Agricultural Journal 3 (3): 163-170, 2008
ISSN: 1816-9155

Efficacy of Some Plant Extracts on the *In vitro* and *In vivo* Control of *Xanthomonas campestris* Pv. *Vesicatoria*

E.U. Opara and R.C. Wokocha
Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria

Abstract: The efficacy of 12 aqueous plant extracts to inhibit the growth of bacterial spot pathogen (*Xanthomonas campestris pv. vesicatoria*) was tested both *in vitro* and *in vivo*. Data obtained showed that in the *in vitro* experiment, all the plant extracts assayed inhibited the growth of the bacterium to varying degrees when compared with the untreated control. *Azadirachta indica* seed aqueous extract was the most effective (40.33%) while the least effective was *Chromolaena odorata* extract (17.33%). However, in the *in vivo* experiment, *A. indica* seed, *Piper guineense and Citrus sinensis* extracts performed better than other extracts while *C. odorata* was the least effective. When seedlings were artificially inoculated and subsequently treated with the extracts, results showed that *P. guineense, A. indica* and *Allium cepa* performed better than other extracts. This experiment shows that there is an advantage in using some local herbs and plant botanicals as antibacterial agents considering their availability and environmental friendliness when compared with synthetic chemicals.

Key words: *Xanthomonas campestris pv. vesicatoria*, plant extracts, antibacterial agents, efficacy, control

INTRODUCTION

The use of botanicals and antimicrobial agents of plant origin is a time-honored practice for control of plant diseases and pests. The necessity to develop a non-toxic, safe and biodegradable alternative to synthetic bactericides has in recent years led to a concerted effort at developing new sources from plant parts. The humid tropics, especially the rainforest ecological zones are endowed with abundant flora of families of plants and herbs with untapped pesticide potentials (Amadioha, 2002, 2003). Stoll (2000) listed an array of plant families and genera possessing antimicrobial properties, amongst which were *Monodora myristica, Annona Muricata*, *Xylopia aethiopica*, *Garcinia kola*, *Gongronema latifolium*, *Dennitia tripetala*, *Azadirachta indica*, *Piper guineense*, *Zingiber officinale* and host of others. Some workers too Amadioha (2003), Kumar and Pamar (1996) and Prakash and Roa (1997) have listed the following as advantages of plant extracts over synthetic chemicals: Possession of low mammalian toxicity, minimal health hazards and environmental pollution. There is practically no risk of developing pest resistance to these products when used in their natural forms. There are fewer hazards to non-target organisms but resurgence has not been reported in the use of botanicals. No adverse effect on plant growth, seed viability or food quality has been reported. Botanicals are less expensive and easily available because of their natural occurrence in abundance. Aqueous extracts of some plants have been used in laboratory bioassays (John and James, 2004).

These plants include *Allium cepa* (onion), a biennial herb of Liliaceae family used commonly as spice for flavoring food. *Allium sativum* L. (garlic), another biennial herb of Liliaceae family and the second most widely use *allium* after *A. cepa*; it is also used as condiments for flavoring foods. The uninjured bulb contains colorless, water-soluble amino acids; allium which can be hydrolyzed to allian containing diallyl disulphide (Vijalakshmi *et al*., 1996). Stoll (1998) reported the bactericidal properties of *Azadirachta indica* A Juss (neem), a fast growing tree of the family Meliaceae is a medicinal plant with insecticidal, nematicidal, antifugal and bactericidal properties. It occupies a foremost status among all the plants exploited so far for bio-efficacy against pests and diseases (Kumar and Pamar, 1996). The primary antimicrobial constituents are azadiractin A and B. In addition, neem contains a number of other chemical substances which include salannin, meliantriol, azadirachtannin A, cinnamoyl, isoazadirohide, nimbin/ nimbidin which seem to haveantiviral effects as well and vilasinim as isolated from the leaf and azadirone from the

Corresponding Author: E.U. Opara, Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria

seed. Some substances support each other, thus creating synergistic effect (Stoll, 2000). *Cymbopogon citratus* (D.C) Stapf (lemon grass). The plant belongs to the family Poaceae. It contains essential oil with 75-80% of aldehydes consisting of citral, which can be converted to ionone. ß-ionone is used for synthesis of vitamin A. *Garcinia kola* Henkel (bitter cola) is a perennial tree in the family Guttiferae with whorled leathery leaves. The seeds are chewed as stimulants and for other various medicinal values. Traditionally, the seeds are believed to repel snakes. *Ocimum gratissimum* L. (sweet basil) is in the family Labiatae. It contains strongly scented essential oils mainly, which is the active ingredient responsible for the bactericidal qualities. *Piper guineense* Schum and Thonn (black pepper), a climber of the family Piperaceae is highly spicy and provides oils, used as aromatics in the alcohol and medicine industries. The fruits and seeds contain pungent piperine, resin and essential oil, which contribute to the bactericidal properties. *Zingiber officinale* Rosc (ginger) is rhizome of the family Zingiberaceae. The rhizome yields essential oil, oleoresin, consisting 1-3% volatile oil of which the chief constituent is zingiberene and zingerone, which serve as the active ingredient against microorganisms and pests (Benjilali *et al*., 1984). In this investigation therefore, efficacy of some of these botanicals as antimicrobial agents was assessed both in culture and in the field.

