (WAT), there were significant effects ($p \leq 0.05$) among plant extracts, SDW and streptomycin. Number of leaves/plant at 2WAT, in Roma VF cultivar, ranged from 37.67 to 68.78. Plots sprayed with streptomycin had lowest number of leaves/plant (37.67) and significantly lower ($p \leq 0.05$) from values obtained from plots sprayed with plant

extracts and SDW. *A. indica* at 25% had the highest number of leaves (68.78). This might be due to the toxic effect of streptomycin on vegetative growth at tender age of the tomato plant. Plant height (cm) and number of leaves at all levels of concentrations of plant extracts in both cultivars were comparable.

Table-2 highlights the effects of plant extracts on incidence and severity of bacterial speck on Gboko and Roma VF cultivars. When Gboko was sprayed with *Moringa oleifera*, *Azadirachta indica* and *Chromolaena odorata* extracts at 12.50% and 25% w/v concentrations, there was a significant reduction ($p \leq 0.05$) of bacterial speck disease incidence and were comparable with streptomycin. Plots sprayed with SDW had the highest significant ($p \leq 0.05$) disease incidence of 100%. Gboko was severely affected when 12.50% concentration of plant extracts were applied. Streptomycin significantly reduced ($p \leq 0.05$) the disease severity with mean value of 1.15, which was not different ($p \geq 0.05$) from 1.60 disease severity when *C. odorata* was applied. Disease incidence from plots contained Roma VF ranged from 4.47 to 100%. Among the extracts, *M. oleifera* significantly reduced ($p \leq 0.05$) disease incidence to 33.33% followed by *A. indica* (41.70%) and *C. odorata* (42.68%) at 25%. These values were not significantly different ($p \geq 0.05$) from 44.47% disease incidence recorded when streptomycin was applied. The same trend was observed in disease severity. Effect of application of plant extracts at 25% concentration on tomato bacterial speck disease incidence was comparable to the effect of streptomycin applied at 0.3% concentration. The superiority of performance of the plant extracts used in this study at 25% might be because of higher concentrations of antibacterial compounds in the extracts over 12.50% concentration. Ganiyu *et al.*^{4,19} affirmed the presence of antibacterial compounds in plant extracts such as *Mangifera indica*, *Azadirachta indica*, *Carica papaya* and *Acalypha wilkiesiana*. Application of *Azadirachta indica* extract used by

Enikuomehin²⁰ on sesame reduced the number of lesions on infected leaves thereby limiting disease development.

Among the plant extracts, when *C. odorata* was applied at 25% concentration, highest number of fruits/plant (99.01) was recorded in plots containing Gboko, which was not significantly different ($p \geq 0.05$) from 89.67 recorded when *M. oleifera* was applied at the same concentration. These values were not different ($p \geq 0.05$) from number of fruits (104.25) obtained when streptomycin was applied (Table-3). Weight of fruits recorded in plots contained Gboko cultivars ranged from 0.05 to 0.24 kg. Plots that received streptomycin had the highest weight of fruits, which was not significantly different ($p \geq 0.05$) from 0.22 and 0.20kg/plant observed in plots sprayed with *C. odorata* and *M. oleifera* at 25% concentration, respectively. Plots sprayed with SDW performed least in all the treatments with the mean weight value of 0.05kg/plant. Number of fruits/plant recorded for Roma VF ranged from 5.67 to 153.00. Treatments performed significantly different ($p \leq 0.05$) from one another. Extract of *C. odorata* at 25% application on Roma VF brought about 101.05 number of fruits/plant, which was not significantly different ($p \geq 0.05$) from the highest number of fruits/plant (153.00) obtained from plots sprayed with streptomycin. In similar manner, plots that received *M. oleifera* and *A. indica* at 25% had 93.12 and 98.11 number of fruits/plant respectively, and were not significantly different ($p \geq 0.05$) from each other. Spray of SDW brought about the least significant ($p \leq 0.05$) number of fruits (5.67) on Roma VF. Weights of fruits in Roma VF ranged from 0.04 to 0.29kg/plant. Among extracts, 25% concentration performed better than 12.50% concentration. In this study, at 25% concentration, *M. oleifera*, *A. indica* and *C. odorata* improved fruits weight of tomato. Green plants can provide valuable source of pesticides²¹. In addition, Popoola *et al.*²² reiterated that water extract from *Lawsonia inermis* could be a substitute for chemical pesticides for the management of bacterial blight of cotton seedlings.

Table-1: Effects of plant extracts on the agronomic parameters of two tomato varieties.

