JOURNAL OF SUSTAINABLE AGRICULTURE AND THE ENVIRONMENT

E-mail: jsae@mouau.edu.ng. Website: www.mouau.edu.ng.

Studies on Bacterial Leaf Spot Disease of Yam (*Dioscorea* Spp) Induced by (*Xanthomonas campestris*) Using Some Plant Extracts in Umudike, South Eastern Nigeria

E. E. Umoh and E. U.Opara

Department of Plant Health Management,
Michael Okpara University of Agriculture, Umudike,
Abia State, Nigeria.
Corresponding Author: Email: ezek4umoh@yahoo.com

Abstract

A survey, field experiment and screen house trial were carried out to assess the incidence and severity of bacterial leaf spot caused by *Xanthomonas campestris* on three yam species (*Dioscoreaalata*, *Dioscorearotundata* and *Dioscoreadumentorum*) in Umudike. Bacterial leaf spot disease of yam was surveyed in the field during the 2016 cropping season at National Root Crops and Research Institutes farm Umudike. Based on varietal performance. *D. alata* (1166) variety recorded (4.58,5.67, 7.33, 8.08, 9.75 and 37.50%, 41.67%, 50.00%, 54.17%, 62.50%) for disease severity and incidence respectively, *D. rotundata* (Nwa) variety recorded (1.50, 2.75, 2.75, 3.58, 8.83 and 16.67%, 33.33%, 54.17%,66.67%,75.00%) for disease severity and incidence respectively and *D. dumentorum* (Ekpe) variety recorded (1.58, 2.58,3.17, 4.58, 9.00 and20.83%, 37.50%, 29.10%, 79.17%, 87.50%) for disease severity and incidence respectively. These three varieties (1166 for *D. alata*, Nwa for *D. rotundata*, and Ekpe for *D. dumentorum*) were all observed to be more susceptible than other yam varieties and the disease incidence and severity increased with amount of rainfall. Infectedleaves were taken to the plant pathology laboratory for isolation and identification of *X. campestris* after pathogenicity test in the screen house. Both field and screen house trials showed the potential of water leaf extracts of *Azadirachtaindica, Eucalyptus officinalis, Cymbopogon citratus, Ocimum gratissimum* in controlling the bacterial leaf spot. Results obtained showed that all the plant extractsassessed reduced the disease incidence and severity when compared with the control (sterile water). *A. indica* recorded the best performance in growth and yield attributes (5.47kg) followed by *C. citratus* (4.34kg). Indicating that extracts of *Azadirachtaindica, Eucalyptus officinalis, Cymbopogon citratus, Ocimum gratissimum* could offer a safe alternative in management of leaf spot disease in Umudike caused by *X. campestris*.

Keywords: Pathogenicity test, Isolation, Yam, Leaf spot, *Xanthomonas campestris*

Introduction

Yam (*Dioscorea* spp) belongs to the family Dioscoreaceae with more than six hundred (600) species of yam (Kay, 1987). Some of the cultivated species are *Dioscorea rotundata* (White yam), *D. dumetorum* (Bitter yam), *D. cayensis* (Yellow yam), *D. alata*

(Water yam), *D. esculenta* (Chinese yam) and *D. bulbifera* (Aerial yam). Yam is one of the most highly regarded food products in tropical countries of West Africa which has been integrated into the social, economic, cultural and religious lives of communities where yam is grown (Okigbo and Ogbonnaya, 2006).Despite the great importance of this crop, effort of farmers to increase or sustain yields is greatly affected by a number of constraints including diseases and insect pests Bilgrami and Dube, 1976. Leaf spot is the most commonly encountered foliar disease of yam in the field. Leaf spot disease reduces the photosynthetic area of the plants and consequently, the amount of assimilate that goes into the sink (Hahn and Hozyo, 1984).

Nutritional Value of Yam

Yam is considered to be the most nutritious of the tropical root crops (Wanasundera and Ravindran 1994). It contains approximately four times as much protein as cassava, and is the only major root crop that exceeds rice in protein content in proportion to digestible energy (Bradbury and Holloway 1988). The amino acid composition of yam protein is suboptimal in sulphur-containing amino acids (cysteine and methionine), but the overall rating for essential amino acids is high and superior to sweetpotato (Bhandari *et al*., 2003). Yam is also a good source of vitamins A and C, and of fibre and minerals. Its relatively low calcium content is related to low concentrations of calcium oxalate, an anti-nutritional factor (Bradbury and Holloway 1988). It is also low in the anti-nutrients phytate (Wanasundera and Ravindran 1994) and trypsin inhibitor (Bradbury and Holloway 1988). A number of authors (Bradbury and Holloway 1988; Wanasundera and Ravindran 1994) have commented on the variability in protein content within yam species, indicating potential for selection for high protein content. However, some of this variability would be due to varying degrees of nitrogen deficiency in the tubers sampled (Bradbury and Holloway 1988). Improving nitrogen nutrition of yams will increase protein production. However, the relative contribution of nitrogen nutrition and genotype to the observed range of protein content has not been determined.

Diseases of Yam in Nigeria

Bacterial leaf spot diseases of yam can be caused by bacteria, together with other organism such as fungi, virus and foliar nematodes, *Aphelenchoides*. Nematodes are of particular concern because, apart from causing significant reduction in tuber yield and quality, they facilitate fungal and bacterial attacks(Douglas, 2012). Common bacteria associated with leaf spots are *Pseudomonas* and *Xanthomonas*. *Xanthomonas* and *Pseudomonas* genera bacteria overwinter in plant debris, but cannot survive for long in soil or water alone. Bacteria infect foliage, fruit, and stems, but require openings such as lesions to get inside the plant because they are relatively weak pathogens. Commonly, insects create injuries from feeding on plants, which the bacteria take advantage of. The pathogen (*Xanthomonas*) itself is seedborne, which can then spread to other nearby plants after the seedling begins to grow through splashing water and overhead irrigation. Spread of the disease is moderately fast if water splashing is highly prevalent. However, this pathogen (*Xanthomonas*) is highly dependent on cool and wet conditions, so if these conditions are not met, the pathogen's distribution will be highly deterred (Wagner, 2004).