MATERIALS AND METHODS

Plant materials: In this investigation 12 locally available plant parts were evaluated for their ability to inhibit the growth of the bacterium responsible for bacterial spot symptoms both *in vivo* and *in vitro*. The 12 test plant parts selected for assessment were as follows: neem seed and leaf (*Azadirachta indica*. A Juss), bitter kola seed (*Garcinia kola* Henkel), ginger stem (*Zingiber officinale*, Rosc) 'sweet basil' leaf (*Ocimum gratissimum* L.), garlic bulb (*Allium sativum* L.), piper seed (*Piper guineense* Schum and Thonn), lemon grass (*Cymbopogon citratus* (D.C) Stapf, orange peel (*Citrus sinesis* Malta), bitter leaf (*Vernonia amygdalina* L.), siam weed leaf (*Chromolaena odorata* L.), 'utazi' leaf (*Gongronema latifolium*). The antibiotics Streptomycin sulphate and sterile distilled water served as controls.

Preparation of plant aqueous extracts: The selected parts of the test plants above were sun dried and ground into fine powder using sterile hand grinder (Amadioha, 2004). Then 3, 5 and 10% extracts were prepared by dissolving 30, 50 and 100 g, respectively of the powdered plant extracts in 1 L distilled water and soaked for 24 h, then filtered with muslin cloth.

Extraction of oils: For *Azadirachta indica* seed and *Citrus sinesis* peels and *piper guineense*, their oil components were extracted employing a modified method of Amadioha (Benjilali *et al*., 1984; Amadioha, 2002). A total of 300 g of the powdered extract were used for extraction with n-hexane at 60°C in a soxhlet extractor for 8 h. At this stage a drop of hexane on a filter paper left no residual oil spot. However, hexane was removed in a rotavapour under reduced pressure at 60°C to yield oil.

Preparation of bacterial inoculum: The bacterial culture (*Xanthomonas campestris* pv. *vesicatoria*) used in this test was isolated from infected tomato fruit in the field during the 2000/2001 cropping season. The method of isolation used was according Bradbury (1970) in which the infected fruits were surface sterilized with 70% ethanol and washed thoroughly with sterile water. A small piece of the diseased tissue was cut from the boundary between the diseased and the healthy tissue with a sterile scalpel. The tissue was then washed thoroughly in sterile water twice before placing in Petri dish with a drop of water and then crushed with sterile needles and allowed to stay for 30 min. The suspension obtained was then streaked on Sucrose Peptone Agar (SPA). The streaked media were placed upside down in the incubator and the temperature maintained at 27°C for a period of 48 h. A pure culture was obtained after sub culturing twice. The bacterial suspension of fresh colonies (24 h old) from SPA in distilled water was obtained by suspending the young active colonies in sterile distilled water to give a concentration of approximately 10^8 cells mL^{-1} (cfu mL^{-1}) adjusted with a haemocytometer.

Phytotoxicity test of the plant extracts on tomato seedlings: To determine if botanicals could be harmful to plants at the concentrations in which they are thought to be toxic against the pathogen, phytotoxicity test was deemed necessary before trails with the crops. For this reason the botanicals selected for use as antimicrobial agents were assessed for phytotoxicity effect at 2 stages of crop growth; at germination and seedlings stages.

Germination test: Hundred seeds of tomato (Roma F) seeds were placed in a sterile Petri dish with sterile filter paper (Whatman No.1) and replicated 5 times so that each treatment had a minimum of 500 seeds. Using sterile syringe 5 mL each of the eleven aqueous plant extracts was applied at different concentrations (30, 50 and 100 L^{-1} while streptomycin was tested at 0.1, 0.5 and 1.0 g L^{-1} active ingredient). The filter paper was previously moistened with sterile water before adding the extracts. There was a control, which was treated with sterile

distilled water while Streptomycin served as a standard with which to compare the performance of the extracts. The Petri Dishes had their lids covered and incubated for seven days at room temperature. After 7 days of incubation the percentage seed germination was recorded.

Phytotoxicity test of vegetative growth: Tomato seeds were first raised in clean nursery boxes containing heat sterilized soil before transplanting one per a pot, 2 weeks after germination and arranged in Completely Randomized block Design (CRD) with 5 replications. When the seedlings were 2 weeks old (after transplanting) the treatments were applied using the same concentrations (30, 50 and 100 g L^{-1}) as in the germination test. However, method of application was by foliar spray using a hand sprayer. The plants were allowed to grow for 2 weeks after which plants were assessed for leaf damage, stunted growth or growth inhibition by taking some growth parameters like plant height and leaf shape. Leaf assessment key adopted was that of Bird and Hardley (1958) as follows; Entire leaf in good shape, One-third of leaf area distorted, One-half of leaf area completely distorted and Whole leaf area completely distorted.

Effect of plant extracts and oils on the pathogen *in vitro*

Agar plant method: The aqueous extracts were used at two concentrations; 30 and 50 g L^{-1}. The culture medium used was Nutrient Yeast extracts Agar (NYA) consisting of (mg L^{-1}): beef extract powder 1.0, yeast extract 2.0; peptone 5.0, NaCl 5.0 and agar 15.0, adjusted to pH 7.4 using dilute solution of NaOH.