Plant extract	Gboko†				Roma VF†			
	2WAT	4WAT	2WAT	4WAT	2WAT	4WAT	2WAT	4WAT ^X
<i>M. oleifera</i> (12.5%)	19.11 ^a	30.94 ^a	70.67 ^a	182.83 ^a	15.13 ^a	34.53 ^a	50.83 ^b	126.00 ^a
<i>M. oleifera</i> (25%)	18.05 ^a	38.95 ^a	66.55 ^a	179.00 ^a	16.25 ^a	33.11 ^a	57.53 ^b	121.67 ^a
<i>A. indica</i> (12.5%)	15.20 ^a	31.09 ^a	68.14 ^a	146.92 ^a	11.87 ^a	28.43 ^a	60.11 ^a	132.00 ^a
<i>A. indica</i> (25%)	16.19 ^a	32.67 ^a	69.57 ^a	151.05 ^a	13.55 ^a	30.43 ^a	68.78 ^a	130.25 ^a
<i>C. odorata</i> (12.5%)	17.65 ^a	33.98 ^a	69.77 ^a	168.08 ^a	17.03 ^a	33.95 ^a	57.87 ^b	130.50 ^a
<i>C. odorata</i> (25%)	18.22 ^a	35.95 ^a	65.98 ^a	172.05 ^a	16.33 ^a	34.02 ^a	60.95 ^a	119.01 ^a
SDW ^Y	16.50 ^a	30.42 ^a	66.67 ^a	130.83 ^a	16.83 ^a	31.50 ^a	62.78 ^a	115.00 ^a
Streptomycin (0.3%)	18.11 ^a	32.09 ^a	56.33 ^a	139.83 ^a	17.13 ^a	30.00 ^a	37.67 ^c	103.00 ^a

†Means followed by the same superscript (abcd) within a column are not significantly different ($p \geq 0.05$) according to Duncan Multiple Range Test (DMRT). ^XWAT: Weeks after transplanting, ^YSDW: Sterile distilled water.

Table-2: Effects of plant extracts on incidence and severity of bacterial speck of tomato.

Plant extract	Gboko†	Severity	Roma VF†	Severity
	Incidence (%)		Incidence (%)	
<i>M. oleifera</i> (12.5%)	30.60 ^c	4.33 ^b	50.03 ^{bc}	2.33 ^c
<i>M. oleifera</i> (25%)	33.33 ^c	2.00 ^d	33.33 ^d	1.33 ^d
<i>A. indica</i> (12.5%)	38.93 ^c	4.00 ^b	66.70 ^b	3.33 ^b
<i>A. indica</i> (25%)	33.34 ^c	3.33 ^c	41.70 ^c	2.33 ^c
<i>C. odorata</i> (12.5%)	43.33 ^b	4.00 ^b	69.47 ^b	3.00 ^b
<i>C. odorata</i> (25%)	32.80 ^c	1.60 ^{de}	42.68 ^c	2.33 ^c
SDW ^Y	100.00 ^a	5.00 ^a	100.00 ^a	5.00 ^a
Streptomycin (0.3%)	44.47 ^b	1.15 ^{de}	44.47 ^c	1.06 ^d

†Means followed by the same superscript (abcd) within a column are not significantly different ($p \geq 0.05$) according to Duncan Multiple Range Test (DMRT), ^YSDW: Sterile distilled water.

Table-3: Effects of plant extracts on the yield of tomato.

Plant extract	Gboko†		Roma VF†	
	Number of fruits/plant	Weight of fruits (kg)/plant	Number of fruits/plant	Weight of fruits (kg)/plant
<i>M. oleifera</i> (12.5%)	64.66 ^c	0.11 ^c	75.16 ^c	0.18 ^b
<i>M. oleifera</i> (25%)	89.67 ^a	0.20 ^a	93.12 ^b	0.21 ^{ab}
<i>A. indica</i> (12.5%)	63.58 ^c	0.11 ^c	73.83 ^c	0.18 ^b
<i>A. indica</i> (25%)	73.55 ^b	0.18 ^b	98.11 ^b	0.23 ^a
<i>C. odorata</i> (12.5%)	67.92 ^c	0.12 ^c	72.80 ^c	0.19 ^b
<i>C. odorata</i> (25%)	99.01 ^a	0.22 ^a	101.05 ^a	0.27 ^a
Sterile distilled water	4.17 ^d	0.05 ^d	5.67 ^d	0.04 ^c
Streptomycin (0.3%)	104.25 ^a	0.24 ^a	153.00 ^a	0.29 ^a

†Means followed by the same superscript (a,b,c,d) within a column are not significantly different ($p \geq 0.05$) according to Duncan Multiple Range Test (DMRT),

Conclusion

Present study revealed that cold water extracts from *M. oleifera*, *A. indica* and *C. odorata* at 25% could curtail incidence and severity of bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato*. They could be used as substitutes by resource-poor farmers as alternative to synthetic chemicals.

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