Yam anthracnose disease

Yam dieback, or anthracnose, is caused by the fungus *Colletotrichum gloeosporioides.* It is probably present in all the countries of the region and is often a major problem where yam *(Dioscorea* spp.) is grown intensively. However, water yam *(D. alata)* is thought to be more susceptible to anthracnose than other yams. In some countries, yam dieback is thought to be caused by lightning. This is because after heavy rain, the disease on some varieties increases rapidly from occasional leaf spots to extensive blackening of leaves and stems, and plants may die. On susceptible yam cultivars, symptoms appeared at first as small dark brown or black lesion on the leaves, petioles and stems. The lesion is often surrounded by a chlorotic halo enlarged and coalesces, resulting in extensive necrosis of the leaves and die-back of the stem (Amusa, 1991; 1997).

Water yam virus disease (*Dioscorea alata* virus)

The disease is more commonly found on *D. alata*. Symptoms included chlorosis vein banding, flecking and leaf puckering. The

organism has not been characterized. (IITA,1993).

Nematode Diseases

Foliar symptoms of nematode infections on food yams are occasionally observed. Early yellowing, and leaf fall termination of vine growth have been seen on *D. rotundata* infected with *M. incognita,* but infections only rarely reduces total tuber yield of these yams (Nwauzer and Fawole, 1981).

Management of Diseases of Yam

Cultural, physical and chemical control methods

Cultural control measures such as the removal of weeds that may be alternative hosts, planting barrier crops of maize, avoiding damage to tubers at harvest, early staking, and ploughing-in plant residues immediately after harvest are likely to reduce disease development. Before planting, try to ensure that the seeds are disease-free. This is the best method to avoid initial infection and further spread of infection. Using sanitized tools will significantly reduce the chance of bacteria distribution from plant to plant while working with crops. Avoid the use of sprinkler irrigation as sprinklers increase the amount of splashing water amongst crops (Herren, 1994).

Chemical control is difficult and costs are high. Weekly benomyl treatments alternating with applications of copper, dithiocarbamates or daconil have been tested. Resistance to benomyl is reported. Fungicides can delay the onset of epidemics, but cannot prevent them developing during the rainy season. If foliar sprays are used they should be applied before symptoms of anthracnose appear in the crop, and weekly during the growing season. In addition on preventing spread of the disease, you can use a copper fungicide on crops. This has limited management use unless it is applied early in the disease cycle. When it comes to bacterial leaf spot, there aren't many chemical methods of control. Due to the lack of bactericides available, chemical controls aren't the best option. Note that fungicides cannot be used to control bacteria. If managed correctly, cultural controls can be very effective to eliminate or reduce spread of the disease (Wagner, 2004).

Use of plant extracts in plant disease management

There are several local plant species whose extracts or biocides have proved efficacious in protecting crops example yam before and after harvest. Ecologically oriented plant protection is critical to develop also to apply economically sustainable pest and disease management techniques to avoid the use of synthetic pesticides (Herren, 1994).Plant extracts are parts of plants which are in solution of water, alcohol and nutrient medium. Certain chemicals contained in plants are toxic to agricultural pest and diseases (Amadioha, 2004; Emeasor *et al.*, 2005).

The most popular among them is neem (*Azadirachta indica*). Opara and Wokocha (2008), observed that aqueous extracts of *Azadirachta indica* seed, *Piper guineensis, Citrus sinensis* and *Chromolaena odorata* were effective in inhibiting the growth of bacterial spot pathogen (*Xanthomonas campestris pv vesicatoria*) *in vitro* and *in vivo*. Mbadianya *et al* (2013) reported that ethanolic extracts of *Carica papaya* and *Azadirachta indica* leaves, *Zingiber officinale* stem and *Garcinia kola* seeds at 0.030g/ml, 0.120g/ml, 0.060g/ml and 0.250g/ml concentrations inhibit the growth of the fungus *Helminthosporium infestans*, causal organism of leaf spot disease in egg plant at different degrees when compared with the untreated control 14 days after inoculation.

Therefore the objectives of this research work include;

I. To conduct a survey to establish the incidence (occurrence) and severity of yam leaf spot disease in the field in Umudike.
II. To isolate and identify the causal organisms of yam leaf spot disease.
III. To evaluate the efficacy of the botanicals on leaf spot disease.

Materials and Methods

Yam varieties used; for *D. rotundata* (chili, Asaga, Okposi, Nwa, Aro), for *D. alata* (1423, 3577, 00060, 1166, Um680), for *D. dumentorum* (89/2665, Amula, 97/00840, Ekpe, Aluma).

Field Survey

Three yam (*Dioscorea* spp) farms at two different locations (National Root Crops and Research Institutes

and Michael Okpara University of Agriculture, Umudike were assessed for the bacterial leaf spot incidence and severity on leaves of test yam in the selected farms. In each farm location, sixty plants were randomly tagged for data collection which was done at one week interval. Percentage disease incidence was calculated using the formula;

$$\textbf{Disease Incidence (\%)} = \frac{\text{Number of plants infected in the sampled area}}{\text{Total number of plants assessed in the sampled area}} \times \frac{100}{1}$$

Assessment of disease severity on leaves was by using the rating on a scale of 1-10 (Opara *et al*, 2013).

Where;

1 = No leaf spot/lesion
2 = 1 or 2 lesions on leaf surface
3 = ¼ or 25% of leaves covered with lesions/spot
4 = ½ or 50% of leaves covered with lesions/spot
5 = above 50% of leaves covered with lesions/spot
6 = lesion covered 75% or ¾ and lesions coalesce to form blight (large dead area).
7 = above 75% or ¾ covered and leaf margin twist or curve inwards
8 = above 75 or ¾ but leaves turns yellow or chlorotic blight
9 = leaves surface turned from yellow to dark brown blight (almost dead)
10 = leaves surface completely dark/ black, dead and turn apart and dry or fall off.