After autoclaving, the medium was allowed to cool down to 45°C before aseptically adding 5 mL each of the twelve aqueous extracts and oils separately to 15 mL of the medium and stirred to mix thoroughly. Each medium mixture was dispensed into Petri dishes and allowed to solidify. A second set of Petri dishes contained 15 mL of the medium mixed with 5 mL of standard antibiotic (0.1% Streptomycin) while the 3rd set of plates received 5 mL sterile water in the medium without any plant extract, these last 2 sets were used as a standard and control respectively. In all, there were fourteen treatments, each replicated 5 times.

The effect of the extracts and oils on inhibiting the growth of the pathogen was determined using a modified method described by Amadioha (2003, 2004), in which the center of well-solidified surface of each culture plate was inoculated with a disc of 5 mm diameter from a 48 h old bacterial culture. The culture plates were incubated at 28±2°C for 48 h in the laboratory. The tests were repeated three times to ensure consistency and the mean values obtained. Thereafter, the cultures were assessed for colony growth and the percentage growth inhibition or the minimum inhibitory rate recorded. Growth inhibition was obtained by measuring the colony growth diameter, taken as mean of the widest and the shortest diameter. The percentage growth inhibition was determined using the formula adopted by Amadioha (2003, 2004) as follows:

$$\% \text{ growth inhibition} = \frac{dc - dt}{de} \times \frac{100}{1}$$

Where,

dc = Colony diameter of control.

dt = Colony diameter of treated plates.

Disc diffusion sensitivity tests: Antimicrobial sensitivity testing was carried out using the disc diffusion sensitivity test of Cheesbrough (1991). Preparation of antibacterial discs was achieved through the following procedure: Discs (5 mm in diameter) were punctured out from a Whatman No.1 filter paper. Four discs were placed in Petri dish diameter allowing about 4 mm in between discs and then sterilized in hot air oven at 160°C for 1h. After allowing the disc to cool, about 0.1m1 of the sterile aqueous extracts (50 g L^{-1}) or oils (30 g L^{-1}) were pipetted unto each disc. The discs were dried by placing them inside Petri dishes with lids slightly raised in an incubator at 37°C for 1 h.

To assess the extracts for antimicrobial activity, sterile Petri dishes of 9 cm diameter were filled to a depth of 4 mm with 25 mL NYA medium. A loopful of colony suspension from 24 h old culture was evenly spread over the medium using a cotton swab. The inoculated plates were allowed to dry for approximately 5 min, with their lids slightly opened in incubator, kept at 37°C and the anti-microbial discs placed 5 per plate with a needle in such a way that the disc to not trap in air. The plates were then incubated at 28±2°C for 24 h. After incubation, the inhibition zone diameters were measured to the nearest millimeter (mm) with a ruler, while the mean were calculated and analyzed statistically; using Analysis of Variance (ANOVA) and the treatment means separated using Fisher's Least Significance Difference (LSD) at 5% level.

Eefect of plant extracts on bacterial spot disease *in vivo*

Field trial: The efficacy of the 12 plant aqueous extracts in reducing bacterial spot disease caused by *Xanthomonas campestris pv. vesicatoria* was evaluated in the field for 2 cropping seasons in 2002 and 2003 wet seasons using tomato RomaVF. The plant extracts used

were from neem seed and leaf (*Azadirachta indica.* A Juss), bitter kola seed (*Garcinia kola* Henke*l)*, ginger stem (*Zingiber officinale*, Rosc) 'sweet basil' leaf (*Ocimum gratissimum* L), garlic bulb (*Allium sativum* L.), piper seed (*Piper guineense* Schum and Thonn). Lemon grass (*Cymbopogon citratus* (D.C) Stapf, orange peel (*Citrus sinensis* Malta), bitter leaf (*Vernonia amygdalina* L.) Siam weed leaf (*Chromolaena odorata* L), 'utazi' leaf (*Gongronema latifolium* Benth-Asclepiadaceae). The antibiotics Streptomycin sulphate and sterile distilled water served as a standard and control, respectively.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Plots were 7×4 m^2 and crop spacing 0.5×1 m giving a plant population of 75 per replicate and 225 per treatment. This study was performed under natural infection conditions without artificial inoculation (Graigne and Ahmed, 1996). Aqueous plant extracts were prepared at the rate of 5% powder as earlier described while streptomycin sulphate was applied at the rate of 1% a.i. (active ingredient). Sterile distilled water was used as a control experiment. All treatments were applied as foliar sprays. The first spraying was carried out 10 days after transplant. The second spraying was done 7 days later.

Disease incidence and severity assessment started 7 days after the last spraying followed by weekly assessment up to fruiting stage. About 50 seedlings per plot from the net plots were randomly selected for assessment. Bacterial spot disease incidence was measured as percentage of the plants affected in the 150 randomly selected plants per treatment. Disease severity score was determined based on scale 1-6 (Bird and Hardley, 1958) index as follows: Disease free leaf; One or 2 pin point lesions on leaf; Few scattered lesions on leaf; Large number of lesions on leaf, no coalescing; Large number of lesions on leaf with coalescing and Leaf completely killed.

