Effectiveness of some plant extracts in the management of tomato pith necrosis caused by *Pseudomonas corrugata*

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ABSTRACT

Three plants extract - *Aloe vera, Azadirachta indica* and *Lawsonia inermis* were tested in the laboratory and on the field to establish their effectiveness in the control of *Pseudomonas corrugate* the causative agent of tomato pith necrosis. Streptomycin and water served as positive and negative control respectively. *In-vitro* evaluation of plant extracts to control the growth of the pathogen was carried out prior to field application. The water-extract of the plants were sprayed three weeks after transplanting in a randomized complete block design involving five treatments and three replicates. Results showed that *Aloe vera* extracts led to the least bacterial growth of $11.34x10^7$ cfu/ml. Extracts of *Azadirachta indica* led to the highest bacterial population of $490.66x10^7$ cfu/ml. Among the three plant materials treated *Aloe vera* extracts significantly ($p<0.05$) showed the least disease incidence (40.70%) and severity (38.70%) compared with *Azadirachta indica,* incidence (53.30%) and severity (52.70%) and water (35.00%) and severity (45.60%). Similarly, *Aloe vera* supported better vegetative growth indices in terms of number of branches, plant height and stem girth. However, yield in tomato treated with the three plant extracts did not show statistically significant differences at $p<0.05$. The work concluded that water extracts at 0.5g/ml of *Aloe vera* applied as spray at three weeks after transplanting could be used to manage tomato pith necrosis caused by *Pseudomonas corrugata* and also as possible alternative to chemical pesticides known to be environmentally unsafe.

Keywords: Plants extracts, Streptomycin, Tomato pith necrosis, *Aloe vera, Azadirachta indica, Lawsonia inermis,* and *Pseudomonas corrugata*

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INTRODUCTION

Tomato pith necrosis (TPN) is a sporadic bacterial disease that was first reported in Argentina and in green house in Europe (Saygili *et al*, 2004, Alippi *et al.*, 1993). TPN was also reported as the destructive diseases of tomato (Sahin *et al*., 2005) and has been found to be caused by soil borne *Pseudomonas spp* known as *Pseudomonas corrugata* (Scarlet *et al*., 1978, Moural *et al*, 1994). Likewise Lopez *et al*.,(1994) reported *P.corrugata* as the causative organisms of TPN as high level of aggressiveness and significant yield reduction was recorded.

The presence of this pathogen was observed in the Research and Teaching farm of the University of Agriculture, Abeokuta, Nigeria. The deceptive nature of the disease had made it escape detection over time. It's devastating effect during the 2005/2006 cropping season at the University of Agriculture, Abeokuta in which the whole field resulting to 100% fruit loss to the disease necessitated the study of its possible control.

Since fields with history of soil borne pathogen will have the problem consistently from year to year. *Pseudomonas corrugata* as a soil inhabitant bacterium affects tomato productions output due to its continuous recurrence every growing season. Like other bacterial plant pathogen affecting agricultural crops, TPN often results in considerable damage and serious economic loss worldwide. Observation of this pathogen on tomato field recorded a hidden devastation capable of producing zero yields. The disease progress is a subtle inconspicuous one and can escape early detection. It is the subtle but devastating effect that forms the core of the problem.

According to the past research works, symptoms of TPN in glasshouse tomatoes have been reported to be caused by several other bacterial pathogens such as *Pseudomonas* cichorii (Wilkie and Dye, 1974), *Pseudomonas viridiflava* (Malathrakis and Goumas, 1987; Goumas and Chatzaki, 1998), *Erwinia carotovora spp* (Speights *et al*., 1967; Dhanvanthari and Firks, 1987; Malathrakis and Goumas, 1987) and *Pseudomonas flourescens* biotype" 1 (Malathrakis and Goumas, 1987). Fluorescent *Pseudomonas spp* closely related to *Pseudomonas corrugata* have been identified as causing TPN in France, Spain and Italy (Catara *et al*., 1997; Sutra *et al*., 1997), Greece (Aliviazates, 1984), Turkey (Demir,1990) and Canada (Dhanvanthari, 1990). Yet, another *Pseudomonas* spp whose most of its strains were isolated from *Mediterranea* countries called *Pseudomonas mediterranean* sp. nov have been discovered to have a closer similarities to *P. corrugata* in terms of microbiological properties, disease infections and symptoms (Catara *et al*., 1997, 2000; Sutral *et al*., 1997).