Disease severity was calculated using the formula.

$$\textbf{Disease severity} = \frac{\text{Sum of individual ratings}}{\text{Total number of plants examined}} \times \frac{100}{1}$$

Collection of Diseased Specimens

Leaves of yam (*D. alata, D. rotundata, D. dumentorum*) showing symptoms of leaf spot disease were randomly collected, and packaged separately in well labeled envelopes and was taken to the plant pathology laboratory of Plant Health Management, Michael Okpara University of Agriculture

Umudike for isolation of the causal organisms.

Preparation of culture medium

The culture medium was prepared by adding 7g of nutrient agar (NA) in 250ml of distilled water in a conical flask. This was shaken properly to obtain an even mixture. The mixture was autoclaved for 30 minutes at 121^{o}C, allowed to cool to 45^{o}C and 15ml were dispensed into sterilize Petridishes. The agar plates was allowed to solidify and turned upside down for the evaporated moisture to dry.

Isolation and Identification of the pathogen

Leaves showing symptom of leaf spot disease collected from the field were washed under a running tap water, surface-sterilized in 0.5% sodium hypochlorite solution by dipping plant material for 2-5 minutes. The plant materials were rinsed a few times in sterile distilled water to remove trace of disinfectants. Small sections of about 1-2mm was taken from the advancing edge of the lesion with a sterile scalpel and macerated in a drop of water with a glass rod to form a suspension. This was allowed to stand for 15minutes for the bacterial cells to multiply. The suspensions were streaked unto the solidified agar plates with the aid of a flamed red and cooled wire loop and plates were incubated at 30-32^{o}C for 24 hours. Single colonies from the culture were subcultured to obtain pure culture of the respective colonies.

Pathogenicity Test

Seedlings of the three yam varieties (*D. alata*, *D. rotundata*, *D. dumentorum*) was raised in 23cm diameter perforated polyethene bags filled with sterilized soil up to 10cm from the brim. Isolates identified from the samples were singly used to spray-inoculate the leaves of the yam seedlings after sprouting at 8 weeks after planting. The bacterial inoculum was hand sprayed on the leaves until run-off, the seedlings were covered with a transparent polyethene bags to maintain high humidity (70-80%). The inoculated seedlings were uncovered after 48 hours and observed daily for symptoms of leaf spots disease. Re-isolation was made from the diseased leaves.

Preparation of Bacterial Inoculum

Bacterial inoculum was prepared by washing bacterial colonies on agar plates with sterile distilled water into McCartney bottles and adjusting the density of the

inoculum to 10^8cfu/ml using haemocytometer.

Preparation of Aqueous Extracts

The plants materials used were leaves of *Ocimum gratissimum*, *Eucalyptus officinalis*, *Cymbopogon citratus*, and *Azadirachta indica* which were sourced locally within Michael Okpara University of Agriculture, Umudike, and National Root Crops Research Institute (NRCRI), Umudike. Fresh leaves of these plant materials were thoroughly washed in a running tap water and rinsed with sterile distilled water. The samples were air dried in the laboratory at room temperature. Each plant material was weighed (100g) and ground into paste using sterilized manual grinder. Cold water extraction was prepared by mixing the paste in 500ml of sterile distilled water in a 1000ml beaker and stirred using a sterile glass rod, and then allowed for 24hrs for the extraction of the active ingredients before being filtered using four-fold muslin cloth into a conical flask as described by Wokocha and Okereke (2005).

Field experiment

Experimental Site

The experiment was carried out at the National Root Crop Research Institutes Umudike farm. Umudike is located at Latitude 5° 28' North and Longitude 7° 35' East and an Altitude of 122m above sea level with an average annual rainfall of 1916mm, and relative humidity of 76% and temperature range of 19-35°C (NRCRI, 2010).

Field Preparation and Lay Out

Land was manually cleared and prepared into ridges. Sprouted yam seedlings were sown one per hole at a spacing of 1m × 1m. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replicates for each variety. Each block measured 17m × 5m, containing five (5) plots of 5m × 3m each while space between blocks and plots were 1m and 0.5m respectively. The total area for the experiment was 19m×17m ($323m^2$). Regular weeding was done when necessary using hoe. Inorganic Fertilizer (NPK 15:15:15) was applied two months after planting at the rate of 400kg/ha.

Application of the Plant Extracts in the field

Hand sprayer (model: 418) was used to apply the plant extracts. Each of leaf extracts were separately applied at two weeks interval and three times at natural

manifestation of the disease symptom.

Screen House Experiment

Inoculation of Yam Seedlings

The experiment was laid out in a completely randomized design (CRD) consisting of five treatments and three replications. The plant materials used as water extracts include *O. gratissimum, E. officinalis*, *C. citratus*, and *A. indica* while sterile distilled water served as control**.** Yam seedlings were planted in a sterilized soil of about 9mm diameter by placing the sprouted seedling into perforated polyethene bags placed on the floor in the screen house at the rate of one seedling per polyethene bags.15 polyethene bag were laid out for each variety of *D. alata, D. rotundata, and D. dumentorum*. The bacterial inoculum (10^8cfu/ml) was used to spray on the leaves using hand sprayeruntil run-off on each of the yam seedlings of the three varieties. A small bamboo stake was used to support the sprouting sets from each polyethene bags. Polyethene bags were watered at two days interval.

Application of the Plant Extracts

Hand-held sprayer was used to apply the plant extracts. The water leaf extracts of each of the plant materials were separately applied at two weeks interval after inoculation in the screen house.

Data Collection

Assessment of Disease Incidence, Disease Severity and Growth Parameters

Both screen house and field experiments were examined for disease incidence, disease severity and growth parameters at one week interval for six weeks. Assessments were done per pot in the screen house trial while in the field experiment, four plants were sampled and tagged at random for assessment per plot. The total number of plants infected both in screen house trial and field experiments were counted and the percentage disease incidence and disease severity were determine using the formula and scale started in the survey above.

Assessment of growth parameters

Total number of leaves: These were obtained by counting the total number of leaves on plants tagged for assessment.

Vine length (cm): This was done using a measuring tape

Vine girth (cm): This was done by using measuring tape

Data Analysis

All the data collected were statistically analyzed using SAS model (2009) in the Analysis of Variance (ANOVA) procedure and significant means were separated using Fishers Least Significant Difference (LSD) at 5% level of probability (Steel and Torrie, 1997).