Plant growth parameters considered include plant height (cm), stem diameter (cm), number of leaves, number of branches and number of flowers. Also yield in kg ha^{-1} was recoded per plot and treatment means were statistically analyzed using Fishers' Least Significance Difference (LSD) at 5% level of significance.

RESULTS

Phytotoxicity test of the different plant extracts and botanicals on tomato crops

Effect of plant extract concentrations on seed germination: The effect of three concentrations (30, 50, 100 g L^{-1}) of 12 plant extracts along with streptomycin and distilled water on germination of tomato seed are shown on Table 1. The data showed that at 30 g L^{-1} there was no significant differences ($p \leq 0.05$) among the extracts of *A. sativum, C. odorata, C. sinensis P. guineense*, *G. latifolium* and distilled water which served as controls at 30 g L^{-1}. However, differences exist between the means of *O. gratissimum* (88.77%), A. *indica* leaf (88.60%), *G. kola* (88.47%) and distilled water (88.31%). While *A. indica* seed (87.98%), Streptomycin (87.87%), *Z. officinale* (87.83%) performed lower than and distilled water and their means were significantly different at $p \leq 0.05$. At 50 g L^{-1} *G. latifolium* leaf extract had highest (89.19%) in terms germination % followed by *A. sativum* (88.58%) the least at 50 g L^{-1} was streptomycin (87.73%) followed by *A. indica* seed (87.98) and their differences were statistically significant ($p \leq 0.05$). When the concentration was increased to, 100 g L^{-1} *A. indica* leaf extract had the highest percentage germination (88.77%) even better than distilled water (88.60%) but there were no significant differences among them ($p \leq 0.05$). The least percentage germination was recorded by streptomycin (87.60%) followed by *Z. officinale* (87.77%) and there was differences between their mean and that of the control (distilled water).

Effect of the extract concentration on leaf development: From the data obtained (Table 1) at 30 g L^{-1} concentration, all the extracts tested did not differ significantly ($p \leq 0.05$) from the control (1.00) except for *A. indica* seed (1.02) *P. guineense* (1.02) and streptomycin (1.03). The result at 50 g L^{-1} followed similar trend as in 30 g L^{-1}; streptomycin, *P. guineense, C. sinensis* and A. *sativum* had the same leaf deformity value (1.02) which differed significantly ($p \leq 0.05$) from that of distilled water, *C. odorata*, *A. indica* leaf, *C. citratus*, *G. kola* and *G. latifolium* with the same score (1.00). While at 100 g L^{-1} the result showed that streptomycin had the highest leaf deformity (1.06) which differed from the control (distilled water) and the extracts followed by *A. sativum*, *A. indica* and *G. kola* and *P. guineense* (1.02) and their values significantly differed ($p \leq 0.05$) from that of the control. The rest extracts did not differ significantly ($p \leq 0.05$) from the control except *C. citratus*, *V. amygdalina* and *Z. officinale*.

Effect of the concentration on plant height: The results of twelve plant extracts tested at the three concentrations (30, 50 and 100 g L^{-1}) on plant height are shown in Table 1. At 30 g L^{-1} *A. indica* leaf extract performed best (21.56 cm) followed by *A. indica* seed extract (21.43 cm) and the difference was significant ($p \leq 0.05$) from that of control (21.11 cm). Other extracts including streptomycin were significantly better than the control except *G. kola*

Table 1: Phytotoxicity test of different concentration of plant extracts on seed germination and crop growth

	25 g L^{-1} Concentration			50 g L^{-1} Concentration			100 g L^{-1} Concentration		
	Germination (%)	Leaf deform	Plant height (cm)	Germination (%)	Leaf deform	Plant height (cm)	Germination (%)	Leaf deform	Plant height (cm)
A. sativum	88.26±0.18	1.01±0.006	21.10±0.33	88.58±0.72	1.02±0.003	21.33±0.12	88.33±0.33	1.02±0.003	22.00±0.27
A. indica seed	87.98±0.44	1.02±0.008	21.43±0.93	87.98±0.44	1.01±0.006	21.23±0.93	87.83±±0.49	1.02±0.003	22.00±0.27
A. indica leaf	88.60±0.72	1.00±0.000	21.56±0.26	88.05±0.24	1.00±0.000	21.05±0.33	88.77±0.37	1.00±0.000	21.60±±0.93
C. odorata leaf	88.12±0.24	1.00±0.000	21.15±0.12	88.33±0.29	1.00±0.000	21.15±0.27	88.43±0.28	1.00±0.000	21.96±±0.11
C. sinensis peel	88.32±0.28	1.00±0.000	21.33±0.12	88.31±0.28	1.02±0.008	21.23±0.12	88.31±0.28	1.00±0.000	21.67±0.60
C. citrates leaf	87.77±0.37	1.01±0.006	21.26±0.60	88.33±0.33	1.00±0.000	21.08±0.60	87.86±0.49	1.01±0.006	21.27±0.93
G. kola seed	88.47±0.28	1.01±0.006	21.00±0.33	88.43±0.23	1.00±0.000	21.03±0.33	88.26±0.18	1.02±0.003	21.93±0.16
G. latifolium	88.33±0.33	1.00±0.000	21.25±0.15	89.19±0.21	1.00±0.000	21.03±0.15	88.16±0.24	1.00±0.000	21.89±0.26
O. gratissimum	88.77±0.37	1.00±0.000	21.09±0.16	87.95±0.49	1.01±0.006	21.18±0.16	88.32±0.28	1.00±0.000	21.81±0.33
P. guineense	88.33±0.33	1.02±0.003	21.08±0.15	86.26±0.21	1.02±0.003	21.30±0.15	88.12±0.24	1.02±0.003	22.27±0.93
V. amygdalina	88.27±0.37	1.00±0.000	21.03±0.16	88.35±0.18	1.01±0.005	21.01±0.16	88.32±0.33	1.01±0.006	21.26±0.11
Z. officinale	87.83±0.49	1.01±0.006	21.01±0.60	88.27±0.37	1.01±0.008	21.08±0.60	87.77±0.49	1.01±0.006	21.85±0.29
Streptomycin	87.87±0.49	1.03±0.008	21.24±0.00	87.7±3±0.37	1.02±±0.003	21.00±0.00	87.60±0.37	1.06±0.008	20.83±0.60
Distilled water	88.31±0.28	1.00±0.000	21.11±0.11	87.07±0.12	1.00±0.000	21.40±0.11	88.60±0.72	1.00±0.000	21.53±0.37
SE	0.280	0.008	0.119	0.246	0.005	0.927	0.264	0.006	0.401

