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Studies on the Control of Bacterial Spot Disease of *Telfairia occidentalis* (HOOK f.) Using some Organic Products in Umudike, Humid South Eastern Nigeria.

***Okoronkwo, U. A[1] and Opara, E.U[2]**
Department Of Plant Health Management, ; Michael Okpara University of Agriculture, Umudike.
***Corresponding Author: uduakrobert59@gmail.com**

Abstract:

A research was conducted in the Research and Training Farm of Michael Okpara University of Agriculture Umudike, Abia State, Nigeria during 2024 cropping season to investigate the impact of some organic products on the control of bacterial spot disease of fluted pumpkin. The experiment was arranged in a randomized complete block design (RCBD) with four replicates. The organic products from plantain stem, Roselle, SuperGro and a control (water) were applied at 20ml/plant two weeks after germination and data was collected for 20weeks of planting. The parameters assessed were disease incidence, disease severity, growth and yield parameters. Samples of diseased leaves were taken from field to the laboratory for pathogenicity test, isolation, identification and characterization of the pathogen. Fisher's Least Significant Difference (FLSD) was used to separate the means at 5% probability level. Results obtained showed that SuperGro + Super10 scored 16.31% for disease incidence and 1.44 for disease severity, plantain fluid scored 21.47% for disease incidence and 1.53 for severity which were significantly (P≤0.05) better than the control (with 50.74% disease incidence and 4.21 severity). Generally all the organic amendments were observed to enhance growth in all the parameters assessed (vine length, number of leaves and number of branches) when compared with the non-treated plants (control). For example, plant treated with plantain fluid gave best vine length (154.83cm) followed by SuperGro and super10 (135.33cm) and were statistically higher than the control (109.92cm). Results obtained from pathogenicity test showed that the organism could induce bacterial spot on the seedlings and the organism from infected leaves was identified as Xanthomonas spp. This study demonstrated the efficacy and potential of some organic products in reduction of bacterial leaf spot of fluted pumpkin and should be utilized by farmers. Hence, this study recommends use of plantain fluid, roselle extract and SuperGrow+ Super10 in growing fluted pumpkin for disease free leaves and long vines. It also recommends especially the use of roselle extract and supergrow+ super10 to enhance increased number of branches and leafy yield of fluted pumpkin at harvest since they are readily available and cost effective.

Keywords: Organic products, *Hibiscus sabdariffa* calyx, Bacterial spot, Disease incidence, Disease severity.

Introduction: Fluted pumpkin with the scientific name *Telfairia occidentalis* (Hook f.) belongs to the family Cucurbitaceae. It is one of the basic vegetable crops of major economic importance in the South South and South Eastern Nigeria. Fluted pumpkin is drought tolerant and perennial (Opara, E. U. and Robert, U. A., *2019*). It occurs mostly in cultivated form in various parts of Southern Nigeria for its edible and nutritious leaves which are mainly used as vegetables in Nigerian foods (Burkil, 2004; Opara *et al.*, 2019). The seeds of *T. occidentalis* (fluted pumpkin) have high content of fat and protein and thus contribute to a well balanced diet (Okon and Udoffot, 2012). The nutritional values of *T. occidentalis* are enormous. The leaf composition per 100 g edible portion contains water 86.4 g, energy 147kJ (47 kcal), protein 2.9g, fat 1.8g, carbohydrate 7.0g and fibre 1.7g (Gruben and Benton, 2004). According to theses authors, seed size of this crop is said to affects vigour and germination of seedlings. Large seeds are reported to show good growth potential compared to the smaller seeds. Before the fruits are displayed for sales, they are graded according to size. While in the market or store, they are placed in heaps and sold as heaps or singly. Seeds are left in the fruits until they are used for planting or consumption. Fluted pumpkin is prone to attacked by number of pathogens. Among these, *Xanthomonas campestris pv. cucurbitae* causing bacterial spot disease is an important pathogen emerging in recent times, which reduces the leaf quality, market value, and yields of the crop and hence constitute constraints to the cultivation of *T. occidentalis.* Conventional control methods, including host resistance, crop rotation, and chemical methods, may be limited or may elicit negative effects on food safety and environment (Messiha, N. A. S., Van, A. D., Diepeningen, and M. Wenneker, 2007). Some biological methods previously used include the use agricultural effluents, use of agricultural waste ashes as well as some organic products sourced from the local environments.