Results

Leaf spot Incidence and severity on (*D. rotundata*) at Umudike in 2016 cropping season.

The result of the survey of leaf spot disease in Umudike on *D. rotundata* is represented in Figure (1, and 2). Disease severity on the varieties shows no difference in terms of disease infestation for the five varieties (chili, Asaga, Okposi, Nwa, Aro) at week one. All the varieties were infected with leaf spot disease in an increasing trend across the weeks for both disease severity and incidence.

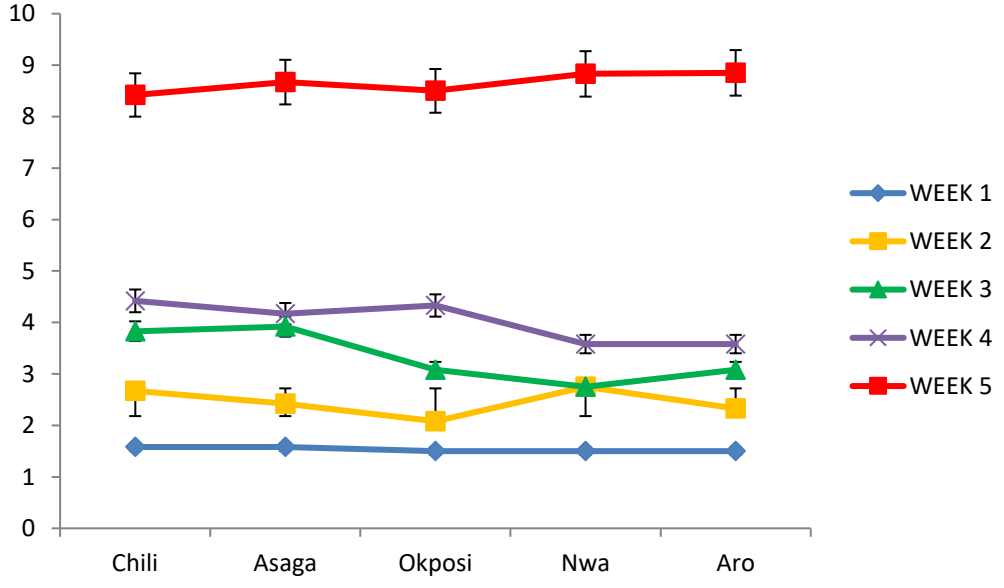

Fig. 1: Leaf spot severity on (*D. rotundata*) at Umudike in 2016 cropping season.

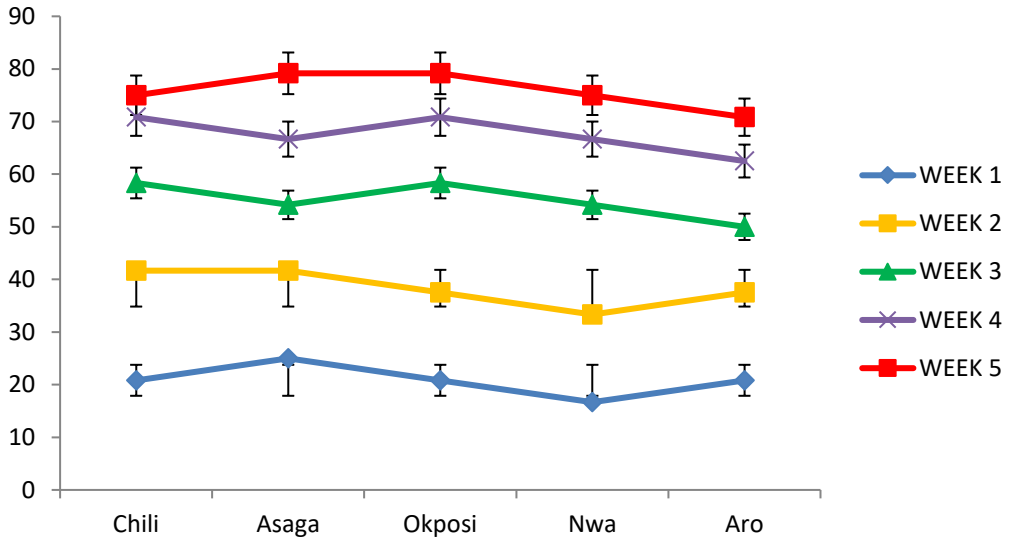

Fig.2: Effect of Disease Incidence on Yam (*D. rotundata*)Varieties in Umudike in 2016

Leaf spot Incidence and severity on (*D. alata*) at Umudike in 2016 cropping season.

The result of the survey of leaf spot disease in Umudike on *D. alata* is presented in Figure(3, and 4). For disease severity all the varieties (1423, 3577,00060,1166 and Um680) recorded almost the same mean value across the weeks. Um680 variety recorded highest mean value for disease incidence at week 5. Whereas other varieties almost recorded the same mean value in terms of disease incidence.

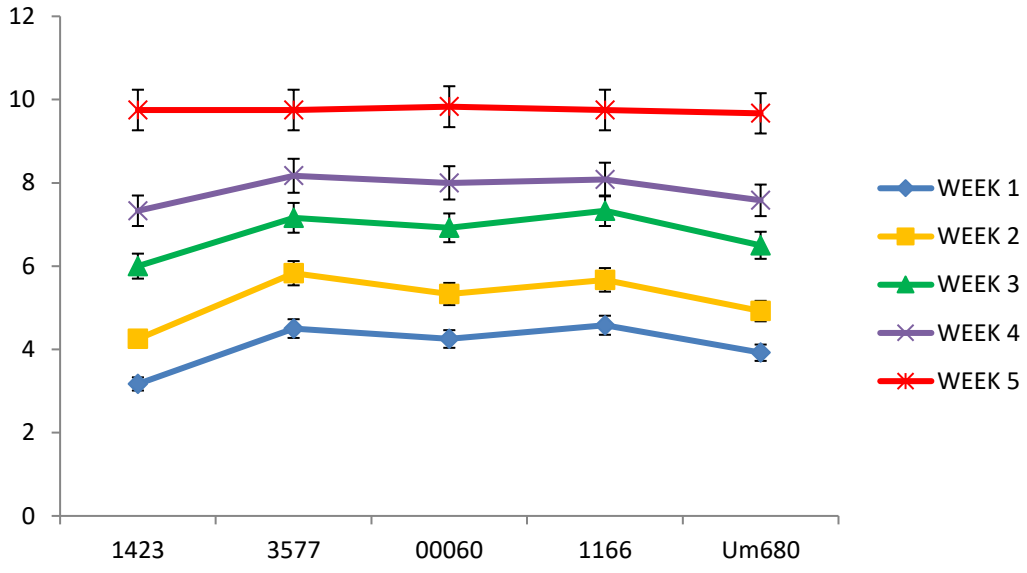

Fig.3: Effect of Disease severity on Yam (*D. alata*)Varieties in Umudike in 2016