Table 2: Effect of plant extracts on the bacterial spot pathogen using the agar plant method

Plant extract (Botanical)	Concentration (g L^{-1})	Rate of inhibition (%)
A. indica seed oil	30	40.35^{B}
A. indica leaf	50	22.50^{EFG}
G. Kola seed	50	18.33^{G}
V. amygdalina leaf	50	20.33^{FG}
Z. officinale stem	50	20.50^{FG}
O. gratissimum leaf	50	18.33^{G}
A. sativum bulb	50	25.33^{EF}
P. guineense seed oil	50	33.00^{CD}
C. citratus leaf	50	27.33^{ED}
C. sinensis peel oil	30	34.83^{BC}
G. latifolium leaf	50	18.50^{G}
C. odorata leaf	50	17.33^{G}
Streptomycin (antibiotic)	5	95.0^{A}
Distilled water (Control)	-	0.0^{H}

Table 3: Diffusion sensitivity test of different plant extracts against bacterial pathogen *In vitro*

Plant extract (Botanical)	Concentration (g L^{-1})	Growth of inhibition (%)
A. indica seed oil	30	66.80±5.25
A. indica leaf	50	42.46±3.15
G. Kola seed	50	49.35±4.35
V. amygdalina leaf	50	48.98±2.55
Z. officinale stem	50	44.69±0.29
O. gratissimum leaf	50	47.02±2.80
A. sativum bulb	50	62.62±5.09
P. guineense seed oil	50	45.72±3.52
C. citratus leaf	50	52.75±2.90
C. sinensis peel oil	30	44.04±6.00
G. latifolium leaf	50	40.64±4.24
C. odorata leaf	50	40.64±3.19
Streptomycin (antibiotic)	5	98.96±7.02
Distilled water (Control)	-	0.00±0.00
SE		11.014

(21.00 cm), *Z. officinale* (21.01 cm) which did not differ from the control experiment at $p \leq 0.05$. At 50 g L^{-1} there were no differences among the means of the various treatments. However, *A. sativum* had highest plant height (21.33 cm) followed *P. guineense* (21.30 cm), the lowest plant height was scored by streptomycin (21.00 cm) followed by *G. kola* and *G. latifolium* (21.03 cm). The data obtained at 100 L^{-1} on plant height shows that the highest was *P. guineense* (22.27 cm) followed by *A. sativum* (22.00 cm) and *C. odorata* (21.96m) their values differed significantly ($p \leq 0.05$) from that of control (21.53 cm), the rest did not differ ($p \leq 0.05$) from the control except streptomycin (20.83 cm).

Effects of plant extracts on the growth of the pathogen *in vitro*

Agar plate method: The results of the effect of different extract on growth of the pathogen in the laboratory using the agar plate medium is shown on Table 2. The data obtained showed that all the plant extracts and oils inhibited the growth of the test bacterium (*Xanthomonas campestris pv. vesicatoria*) to varying degree. *Azadirachta indica* seed oil (40.33%) was the second superior after streptomycin (95%) and the value differed significantly ($p \leq 0.05$) from the other plant extracts. *Citrus* peel oil came third (34.8%). The least inhibitory value was recorded with *Chromolaena odorata* leaf (17. 33%) and this was significantly better than the control (0.00%) which did not inhibit the pathogen.

Disc diffusion sensitivity test: The various plant extracts and oils exhibited different degrees of inhibition against the bacterium using the paper disc method. The results obtained in relation to twelve plant extracts microbial effects are presented in Table 3. Among the extracts evaluated *A. indica* seed oil had the highest growth inhibition (66.8%) next to the standard antibiotic (98.96%), followed by *A. sativum* (62.62%), *C. citratus* (52.75%) and *G. kola* (49.35%). The least growth inhibition % was recorded by *C. odorata* leaf and *G. latifolium* (40.64%) that performed better than distilled water, the control.