Pseudomonas was isolated from tomato plants with pith necrosis

collected from different areas in Spain and from pepper plants with pith necrosis in Tenerife (Cannary Islands). Cultural, biochemical and physiological characteristics of isolates from both hosts were similar to those of the type strain of *P. corrugata* (Alippi *et al*.,2003). Inoculation of pepper and tomato plants with isolates from both host caused similar pith necrosis. Therefore, *P. corrugata* is considered to be ubiquitous bacterium with a broad host range, causing pith necrosis mainly on tomato, but also on pepper (Lopez *et al*., 1994) and chrysanthemum (Fiori, 1992) with the same symptomatology.

Pseudomonas corrugata apart from being the causal agent of Tomato Pith Necrosis is also used in the biological control of plant pathogenic bacteria and fungi. Potentially, it could be used in other fields such as the production of commercial biomolecules with a wide range of application including bioremediation (Catara, 2007), since its host range is limited to tomatoes and a few cultivars of pepper, advantages of its root colonizing ability and antimicrobial properties could be used.

Generally, bacteria are becoming more difficult to control because bactericides in present-day use are not as effective as they have been in the past (Zalom,2007). Development of copper tolerant and copper- resistant strains have caused major difficulty in controlling bacterial plant pathogen with copper compounds. Tolerance and resistance to copper has been reported for many bacterial pathogens affecting important crops which also includes tomato (Jones *et al*., 1991; Bender and Cooksey, 1987).

The future role of pesticides in agriculture is increasingly threatened by several factors including the development of pest resistance, increasing concerns about food safety and environmental accumulation of toxic compounds which has led to other alternative disease control that is environmentally friendly (Cooksey and Azad,1992).

Research work carried out on developing biologically based management strategies for the control of soil borne pathogen in place of chemical method supports the use of biocontrol agents, plant derived natural products and plant extracts (Lakshman and Dilip 2006). Investigation on natural antimicrobial products has demonstrated the effectiveness of botanical formulation that inhibits common plant pathogenic bacteria. This specific formulation was also found to be highly effective in controlling the agent and soil borne wilt pathogen *Ralstoria solanacearum*. e.t.c. This botanical formulation is a broad-based control strategy of soil borne pathogens. Owolade *et al* (2003) works on the efficacy of aqueous extracts from the leaves of *Carica papaya, Tithonia diversifolia* and *Acalypha ciliate* as potential biofungicide against *Collectotrichum capsci*. The extracts reduced disease incidence and severity both *in-vitro* and *in-vivo*. Thus, revealing the efficacy of

plant extracts in controlling fungal diseases. Therefore, this research study investigates the effectiveness of some plant extracts in the management of tomato pith necrosis caused by *Pseudomonas corrugata*.

MATERIALS AND METHODS

Research Location:

The experiment was carried out in the Department of Crop Protection laboratory and on the Experimental Plot, University of Agriculture, Abeokuta.

Source of plant materials:

Lawsonia inermis was obtained from Abeokuta environment, Ogun state, while *Azadirachta indica* and *Aloe vera* were obtained from different locations in University of Agriculture, Abeokuta and University of Ibadan. Streptomycin was used as the positive control while sterile distilled water served as negative control.