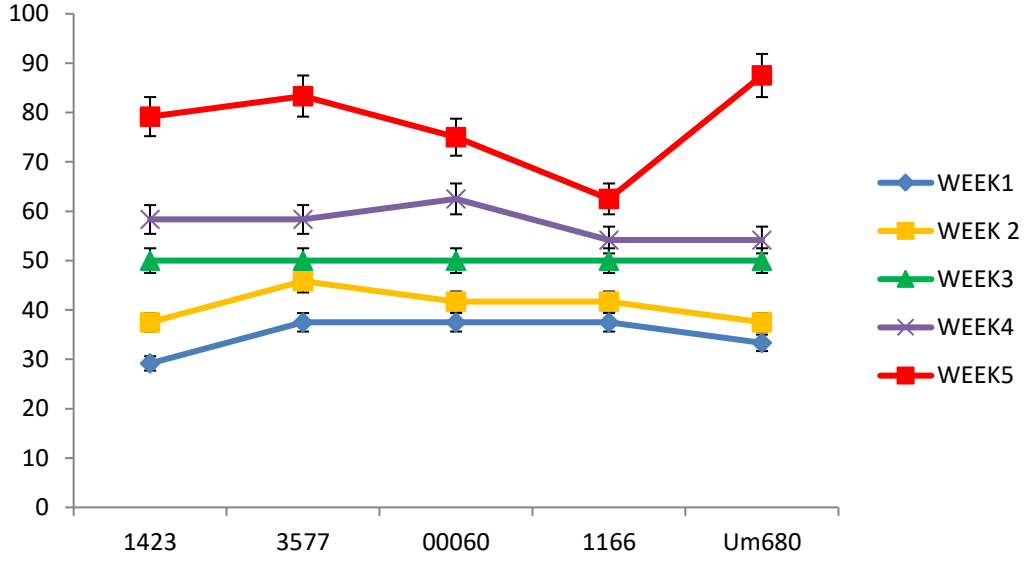

Fig.4: Effect of Disease Incidence on Yam (*D. alata*)Varieties in Umudike in 2016

Leaf spot Incidence and severity on (*D. dumentorum*) at Umudike in 2016 cropping season

The result of the survey of leaf spot disease in Umudike on *D. dumentorum* is presented in figure (5, and 6).The disease trend for severity shows almost the same mean value across all the varieties (89/2665, Amula, 97/00840, Ekpe, Aluma). For disease incidence, 89/2665 variety recorded the highest mean value for week 5, Ekpe recorded highest mean value for disease incidence in week 4. Other varieties recorded almost the same mean value across the weeks.

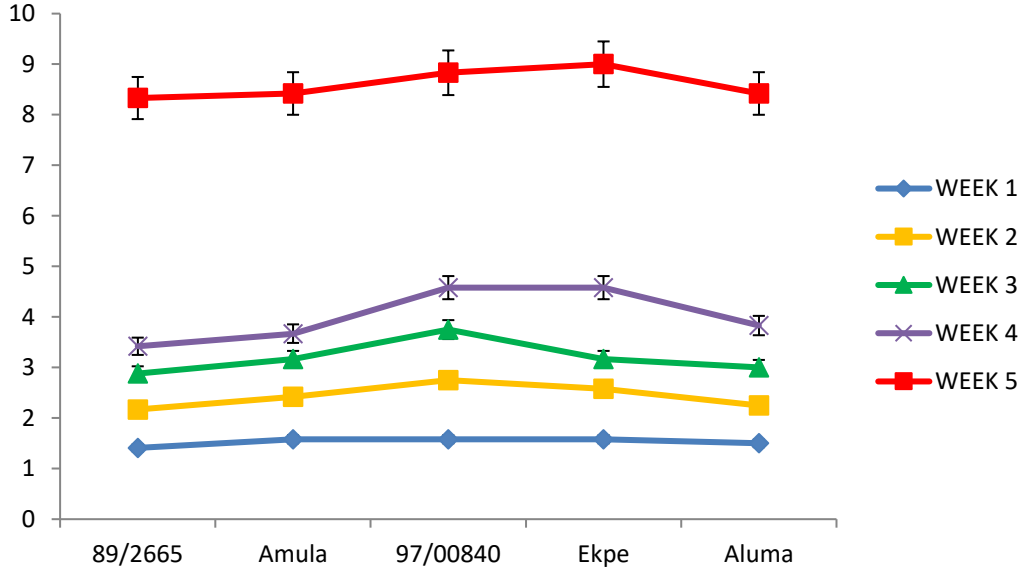

Fig.5: Effect of Disease severity on Yam (*D. dumentorum*)Varieties in Umudike in 2016