Table 4: Effect of different plant extracts on disease severity, growth and yield of tomato during the 2002 cropping season

Plant extract	Disease severity	Plant Ht. (cm)	Plant Dia.(cm)	No. Leaf	No. Branch	No. Flower	Fruit wt. (g)
A. indica seed	3.05±0.13	72.45±0.12	3.16±0.02	28.67±0.19	9.00±0.76	7.84±0.48	39.34±0.77
A. indica leaf	3.90±0.12	73.09±2.06	2.32±0.04	18.84±0.48	6.16±0.67	6.33±0.58	27.66±1.34
G. kola seed	3.45±0.07	80.75±5.24	2.77±0.02	21.17±2.02	9.00±0.76	9.33±0.58	33.67±2.11
V. amygdalina leaf	4.04±0.06	66.50±8.75	2.50±0.29	17.17±0.09	6.17±0.67	8.83±0.09	28.34±1.34
Z. officinale stem	3.46±0.02	75.90±1.31	5.70±0.67	19.17±1.24	9.67±1.15	8.17±0.09	36.0±2.50
O. gratissimum leaf	3.95±0.02	55.00±5.67	2.63±0.28	15.00±1.34	5.33±0.77	7.17±0.28	27.00±0.58
A. sativum bulb	3.38±0.21	70.75±0.76	3.12±0.20	23.17±1.24	7.66±1.34	6.66±0.00	40.33±0.77
P. guineense seed	3.09±0.09	72.50±2.98	2.77±0.00	20.50±1.05	9.16±1.82	11.33±0.57	44.50±1.63
C. citrates leaf	4.00±0.24	65.67±1.15	2.72±0.14	20.17±0.67	8.50±1.25	13.67±1.73	30.50±0.05
C. sinensis peel	3.13±0.07	78.84±5.86	2.63±0.58	21.00±0.19	8.33±1.35	13.17±0.02	39.84±0.48
G. latifolium leaf	4.35±0.05	65.67±0.19	2.63±0.27	22.00±0.19	7.50±0.86	10.16±1.63	28.00±0.57
C. odorata leaf	4.13±0.07	60.83±2.59	2.50±0.01	19.00±0.38	6.33±0.58	6.83±0.09	27.57±1.15
Streptomycin	2.42±0.19	79.50±0.47	3.28±0.03	26.17±0.09	13.17±1.06	15.00±1.15	47.00±1.34
Distilled water	4.79±0.27	53.84±1.05	2.30±0.21	14.67±0.57	3.00±0.00	6.00±0.58	24.17±0.67
Mean	3.704	64.410	2.599	20.510	7.785	9.488	33.892
SE	0.288	5.738	0.382	1.722	0.882	1.938	1.206

Table 5: Effect of different plant extracts on disease severity, growth and yield of tomato during the 2002 cropping season

Plant extract	Disease severity	Plant ht. (cm)	Plant dia.(cm)	No. Leaf	No. Branch	No. Flower	Fruit wt. (g)
A. indica seed	3.28±0.13	73.17±0.12	3.20±0.02	29.00±0.19	10.33±0.76	8.67±0.48	38.00±0.77
A. indica leaf	4.08±0.12	69.50±2.06	2.23±0.04	19.67±0.48	7.33±0.67	5.33±0.58	25.33±1.34
G. kola seed	3.57±0.07	71.75±5.24	2.73±0.02	17.67±2.02	10.33±0.76	8.33±0.58	30.00±2.11
V. amygdalina leaf	3.92±0.06	51.50±8.75	2.00±0.29	17.00±0.09	7.17±0.67	8.67±0.09	26.34±1.34
Z. officinale stem	3.50±0.02	78.17±1.31	2.53±0.67	17.00±1.24	11.66±1.15	8.33±0.09	31.67±2.50
O. gratissimum leaf	4.00±0.02	45.17±5.67	2.13±0.28	12.67±1.34	6.67±0.77	6.67±0.28	26.00±0.58
A. sativum bulb	3.75±0.21	62.50±0.76	2.76±0.20	21.0±1.24	10.0±1.34	6.67±0.00	39.00±0.77
P. guineense seed	3.25±0.09	67.33±2.98	2.76±0.00	22.33±1.05	12.33±1.82	12.33±0.57	41.67±1.63
C. citrates leaf	3.58±0.24	63.67±1.15	2.47±0.14	19.0±0.67	10.67±1.25	16.67±1.73	28.67±0.05
C. sinensis peel	3.25±0.07	68.68±5.86	2.53±0.58	21.33±0.19	10.67±1.35	9.67±0.02	39.00±0.48
G. latifolium leaf	4.25±0.05	65.33±0.19	2.16±0.27	21.67±0.19	9.00±0.86	13.0±1.63	27.00±0.57
C. odorata leaf	4.00±0.07	56.33±2.59	2.47±0.01	19.67±0.38	7.33±0.58	7.00±0.09	25.67±1.15
Streptomycin	2.75±0.19	78.67±0.47	3.33±0.03	26.33±0.09	15.00±1.06	17.00±1.15	44.67±1.34
Distilled water	4.33±0.27	52.00±1.05	1.93±0.21	13.67±0.57	3.00±0.00	7.00±0.58	23.00±0.67
Mean	3.68	64.54	2.51	19.85	9.40	9.68	31.83
SE	0.209	5.836	0.362	1.022	0.982	1.988	1.006

Field experiment (natural infection): The results of effect of the different plant extract on disease severity in the non-inoculated tomato seedlings in the field during the 2002 and 2003 cropping seasons are presented on Table 4 and 5.