Source of Tomato seeds:

The tomato seeds used for this research study were obtained from Organic Agriculture Project for Tertiary Institution (OAPTIN) University of Agriculture, Abeokuta Chapter. The viable seeds were sterilized before use. 100g of tomato seed was sterilized using 5% NaOCl for one minute then in 0.5% ethanol for 30 seconds and rinsed with sterile distilled water.

Preparation of plant extracts and preparation of media:

One hundred grammes (100g) of sorted, clean plant leaves were weighed on a sensitive scale. 5% NaOCl was used for the surface sterilization then allowed to dry for a period of 5 minutes. The leaves were rinsed, air-dried and blended in a sterilized electric blender. 200ml sterilized distilled water was used to prepare l00g of each leaf samples, giving a concentration of 0.5g leaf extract/ml. The blended slurry was aseptically sieved with sterilized muslin cloth and hand gloves. For those used in the laboratory, filter membranes were used to obtain sterile plant extracts. Nutrient agar was prepared according to manufacturers' specification and instructions (Hatcher, 1965).

Pathogen Isolation and Identification:

The infected tomato pith was aseptically scrapped into sterile McCartney bottle containing 5ml of sterile distilled water. Streaks were prepared on nutrient agar and kept at 35^0c for 48 hours. Bacterial growths together with the contaminants were observed. The bacteria colony was then sub cultured into fresh nutrient agar pure cultures were subsequently transferred into McCartney bottles containing nutrient agar broth, and allowed to grow. The growth was arrested 48 hours later and kept in the refrigerator as bacterial broth culture. Pathogenicity of different isolates of *P. corrugata* was evaluated in the

screenhouse of crop protection, University of Agriculture, Abeokuta (Data not shown). The most pathogenic *P. corrugata* isolates was used for this study.

Preparation of Standard Inoculum of Pathogenic *P. corrugata*:

Little amount of sterile distilled water was added to the surface of pure bacteria cultured plates. Fresh Cultured plates of nutrient agar were streaked with *Pseudomonas corrugata* from bacterial broth cultures and incubated for 48 hours. A 5ml of sterile distilled water was added to the surface of culture plates. Bacterial colonies were dislodged into solution with sterile L-shaped glass rod. The suspension was collected and kept in sterile bottle as stock inoculum while 1ml of stock inoculum was subjected to serial dilution up to 10^7 dilution factor. The 10^7 plate was cultured, the colonies were counted and recorded as cfu/ml, having corrected for dilution factor.

In-vitro control of *Pseudomonas corrugata* using plant extracts:

There were five treatments viz; *Lawsonia inermis, Azadirachta indica, Aloe vera,* Streptomycin was used as positive control while sterile distilled water was used as negative control. All the treatments were conducted in three replicates making a total of 15 plates under observation. Half milliliter (10^7c.f.u/ml) inoculum was pipetted into each of the experimental plates. This was mixed with 0.5ml of plant extracts, streptomycin and distilled water. Nutrients agar was poured on all and each plate was swirled for proper mixing of the content. After setting of the plates, they were arranged in the incubator in a completely randomized design. Colony forming units (cfu/ml) were counted and recorded after 48 hours of plating.

In-vivo control of *Pseudomonas corrugata* using plant extracts:

Sandy-loam soil was sterilized with electric soil sterilizer for 90 minutes. It was then packed into sacks and aseptically stored for two weeks before use. The sterilized soil was spread on the nursery trays and properly moistened. Beske variety of tomato seeds were sowed and nurtured till the 3rd week in the nursery.

With use of hoe and cutlass, the field was cleared, ploughed and made ready for transplanting. The entire area of the plot cultivated was $180m^2$ containing 15 mini-plots of dimensions 2.4m x 2m ($4.8m^2$ area) -each and the space of 1m was observed between each mini-plot. The intra and inter rows spacing within each plot is 50cm x 60cm respectively. The plot was organized in Randomized complete block design (RCBD).