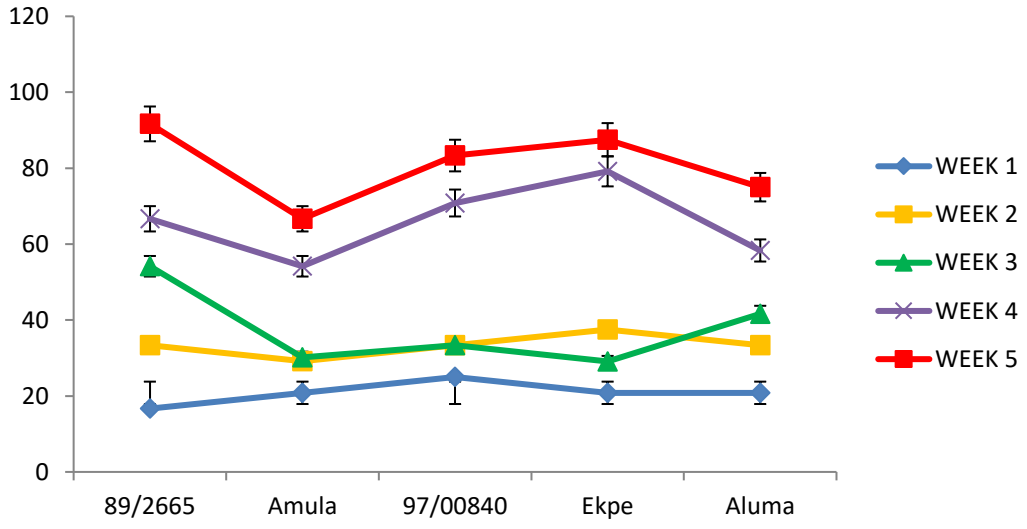

Fig.6: Effect of Disease Incidence on Yam (*D. dumentorum*)Varieties in Umudike in 2016

Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 8 weeks after planting in the Field

The effect of plant extracts on growth parameters, disease severity and incidence is presented in Table 1. *A. indica* (94.44cm) prove to be the best in terms of vine length but not significantly different from *C. citratus* (93.46cm), *O. gratissimum* (92.62cm), *E. officinalis* (91.51cm), but significantly different from the control (Sterile water) (81.29cm). For vine girth, *O. gratissimum* (1.74cm) gave the best treatment followed by *A. indica* (1.71cm) although they did not different significantly from each other. The control recorded least (1.12cm) significantly not different from *E. officinalis* (1.35cm). in terms of number of leaves, *A. indica* recorded the highest mean value of 15.76 followed by *O. gratissimum* (15.68) and *C. citratus* (15.42) although they are not significantly different from each other at ($P \leq 0.05$).

For leaf disease severity and incidence, *A. indica* (18.32, 15.28 %) and *C. citratus* (18.32, 15.28%) prove to be the best in reducing disease severity and incidence respectively followed by *O. gratissimum* (20.14, 22.22%) but they are not significantly different from each other at 5% probability level. Control recorded the highest disease severity and incidence (29.37, 27.78%) respectively.

Table 1: Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 8 weeks

Extracts	V/L(cm)	V/G(cm)	N/Lf	D/Sev(%)	D/Inc(%)
A. indica (leaf)	94.44	1.71	15.76	18.32	15.28
C. citratus	93.46	1.48	15.42	18.32	15.28
O. gratissimum	92.62	1.74	15.68	20.14	22.22
E. officinalis	91.51	1.35	13.73	21.40	26.39
Control	81.29	1.12	12.07	29.37	27.78
$LSD_{0.05}$	4.81	0.32	2.02	1.17	8.60

Legend: NS = Not significant ($P \leq 0.05$)

V/L = Vine length (cm), V/G = vine Girth (cm), N/Lf = Number of leaves, D/sev = Disease severity, D/Inc = Disease Incidence

Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 10 weeks after Planting in the Field

The effect of plant extracts on growth parameters, disease severity and incidence is presented in Table 2. In terms of vine length, *A. indica* recorded the best mean value of 113.33cm although it is not statistically significantly different from *C. citratus* (111.44cm), *E. officinalis* (110.48cm) and *O. gratissimum* (109.61cm) but different from the control (99.33cm). *A. indica* still records the best 2.11cm for vine girth followed by *O. gratissimum* (2.06cm) and *C. citratus* (2.03cm). The least mean value was recorded in the control (1.82cm) and *E. officinalis* (1.87cm). For number of leaves, the highest mean value was recorded with *A. indica* (27.50) treatment which significantly was not different from *C. citratus* and *O. gratissimum* with mean 27.25 and 27.03 respectively. The least mean value was recorded in control (24.08) and *E. officinalis* (25.72) treatments.

In terms of leaf disease severity and incidence *A. indica* proved to be the best in reducing disease severity and incidence (15.68, 25.00%) respectively followed by *C. citratus* (18.28%) (26.39%) and *E. officinalis* (19.90%) (29.19%). The highest disease severity and incidence was recorded in control (23.33%) (36.11%) respectively although it did not differ significantly from *O. gratissimum* (20.81%) (30.50%) respectively.

Table 2: Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 10 weeks

Extracts	V/L(cm)	V/G(cm)	N/Lf	D/Sev(%)	D/Inc(%)
A. indica (leaf)	113.33	2.11	27.50	15.68	25.00
C. citratus	111.44	2.03	27.25	18.28	26.39
O. gratissimum	109.61	2.06	27.03	20.81	30.50
E. officinalis	110.48	1.87	25.72	19.90	29.19
Control	99.33	1.82	24.08	23.33	36.11
$LSD_{0.05}$	4.92	2.02	1.70	2.51	5.93

Legend: NS = Not significant ($P \leq 0.05$)

V/L = Vine length (cm), V/G = vine Girth (cm), N/Lf = Number of leaves, D/sev = Disease severity, D/Inc = Disease Incidence

Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 12 weeks after Planting in the Field

The effect of plant extracts on growth parameters, disease severity and incidence is presented in Table 3. For vine length *A. indica* (124.33cm) recorded the highest mean value but it was not different from *C. citratus* (122.69cm), *E. officinalis* (121.31cm), and *O. gratissim*um (121.00cm). Control has the least mean value (110.03cm). *A. indica* (2.18) proves to be the best in terms of vine girth followed by *O. gratissimum* (2.14cm) and they are not significantly different from each other. Controlrecorded the least (2.04) and *E. officinalis* (2.06cm). The best mean value for number of leaves was recorded in *C. citratus* (38.89) followed by *A. indica* (38.81) and *O. gratissimum* (38.28). Control recorded the least (34.67) and it was significantly different from all other treatment. In terms of disease severity and incidence, *A. indica* (12.76, 27.78%) performed best in controlling disease closely followed by *C. citratus* (18.48, 29.17%). The highest disease severity and incidence was recorded in the control (29.76, 38.89%).