Effect of the plant extract on disease severity: The data obtained on the effects of the twelve plant extracts on disease showed that during the 2002 season that *A. indica* seed extract was superior to other extracts and next to the antibiotic (streptomycin). The least effective and next to distilled water were *G. latifolium* and *C. citratus* leaf extracts (Table 4). In 2003 cropping season, *P. guineense* seed, *C. sinesis* peel and A. *indica* seed extracts performed better than the other extract and their differences were not significant (p = 0.05) when compared with the value scored by streptomycin, but was highly significant from that of distilled water (control), *Z. officinale* stem and *G. kola* seed extracts also performed better than distilled water and their differences were statistically different ($p \leq 0.05$) from that of distilled water (Table 5).

Effect of the plant extracts on tomato growth: In 2002, the data analysis on the effect of the extracts on plant height indicated that the best growth was supported by *C. sinesis* peel extract (89.00 cm) and *G. latifolium* leaf extracts (89.83 cm). For other plant, extracts there were no significant differences between their values and that of distilled water at $p \leq 0.05$ (Table 4). However, during the 2003 cropping season the highest was recorded with Streptomycin (78.67 cm), followed by *Z. officinale* (78.17 cm) and this was significant. The least in that cropping season in terms if plant height was *O. gratissimum* leaf extract (45.17 cm) which did not differ from that of distilled water (Table 5). On plant diameter, there was no significant difference among the different plant extract, but the value for *Z. officinale* stem extract was statistically different ($p \leq 0.05$) from that of distilled water (control). *G. latifolium* leaf, *V. amygdalina* etc. during the 2002 cropping season (Table 4). In the 2003 cropping season *A. indica* seed performed better but differed significantly only from *O. gratissimum* and *V. amygdalina* leaf extract (Table 5).

The data obtained from the effect of the different extract treatments on number of leaves showed that for the two years, *A. indica* seed extract scored highest and this was significantly different from that of distilled water in 2002 (Table 4). However, during the 2003 wet season trial, *O. gratissimum* and *V. amygdalina* leaf extract had values that were not significant from that of distilled water on one hand and those of other plant extracts in 2002 (Table 5). Regarding the effect of the extracts on number of plant branches in the 2002 season, Streptomycin had the highest number of branches (11.33).

This did not differ significantly from those of *G. kola, Z. officinale* and A. *indica* seed extracts but differed significantly from the other extracts (Table 4). In the 2003 season, there were no significant differences among the 14 treatment means (Table 5).

Effect of plant extracts on yield and components: The efficacy of the plant extracts on number of flowers and fruits for 2002 and 2003 are summarized on Table 4 and 5. Analysis obtained in the 2002 wet season on mean number of flowers showed that *C. citrus* peel extract had the highest (16.67) although this value did not differ significantly from those of other plant extracts at $p<0.05$ but differed from those of distilled water (Table 4). In 2003 cropping season, the result obtained showed that the extract of *C. citrates* leaf was not significantly different from that of Streptomycin but the values differed significantly from that of distilled water (Table 5). The records on mean fruit weight per plant showed that during the 2002 cropping season, Streptomycin had the highest weight (49.33 g) and this was significantly different from the records for *P. guineense* seed extract which significantly from those of other extracts at 5% probability (Table 4). During the 2003 cropping season, the result followed similar trend as in 2002 with *P. guineense* seed extract coming next to Streptomycin. The least fruit weight was recoded with *O. gratissimum* extract, which was not significantly different from the control (Table 5).

DISCUSSION

This study shows that at 100 g L^{-1} most of the extracts did not inhibit germination of seed, nor did it significantly affect the crop adversely on the crop growth parameters tested (plant height and leaf development). However, at 50 g L^{-1} there was higher percentage germination but that did not differ significantly with that of 100 g L^{-1} when compared statistically. A better seed germination was achieved at 30 g L^{-1} although; there were on differences with other treatments including the control experiment. The effect of the extracts at 100 g L^{-1} on vegetative growth of the seedlings in terms of leaf and height performance followed the same trend with seed germination tests. However, many workers have tried different ranges on different crops. For instance, Vijayalakshimi *et al.* (1996) apply 24 g L^{-1} against *Xanthononas* species in cowpea while Graigne and Ahmed (1996) applied 50 g L^{-1} against bacterial blight caused by *Pseudomonas phaseolicola* of cowpea. Jagannathan and Sivaparakasam (1996) applied neem oil and extract at 3 and 5%, respectively against sheath rot of rice caused by *Saraclacluim oryzae* while John and James applied aqueous emulsions of neem oil and pepper extracts at 1, 5 and 10% (John and James, 2004). Although, some work has been done on extracts of local herbs found in humid southeastern zone of Nigeria it can be said that using any extract at full strength (100 g L^{-1}) may not be necessary since low concentration (30 g L^{-1}) or half strength (50 g L^{-1}) can achieve the same disease reduction a that of 100 g L^{-1}.