Tomato seedlings were transplanted after 3 weeks in the nursery. This was done at two stands per hole and later thinned to one. Each tomato plant was staked to provide support, holding the plant upright and preventing the fruits from touching ground. Though the field work was carried out during

rainy season but manual irrigation of plot was often done to complement the activities of rain whenever it seized. The field was weeded regularly to avoid the competition of weeds with tomato plants and prevent possibilities of harbouring pests.

Plant extracts prepared at 0.5g/ml concentration were applied at two weeks interval starting from the 3 weeks after transplant. Same rate was applied for streptomycin and sterile water treatment on their respective control plots.

Data were collected once a week and the parameters measured weremean plant height, mean leaf number, mean stem girth, mean number of branches, mean number of fruit per plant, mean disease incidence, mean disease severity and mean percentage mortality. The collected data were subjected to Analysis of variance and means were separated by least significant different (LSD) at 5%.

RESULTS AND DISCUSSION

Effect of plant extracts on the growth parameters of tomato:

Results showed *Aloe vera* as the best promoter of vegetative growth in tomato plant (Table). This was observed significantly (P>/0.05) better performances in the growth parameters measured over other plant extracts and controls. It had significantly (P>/0.05) higher plant height (40.50 cm) and stem girth (2.53 cm). Plant sprayed with water gare significantly (P>/0.05) least performance of growth. There was no significance difference among the treatments in the mean leaf number and mean number of branches. Plant height and stem girth however showed a considerable level of significant difference (Table 1).

Effectiveness of Plant extract as control agent against *Pseudomonas corrugata in–vitro*:

The results showed the effect of plant extracts at 0.5 g/ml in the control of *Pseudomonas corrugata* as indicated by the number of colony forming units (Table 2). *Aloe vera* had significantly (P>/0.05) the best controlling effect on the pathogen, followed by *Lawsonia inermis* and streptomycin. *Azadirachta indica* and water showed a similar level of weakness in inhibition. Effect of *Aloe vera* (cfu/ml of 13.34 x 10^7) was significantly different from that of *Azadirachta indica* with 490.70 x 10^7 cfu/ml bacteria load.

Effect of plant extracts on incidence and severity of tomato pith necrosis:

Table 3 showed the effect of plant extracts on incidence and severity of tomato pith necrosis. The plant extracts gave significantly different disease incidence. The difference was evident in *Azadirachta indica* (53.30%) and streptomycin (35.20%). Disease severity however, showed no significance difference. The

disease incidence for the controls, streptomycin (35.20%) was observed to be lower than the values of the plant extracts. *Aloe vera* had the least value for severity at 38.7%.

Table 4 showed that the use of plant extracts generally did not have any statistically significant (5%) effect on disease incidence and severity. However, the two parameters (incidence and severity) became significantly influenced by the period the plant stayed on the field. Interaction of period and plant extracts also produced significant effect on disease incidence and severity.

The symptoms of tomato pith necrosis was late in *Aloe vera* starting on the 5th week (*Fig. 1*) after transplanting unlike other treatments that expressed symptoms earlier at the 4th week after transplanting. The effect of each plant extract on disease incidence showed *Azadirachta indica* increasing to 100% incidence at the 9th week, and stabilizes in this position to the 10th week. *Aloe vera* led to a steady increase in disease incidence with time, exerting control on the disease at the 9th week (58.4%). *Lawsonia inermis* supported an inconsistent increasing disease incidence caused by the death of the strongly affected plants that have higher scores. It later exerted control reducing the incidence to 66.7% at the 9th and 10th week after transplanting while the control experiment (water) recorded a consistent increase in the disease incidence and reached its peak at the 7^{th} week (66.7%) then started declining with the minimum value at the 9^{th} week (11%), which also is the minimum value recorded for disease incidence among all the treatments.

A similar pattern was observed in disease severity as seen in *Fig. 2 in which Lawsonia inermis* performed significantly (P>/0.05) with lower severity (20%) at the end of the experiment.