Many plants, herbs and spices contain naturally occurring compounds and have been shown to possess antimicrobial activities to many pathogens (Olaleye, 2007). The antimicrobial activity of Roselle (*Hibiscus sabdariffa* L) reported by Nizar, S., Elhadi, M.A., Algali, M.A and Hozeife, M. H. (2013) showed that roselle calyx extract had antimicrobial effect similar to that produced by the controlled drug (Cefuroxime). Osai *et al.*, (2013) reported on the anti-microbial effect of plantain inflorescence and demonstrated that it has the potential to control fungal leaf spot caused by *Phoma sorghina*. Their result showed that the different levels of the organic product from plantain used significantly suppressed the sporulation of the fungus and reduced leaf spot in the fluted pumpkin. **The use of organic amendments such as poultry manure, fly ash, and sewage sludge results in lowering the soil metal toxicity has also been shown to leads to the slow release of nitrogen, phosphorus, and potassium to promote plant growth {Aling, V., Hale, S. E., Martingsen, V. Mulder, J.,and Cornelissen (2014) and Zang, L., Xiang, Y., Jing, Y., and Zang, R. (2019)}. Plants are rich in a wide variety of secondary metabolites such as flavonoids, tannins, alkaloids which have been found invitro to have antimicrobial properties (Nizar *et al.*, 2013). The use of organic products of plant source as antimicrobial agent may provide the best alternative to the wide use of synthetic chemicals to grow crops. In this study the antibacterial activities of some organic products (**Roselle extract, Plantain fluid and Super Grow+super 10) was investigated, along with the effects on agronomic properties of fluted pumpkin.

Materials And Methods: Study Area: The experiment was carried out at the Michael Okpara University of Agriculture Umudike in Abia State. Umudike is located on latitude 5·28ºN and longitude 7.33ºE, with an elevation of 122m above sea level.

Field Preparation and Layout: The land was manually cleared using cutlass and prepared into ridges using spade. *Telfairia occidentalis* seeds were sown two per hole and afterword reduced to one per hole. The experiment was arranged in a randomized complete block design (RCBD) with four replicates. Each replicate measured 28m x 9m while each plot measured 3m x 3m, space between replicates and a plot was 1m and 0.5m respectively. Total area for the experiment was 28m x 15m (420m^2). Weeding was done manually to maintain proper sanitation.

Soil Sampling and Analysis: Samples of soil were collected at random with an auger from the site at a depth of (0-20 cm) before planting, bulked into composite sample and taken to the Soil Science laboratory for analysis to determine the physico-chemical properties of the experimental site. At the laboratory, composite sample was air-dried in a room temperature of 27ºC for three days, crushed and sieved using 2mm aperture. The parameters evaluated include the particle size distribution by hydrometer method (Gee and Bauder, 1986). Soil pH was determined using PyeUnican model MK2 pH meter in a 1:2:5 soil/water suspension ratio. Organic carbon was determined by Walkley-Black wet oxidation method (Nelson and Sommers, 1982). Total nitrogen was determined by micro-Kjeldahl distillation technique as described by Bremmer and Mulvaney (1982). Available phosphorus was determined by Bray No.1 method (IITA, 1979). Exchangeable potassium was determined by flame photometer, while cation exchange capacity (CEC) was determined by Ammonium acetate saturation method (Roades, 1982).

Sources of experimental materials: 5 pods of *Telfairia occidentalis* were procured from Orieugba market Umuahia, Abia State. The pods were break open, seeds removed from the pod and sundried before planting. The treatments consisted of organic products including; Roselle Calyx extract, Plant fluid, SuperGrow.

Source and preparation of the organic products: the materials recommended for this experiment were got by boiling the calyx of *Hibiscus sabdariffa* bought from the local market, collecting the water got from making a pit in the center of a cut plantain stem in the local environments and super Grow and super 10 bought from agro chemical shop at Umuahia, Abia state.