Table 3: Effect of Plant Extracts on Growth Parameter, Disease severity and incidence at 12 weeks after planting

Extracts	V/L(cm)	V/G(cm)	N/Lf	D/Sev(%)	D/Inc(%)
A. indica (leaf)	124.33	2.18	38.81	12.76	27.78
C. citratus	122.69	2.11	38.89	18.48	29.17
O. gratissimum	121.00	2.14	38.28	20.88	33.33
E. officinalis	121.31	2.06	36.78	19.12	31.94
Control	110.03	2.04	34.67	29.76	38.89
$LSD_{0.05}$	4.91	0.05	1.94	2.70	5.50

Legend: V/L = Vine length (cm), V/G = vine Girth (cm), N/Lf = Number of leaves, D/sev = Disease severity, D/Inc = Disease Incidence

Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 8 Weeks after planting in the Screen House

The effect of plant extracts on growth parameters, disease severity and incidence is presented in Table 4. On vine length *A. indica* leaf performed best (117.44cm) but not significantly different from *C. citratu*s (114.56cm) and *E. officinalis* (112.00cm). Control recorded the least mean value for vine length (104.89cm) but significantly different from *O. gratissimum* (107.33cm) and *E. officinalis* (112.00cm). *A. indica* (2.00cm) proves best in terms of vine girth but not statistically different from *C. citrates* (1.99cm) and *O. gratissimum* (1.97cm).*E. officinalis* (1.87cm)significantly shows least mean value but not significantly difference from control [sterile water] (1.89cm) and *O. gratissimum* (1.97cm).

For number of leaf *A. indica* (29.22) still shows superior to other treatments though not significantly difference from *E. officinalis* (27.22). The least number of leaves was recorded in the control [sterile water] (24.22) although it was significantly not different from *C. citrates* (26.33) and *O. gratissimum* (26.56)

The control [sterile water] was least effective in reducing the disease severity and incidence 30.10 and 14.80% respectively, although statistically not significantly different from *O. gratissimum* 16.53% [severity] and

7.40% [incidence]. *C. citratus* (16.05%), *A. indica* (16.05%) and *E. officinalis* (1.00) proved to be the best in terms of reducing disease severity while *A. indica* (0.00%) and *E. officinalis* (0.00%) proved best in reduction of disease incidence.

Table 4: Effect of Plant Extracts on Growth Parameter, Disease severity and incidence at 8 weeks after planting

Extracts	V/L(cm)	V/G(cm)	N/Lf	D/Sev(%)	D/Inc(%)
A. indica (leaf)	117.44	2.00	29.22	16.05	0.00
C. citratus	114.56	1.99	26.33	16.05	3.70
O. gratissimum	107.33	1.97	26.56	16.53	7.40
E. officinalis	112.00	1.88	27.22	16.05	0.00
Control	104.89	1.89	24.22	30.10	14.80
$LSD_{0.05}$	8.54	0.10	2.43	2.33	11.08

Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 10 weeks after planting in the Screen House

The effect of plant extracts on growth parameters, disease severity and incidence is presented in Table 5. On vine length *A. indica* recorded the highest length (128.78cm) but it was not significantly different from *C. citratus* (125.67cm) and *E. officinalis* (123.33cm). The least vine length was recorded in the control (115.56cm) although not significantly different from *O. gratissimum* (119.78cm) and *E. officinalis* (123.33cm). In terms of vine girth *C. citratus* (2.10cm) recorded the highest followed by *A. indica* (2.08cm) and *O. gratissimum* (2.07cm) although they are not significantly different from each other. The least vine girth was recorded in the control (2.00cm) and *E. officinalis* (2.04cm). *A. indica* (40.44) recorded the highest number of leaves which was significantly different from *C. citratus* (37.00), *O. gratissimum* (36.78) and control (34.56) except *E. officinalis*

(38.11). The highest level of disease severity and incidence was recorded in the control (28.13%) and (25.90) respectively followed by *C. citratus* (19.87%, 14.80%) and *O. gratissimum* (19.87%, 14.80%) respectively which there are not statistically different from each other. The least leaf disease severity and incidence was recorded with *A. indica* treatment at 18.08 and 3.70% respectively.

Table 5: Effect of Plant Extracts on Growth parameters, Disease severity and incidence at 10 weeks after planting

Extracts	V/L(cm)	V/G(cm)	N/Lf	D/Sev(%)	D/Inc(%)
A. indica (leaf)	128.78	2.08	40.44	18.08	3.70
C. citratus	125.67	2.10	37.00	19.87	14.80
O. gratissimum	119.78	2.07	36.78	19.87	14.80
E. officinalis	123.33	2.04	38.11	19.06	3.70
Control	115.56	2.00	34.56	28.13	25.90
$LSD_{0.05}$	8.53	0.05	2.55	3.18	13.83

Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 12 weeks after Planting in the Screen House

The effect of plant extracts on growth parameters, disease severity and incidence is presented in Table 6. For vine length, *A. indica* (140.56cm) recorded the highest followed by *C. citratus* (136.22cm) and *E. officinalis* (134.33cm) which there are not significantly different from each other. There was no significant difference in vine girth of the various plant extracts. Significantly higher number of leaves was observed in *A. indica* (51.87). Control (45.22) recorded the least number of leaves which was not significantly different from *C. citratus* (47.33) and *O. gratissimum* (47.33). Control (30.77) and (48.10%) recorded significantly higher.