Effect of plant extract on the yield of tomato:

Result of the effect of various plant extracts on the yield of tomato is presented in Table 5. Plant extract produced significant difference in the mean number of fruits but none was reflected in the Mean weight. Streptomycin had the highest record of yield, 4.0 and 42.0g for the number and weight of fruit per plant respectively. This was followed by *Azadirachta indica* (2.0 and 39.7g), *Aloe vera* (2.0 and 28.3g) and water (1.33 and 16.9g). *Lawsonia inermis* gave significantly (P>/0.05) lowest yield.

In Table 6, treatment (i.e application of plant extracts) did not produce any significant difference in the readings of various growth parameters and yields. The period stayed on the field, however, had significant impact on the growth of tomato.

Table 1: Effect of Plant Extracts on the Growth Parameters of Tomato

Treatment	Mean Number of leaves	Mean Number of branches	Mean Plant height (cm)	Mean Stem girth (cm)
Aloe vera	15.90	2.85	40.50	2.53
A. indica	12.20	2.03	33.80	1.90
L. inermis	16.90	2.34	35.9	1.81
Streptomycin	16.90	2.94	37.6	2.15
Water	11.30	1.74	29.40	1.88
LSD (5%)	Ns	Ns	10.81	0.65

ns – not significant

Table 2: In Vitro Effect of Plant Extract on Bacterial Population,

Plant extracts	Mean Bacterial Population cfu/ml x 10^7
Aloe vera	11.34
Azadirachia indica	490.66
Lawsonia inermis	26.66
Streptomycin	65.34
Water	482.00
LSD (5%)	478.66

Table 3: Effect of Various Plant Extract on Disease Incidence and Severity of Tomato Pith Necrosis.

Plant extracts	Mean Disease incidence (%)	Mean Disease severity (%)
Aloe vera	40.70	38.70
Azadirachta indica	53.30	52.70
Lawsonia inermis	48.00	47.10
Streptomycin	35.20	49.60
Water	35.50	45.60
LSD	15.37	Ns

ns - not significant

Table 4: ANOVA Table Showing the Effect of Treatment, Weeks after Transplanting and the Interaction on Disease Incidence and Severity

Variable	Mean sum of square			
	Treatment	Weeks	Treatment Week	Residual
Disease incidence	1518.80	7459.6* (p<0.001)	178.80* (p=0.004)	715.40
Disease severity	653.40	6896.50* (p<0.001)	1364.00* (p=0.002)	593.80

ns – non significant

Table 5: Effect of Plant Extract on the Mean Yield of Tomato

Treatment	Number of fruit / plant	Weight of fruit (g)
Aloe vera	2.0	28.3
A. indica	2.0	39.7
L. inermis	0.33	3.9
Streptomycin	4.0	42.0
Water	1.33	16.9
LSD (5%)	3.03	Ns

ns – non significant

Table 6: ANOVA Table showing the effect of treatment, weeks after transplanting and their interaction on growth parameters and yield of tomato

Growth parameters and yield of tomato	Mean sum of squares			
	Treatment	Weeks	Treatment. Week	Residual
Number of leaves	163.10	383.50* (p=0.023)	126.20	154.20
Branches	6.43	17.99* (p=0.004)	3.05	5.40
Plant height	419.20	2615.00* (p<0.001)	325.70	353.80
Stem girth	2.10	3.56* (p=0.014)	0.73	1.30
Number of fruits	5.40	N/A	N/A	2.60
Weight of fruits	762.40	N/A	N/A	585.30