Application of waste materials: A recommended rate (1ml per 1lit of water) of SuperGro was used. All the organic products were applied at 20ml/plant two weeks after germination at plant base and subsequent application was done until 18weeks after planting (WAP). Similar application was done using water as control (Opara *et al.*, 2019).

Data Collection And Disease Assessment: Data on growth and yield parameters were collected every two weeks interval after treatment based on: vine length (cm), number of leaves, number of branches and leaves yield weight (g). Percentage disease incidence or the number of infected plants was assessed per plot, the total number of plants and number of infected plants were counted and the percentage disease incidence calculated and the severity determined using the formula below:

Percent Disease Incidence (PDI) = $\frac{\text{No. of plants infected in the sampled area}}{\text{Total no. of plant assessed in the sample area}} \times \frac{100}{1}$

Severity score was based on the scale of 0-5 (According to scale modified by Opara and Wokocha, 2008) as follows:

0 = leaves without spot

1 = A few spots on the leaves, about 5% of the leaves covered

2 = Spots join together to form necrotic lesion, covering about 25%

3 = Spots enlarged and extended to leaf margin or about 50% surface covered

4 = Lesion tear and leaf partially rotten, covering about 75%

5 = Leaf collapsed/completely rotten, turn apart and may fall off covering 100%

Pathogenicity Test and inoculation of seedlings: The method of Goszczynska, Serfontein, and Serfontein., (2010) was employed in carrying out the pathogenicity test. Bacterial inoculums was prepared from 24 hours culture by washing bacterial colonies on agar plates with sterile distilled water into Mccartney bottles and adjusting the density of the inoculums to 108cfu/ml using haemocytometer. Young seedlings of fluted pumpkin were inoculated with the prepared bacterial inoculum concentration of 10^8CFU ml^{-1}using an atomizer. The inoculum was sprayed on the leaves until run-off, the seedling were kept under shade and covered with a transparent polyethene bag to maintain high humidity. The inoculated seedlings were uncovered after 48hours and observed daily for symptoms of leaf spots and re-isolation made from symptomatic leaves.

Statistical Analysis: Data collected subjected to analysis of variance (ANOVA) using SAS 2009 Model. The significant differences between the means were separated by Least Significant Difference (LSD) at 5% probability level (Steel and Torrie, 1980).

Results: Soil Analysis and characterization: The physical and chemical properties of the experimental farm are presented in Table 1. Soil test carried out reveals that the experimental soil is sandy loamy, moderately acidic with pH of 5.1, sand, silt and clay particles are 70.80%, 11.00% and 18.20% respectively. It contained a low nitrogen level of 0.154cmol/kg and exchangeable calcium of 5.20cmol/kg.

Effect of organic product on disease incidence, disease severity and on growth parameters at 20 weeks after planting (20WAP) (Table 2).

a) Disease Incidence and severity: The effect of organic product on disease incidence and disease severity is shown on Table 2. The control had the highest disease incidence and severity (50.74% and 4.21 respectively) and these were significantly different from the treated plants. Plants treated with supergro and super 10 had the least disease incidence (16.31%), and severity (1.44).

b) Vine length: plants treated with plantain fluid had the highest vine length (154.83cm) which was significantly different from the plants treated with roselle extract and the control (120cm and 109cm respectively). The control had the least vine length.

c) Number of leaves: Plants treated with plantain fluid had the highest number of leaves (22.75) and was significantly different from plants treated with supergro and the control of the experiment (18.33 and 17.00 respectively).

d) Number of branches: plants treated with roselle calyx extract had the highest number of branches (8.00), but it was not significantly different from other treatments and the control at 5% probability level.