Table 6: Effect of Plant Extracts on Growth Parameters, Disease severity and incidence at 12 weeks after planting

Extracts	V/L(cm)	V/G(cm)	N/Lf	D/Sev(%)	D/Inc(%)
A. indica (leaf)	140.56	2.18	51.78	20.85	22.20
C. citratus	136.22	2.18	47.33	23.25	25.90
O. gratissimum	130.67	2.14	47.33	25.13	29.60
E. officinalis	134.33	2.13	48.67	24.10	25.90
Control	125.67	2.09	45.22	30.77	48.10
$LSD_{0.05}$	8.73	0.07	2.75	3.00	2.02

Legend:
V/L = Vine length (cm), V/G = vine Girth (cm), N/Lf = Number of leaves, D/sev = Disease severity, D/Inc = Disease Incidence

Effect of Plant Extracts on yield Parameters of yam varieties

The effect of plant extracts on growth parameters, disease severity and incidence is presented in Table 7. In terms of varieties, *D. rotundata* (29.89cm) proved to be the best in Tuber length followed by *D. alata* (28.98cm), but they were not significantly different from each other. *D. dumentorum* (24.37cm) recorded the least tuber length statistically different from the other two varieties .*A. indica* (30.72) recorded the highest tuber length followed by *C. citratus* (29.39cm). Control (24.23cm) recorded the least tuber length which was significantly different from other plant extracts except *E. officinalis* (26.14cm). The tuber diameter of*A. Indica* was significantly higher with mean value 87.09mm which was significantly different from *C. citratus* (85.13mm). Control recorded the least (61.34mm) which was not significantly different from *E. officinalis* (70.88mm) and *O. gratissimum* (71.85mm). The tuber weight of *A. indica* recorded the highest, with mean value of 5.47kg. *C. citratus* (4.34kg) recorded the second best in terms of tuber weight and was significantly not different from *O. gratissimum*, *E. officinalis* and control, which had mean values of 4.04kg, 3.72kg and 3.16kg respectively.

Table 7: Effect of Plant Extracts on yield Parameters of yam varieties at harvest

		TLh(cm)	TDIA(mm)	Tweight(kg)
Varieties	*D. rotundata*	29.89	76.87	4.27
	D. alata	28.98	75.91	4.04
	D.dumentorum	24.37	72.99	4.13
	$LSD_{0.05}$	2.20	NS	NS
Extract*s*	*A. indica*	30.72	87.09	5.47
	C. citratus	29.39	85.13	4.34
	O. gratissimum	27.41	71.85	4.04
	E. officinalis	26.14	70.88	3.72
	Control	24.23	61.34	3.16
	$LSD_{0.05}$	2.83	13.62	1.24

Legend: NS = Not significant ($P \leq 0.05$)
TLh= Tuber length, TDIA= Tuber diameter, Tweight= Tuber weight

Discussion

The results of the survey show that bacterial leaf spot on yam leaves can develop from early stage of about 8 weeks. As the weeks after planting increased, there was a corresponding increase in disease incidence and severity. The rapid increase in disease incidence and gradual progress in severity was in agreement with Agrios (2005) who reported that in the early stage of disease, disease incidence in plant increase rapidly but disease severity on individual plants will be low but subsequently increase with time. The steady increase in incidence and severity of the disease during the growth period could be attributed to increase in inoculum load and virulent of pathogen on its host as the duration of infection increases. This was in line with the report of Wasihum and Flagote (2016). According to Wagner (2004) the pathogen (*Xanthomonas*) itself is seedborne, which can then spread to other nearby plants after the seedling begins to grow through splashing water and overhead irrigation. Spread of the disease is moderately fast if water splashing is highly prevalent. However, this pathogen (*Xanthomonas*) is highly dependent on cool and wet conditions, so if these conditions are not met, the pathogen's distribution will be highly deterred (Wagner, 2004).

The occurrence of the diseases could be attributed to the prevailing climatic conditions in the study area.

Application of several phytochemicals in Agriculture has been found effective in inhibiting the growth of *Xanthomonas* (Bajpai *et al*., 2010a).Certain essential oils obtained from plants stand out as better antibacterial agents than the commonly used synthetic chemical antibacterial agents against plant pathogenic bacteria like *Xanthomonas* species (Bajpai *et al*., 2010a, b; Gyorgyi *et al*., 2004; Nguefack *et al*., 2005).Results from this study show that disease severity and incidence of leaf spot was significantly reduced by the application of different plant materials. Percentage disease incidence and severity was very much lowered with the application of *A. indica* leaf extract while *C. citrates* extract substantially reduced incidence and severity of disease. This confirms with the earlier reports that many plant products contains anti-bacterial and fungi toxic constituents that have the potential to control plant diseases (Emechebe and Alabi (1997), Amadioha, (2000), Enikuomehin and Peter, (2002), Balm, (2003), Bdliya and Dahiru, (2006), Opara and Wokocha, (2008), Okigbo, (2009), Opara and Agugo, (2014).

The consistent best performance of *A. indica* leaf extract agrees with those of Bdliya and Dahiru (2006) who showed that aqueous extracts of neem leaf and seed extract significantly reduced the incidence and severity of tuber soft rot. Opara and Wokocha, (2008) reported that aqueous seed extracts of *A. indica* was most effective and comparable to streptomycin in inhibiting bacterial leaf spot pathogen (*Xanthomonas campestris* Pv*vesicatoria*) on tomato both *in vitro* and *in vivo.*

Amadioha and Obi (1998) reported the Fungi toxic activity of cold water extracts of *A. indica* and *Xylopia aethiopica* on *Collectotrichum lindemuthiamum* in cowpea. *A. indica* may have acted by the production of more antibiotic substances that inhibited the growth of *A. niger* and *F. oxysporium*; this has been reported by Okigbo and Emeka (2010).In related work, Amadioha (2012) observed that *O. gratissimum* ,*Asystacia gangetica* and *piper nigrum* inhibit the radial growth of *Aspergillus niger* and *Botryodiploida theobromae* , causal organisms of storage dry rot disease of yam.

Conclusion

This study was carried out to assess the incidence and severity of bacterial leaf spot of yam in Umudike, South Eastern Nigeria, isolate the causal organism of the disease and ascertain the efficacy of plant materials in managing the disease. The study showed that bacterial leaf spot can occur from the early stage of growth at 8 weeks after planting and persist till harvest. From previous studies, bacterial leaf spot pathogen has already been attributed to *Xanthomonas campestris* as the causal organism in Umudike.

Report of these findings has therefore provided a basis for further studies on bacterial leaf spot disease of yam. The studies further showed that leaf extracts of *A. indica* and *C. citratus* has the potential in the reduction of leaf spot of yam. Due to hazardous effect, and high cost of chemicals, botanical extract of these plant materials could be used as an alternative way of reducing and managing these diseases by small holder farmers.

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