* - Significant

N/A - Not available

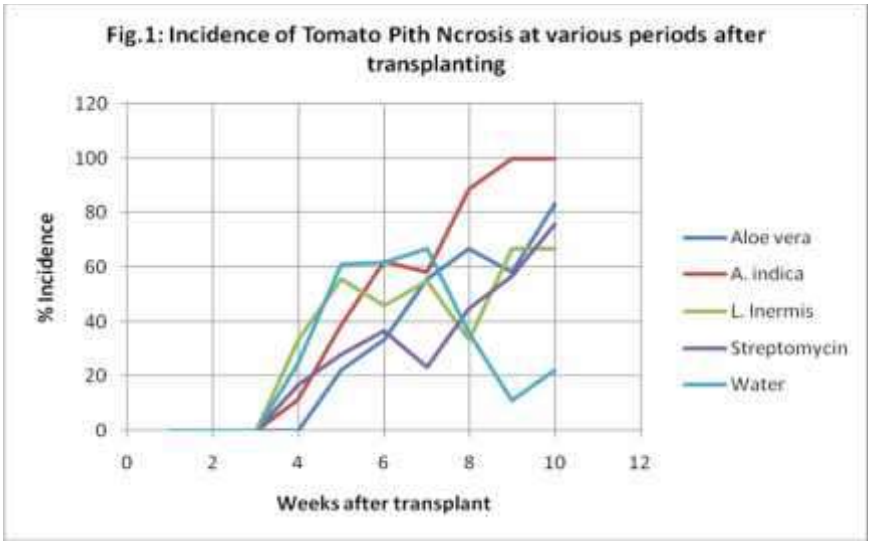


Fig.1: Incidence of Tomato Pith Ncrosis at various periods after transplanting
% Incidence
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Weeks after transplant
Aloe vera
A. indica
L. Inermis
Streptomycin
Water

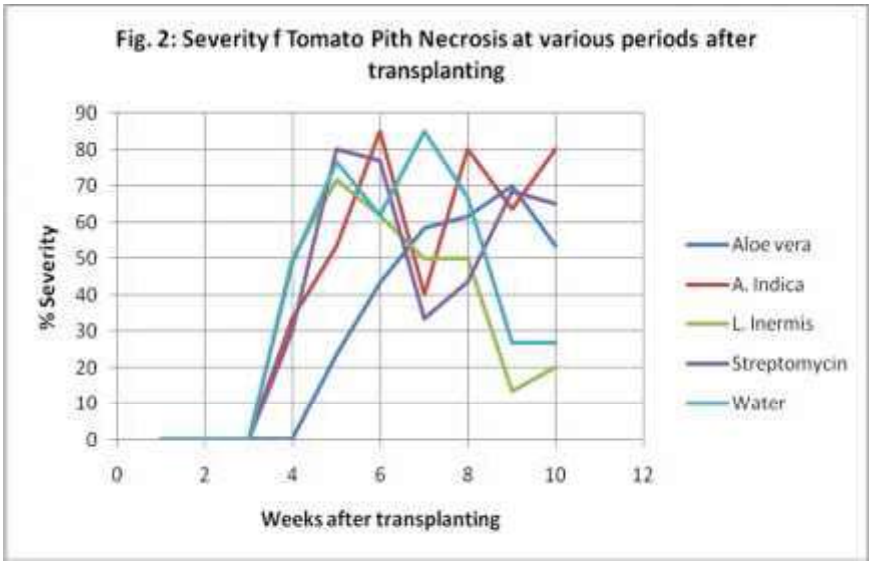
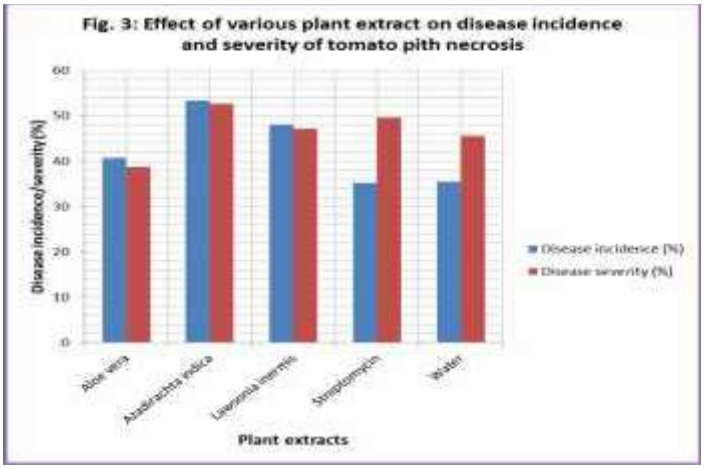