In the agar plate experiment, the 12 test aqueous extracts showed various degrees of inhibition against the bacterial spot pathogen in culture. *A. indica* seed and *C. citrates* peel oil were superior in inhibiting the growth of the bacterium probably because of the diffusible essential oil contents; similar conclusion was made by earlier workers against some pests (Benjilali *et al.*, 1984; Jones and Jones 1985). Similarly, P. *guineense* seed ranked third in inhibitory activity against the test organism for the same reason. Amadioha (2002) reported that the differences in toxicity of different extract were due to the presence of different active compounds in the plant materials. Other workers have also shown that plant extracts contain some antimicrobial substances, which are responsible for the inhibition of the organisms *in vitro* or *in vivo*. Some of the active materials in plants with antimicrobial properties include azadiractin in *A. indica,* piperine in *P. guineense* and zingiberene in *Z. officinale* (Jones and Jones 1985; Fahy and Hayward, 1993; Kumar and Parmar, 1996; Stoll, 2000).

The trend was similar in the disc sensitivity tests conducted using the same extracts and oils. Although, the use of disc diffusion test has been criticized by some workers because it based on human interpretative criteria. The data obtained from the disc diffusion tests are valuable because they distinguished the sensitive from resistant strains of bacterial pathogens. In addition, the disc diffusion test merely indicated that if the organisms are susceptible, then control may be successful if extract concentration similar to those achieved in laboratory could be applied in the field and where resources are not available to determine Minimum Inhibitory Concentration (MIC) the pathologist can rely on disc sensitivity tests

(Cheesbrough, 1991). It is more advantageous that agar plate data are used in conjunction with disc diffusion data in estimation and evaluation of organism as susceptible or resistant to antimicrobial agents (Barley *et al*., 1998). In the present study, the inhibition of *X. campestris* pv. *vesicatoria* by some of the test botanicals similar to the standard antibiotic (Streptomycin) shows that plant extracts can serve as a good alternative to chemical bactericides considering their safety, degradable qualities and cost effectiveness.

REFERENCES

Amadioha, A.C., 2002. Fungitoxic effects of extracts of *Azadirachta indica* against *Cochliobolus miyabeanusi* causing brown spot disease of rice. Arch. Phytopat. Pflanz, 35: 37-42.

Amadioha, A.C., 2003. Evaluation of some Plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. Acta. Phytopathol. Enthomol. Hungarica, 38: 259-265.

Amadioha, A.C., 2004. Control of Black rot of potato caused by *Rhyzoctonia bataticola* using some plant leaf extracts. Arch. Phyyopathol. Plant Prot., 37: 111-117.

Barley, T.A., C. Silvamore, U. Warnery, J.A. Samour and J.A. Naldo, 1998. Antimicronial resistance and MIC of Bacteria Isolated from Bustard In united Arab Emirate. Avian Dis., 42: 690-697.

Benjilali, B., A. Tantaoui-Elaraki, A. Ayadi and M. Hilal, 1984. Method to study the antimcrobial effect of essential oils. Application of the antifungal activity of 6 marocan essence. J. Food Prot., 47: 748-752.

Bird, L.S. and H.H. Hardley, 1958. A statistical study of the inheritance of Stoneville 20 resistance to *Xanthomonas malvacearum* races 1 and 2. Genetics, 43: 750-767.

Bradbury, J.F., 1970. Isolation and preliminary study of bacteria from plants. Rev. Plant Pathol., 49: 213-218.

Cheesbrough, M., 1991. Microbiology, Butterworth, Heinemann. Ltd, Oxford, London, II: 190-205.

Fahy, P.C. and A.C. Hayward, 1983. Media and Methods for Isolation and Diagnostic Test. In: Plant Bacterial Diseases. Fahy, P.C and Persley, G.J. (Eds.), pp: 378.

Graigne, M. and S. Ahmed, 1996. Handbook of plants with control properties. John Wiley and Sons, pp: 355.

Jagannathan, R. and K. Sivaparakasam, 1996. Effect of botanicals on incidence of sheath rot of rice. J. Food Chem., 44: 2147-21150.

John, H.B. and C.L. James, 2004. Effect of formulated plant extracts and oils on population density of *Phytophtora nicotianae* in Soil and Control of Phtophtora blight in the Greenhouse. Plant Dis., 88: 11-16.

Jones, J.B. and J.P. Jones, 1985. The effect of bactericides tank mixing time and spray schedule in bacterial leaf spot of tomato. Proc. Fla. State Hort. Soc., 98: 244-247.

Kumar, J. and B.S. Parmar, 1996. Physiochemical and chemical variation in neem oils and some bioactivity, against *Spodoptera litura F.* J. Agric. Food Chem., 44: 2137-2143.

Prakash and J. Roa, 1997. Botanical Pesticides in Agriculture Lewis Publishers, London, pp: 416.

Stoll, G., 1998. Natural crop protection in the tropics and sub-tropics. Agrecol, Switzerland, pp: 188.

Stoll, G., 2000. Natural Crop Protection in the Tropics; Letting Information come to life. 2nd Edn. Margraf Verlag, pp: 101-139.

Vijayalakshmi, K., B. Subhashini and V.K. Shivani, 1996. Plants in Pest control: Garlic and Onions. Centre for Indian knowledge systems. Hennai/India, pp: 376.