Fig. 2: Severity f Tomato Pith Necrosis at various periods after transplanting
% Severity
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
Weeks after transplanting
Aloe vera
A. Indica
L. Inermis
Streptomycin
Water



The potential of various plant extracts in the control of tomato pith necrosis was evaluated in this study. *Aloe vera* and *Lawsonia inermis* reduce the bacterial population significantly in the in-vitro test, this agrees with Yolanta and Rivka (1995) that uses *Aloe vera* gel at various concentrations to suppressed the germination and mycelia growth of *Penicillium digitatum, P. expansum, Botrytis cinerea*, and *Alternaria alternata* which have been identified as the postharvest fruit pathogens. The inhibitory effect of the *Aloe vera* gel on colony growth was exhibited at 1 µl l^{-1}, when a 67–69% reduction in radial growth was recorded for *P. digitatum, A. alternata*, and *B. cinerea*, and a 19% reduction for *P. expansum* after five days on potato dextrose agar (PDA) at 23 °C. The effect of the gel on disease development in *P. digitatum*-inoculated grapefruit was expressed by both a delay in lesion development and a significant reduction in the incidence of infection following dipping in a concentration of *aloe vera* gel of 10^3 µl l^{-1}. The Antimicrobial activities of *Lawsonia inermis* was compared to that of *Aegle marmelos* and *Albizzia libbeck* by Sudharameshwari and Radhika(2007) against three Gram positive bacteria (*B.cereus, B.subtilis, S. aureus*) and three Gram negative bacteria (*E.coli, P.vulgaris*, and *P.aeruginosa*) by disc diffusion method. Maximum inhibition (3.8cm) was recorded in *Lawsonia inermis* which also showed inhibitory action against all the six pathogen tested. The zone of inhibition of the extracts was compared with the standard antibiotics Streptomycin and Spectinomycin and it was concluded that *Lawsonia inermis* is a promising plant in the development of phytomedicine for antimicrobial properties. Thus, this result conforms with the outcome of this experiment, indicating *L.inermis* as effective after *Aloe vera* in the *in-vitro* experiment.

The effectiveness of the bactericidal ability of the plant extracts were checked on an infected field. A level of significant difference (LSD 5%) was

obtained for the disease incidence. The percentage disease incidence recorded was found to be higher than the average of 15 - 20% reported by Sahin *et al*, (2005). This could be due to the high population of the pathogens in the soil. The disease severity has zero value of significant difference and *Aloe vera* has the least value among the treatments. This shows that the symptom was more severe in the Streptomycin chemical control than in the *Aloe vera* extracts. Uston *et al*, (1997) reported the control of tomato pith necrosis caused by *Pseudomonas cichori*, another strain of *Psendomonas spp*, copper hydroxide was found to be effective in disease reduction from 72% in 2001 to 66% in 2002, this is synonymous to the observation in this study as time was observed to contribute significantly to the rate of incidence and severity of disease. Results showed the plant extracts experiencing decline in value after reaching the peak. This could be attributed to the death of severily affected tomato plants.

Aloe vera was observed to have the best performance, its growth parameters showed a considerable level of increase over the chemical control though Streptomycin has the highest yield.

The outcome of the study shows that the use of plant extracts *Aloe vera* at 0.5g/ml could be a good substitute for chemical pesticide in the management of Tomato Pith Necrosis. Also, this work revealed that the bactericidal effect of *Aloe vera* was potent enough in managing tomato pith necrosis caused by *Pseudomonas corrugata*. This is a cheap and easier method of managing the infection apart from being environmentally friendly. It is therefore recommended to tomato growers for use in protecting the plants.

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