Effect of organic product on growth parameters, disease incidence and severity at 12 weeks after planting (Table 3).

a) Vine length: Plant treated with superGro and super10 had the highest vine length (176.17cm), followed by those treated with roselle calyx extract (174.25cm) and were significantly different from the control (135.25cm).

b) Number of leaves: Plants treated with roselle calyx extract had the highest number of leaves (20.92) though it was significantly different from other treated plants and the control.

c) Number of branches: There was no significant difference in the number of branches.

d) Disease incidence and severity: The control had the highest disease incidence and severity (36.80% and 2.82 respectively) and this was significantly different from all treated plants. Plant treated with roselle calyx extract had the least disease incidence (11.61%) while the least disease severity was observed in plants treated with superGro and super 10.

e) **Effect of organic product on growth parameters at 10 weeks after planting (Table 4).**

a)Vine length: Plants treated with roselle calyx extract had the highest vine length (96.58cm) though it was not significantly different from other treatments and but was significantly different from the control (73.72cm).

b) Number of leaves and branches: At 10WAP, there was no significant difference in the number of leaves. There was no significant difference in the number of branches.

c) Disease incidence and severity: The control had the highest disease incidence and severity (28.41% and 2.18 respectively) and was significantly different from all the treated plants. Plants treated with Supergro and super 10 had the least disease incidence (12.83%) while plants treated with roselle calyx extract had the lowest disease severity (0.86).

Effect of organic product on leaf yield at 8, 12, and 16 weeks after planting (Table 5).

The effect of organic product shown on table 4 reveals that at 8WAP, plants treated with SuperGro and Super 10 had the highest leaf yield (155.42g) which was significantly different from plants treated with Plantain fluid and the control (130.08 and 130.03g respectively).

At 12WAP, plants treated with SuperGro had the highest leaf yield (254.58g), followed by plants treated with roselle calyx extract (242.50) which were significantly different from the control of the experiment (208.08g). At 16WAP, plants treated with SuperGro and super 10 still had the best leaf yield though it was not significantly different from the plants treated with other treatments.

Pathogenicity test: The result of the pathogenicity test conducted shows that the bacterium isolated from *T. occidentalis* could induce symptoms in the leaves, that was visible 7-8days after inoculation and at 14days, the lesions

was drastically increased, coalesced and obvious chlorotic halos developed around the lesions similar to those observed in the field. These symptoms on the contrary, were not observed in untreated crops (control). After another one week, spots and lesions on the upper surface of leaves turned yellowish and much enlarged in the seedlings inoculated with the pathogen.

Biochemical and Cultural Analysis of the Pathogen: The result got from the biochemical and Cultural test conducted is as shown on Table 6 and 7. It was concluded from the results of the pathogenicity test, Physiological and biochemical analysis that the bacterium isolated from leaf of *T. occidentalis* in Umudike, humid south easten Nigeria for *Xanthomonas campestris pv. curcubitae* was similar to other scientific report and the strain identified as *X. campestris pv. curcubitae* (Thapa, 2014; Babadoost, 2002).

Discussion: It was observed in this experiment that incidence of disease increased with time, which is similar to the report by Agrios (2005). Also Agrios (2006) observed that the disease severity on individual plants will be low at the early stage of plant growth but subsequently increase with time, which was in line with this research. The disease symptoms observed in this work, including appearance of small lesions on the underside of leaves, with water soaked dots (yellowish in color) from the upper leaf surface corresponds to previous scientific reports on the disease (Agrios, 2006; Dutta, 2013). The utilization of organic products in improving crop yield had been reported (Osai *et al.*, 2013; Ubalua, 2017; Sylvester and Soh-fong, 2018) and demonstrated in this work. Throughout this research, it was observed that the application of the organic products significantly ($p<0.05$) reduced the disease incidence and severity of bacterial spot of fluted pumpkin compared to the control. Plant materials has earlier been shown to have antimicrobial properties against many pathogens (Olaleye, 2007) and the antimicrobial activity of Roselle extract by Nizar *et al.*,(2013) corresponded to the results in this experiment. However, percentage leaves infected was particularly lower in plants treated with superGro/super 10 and plantain fluid. This may be due to the fact that superGro is said to help drive away insects and pests on the farm as a result of it biochemical contents (oleic acid, dipropylene glycerol metyl ether, linear alcohol ethoxylate etc). Antimicrobial activities of organic products used in this work is in line with earlier scientific reports (Nizar *et al.*, 2013; Osai *et al.*, 2013 and Opara *et al.*, 2013).

Conclusion: This work studied the potency of some organic products on the control of bacterial spot disease of fluted pumpkin in Umudike, humid south eastern Nigeria. This research showed that extracts of roselle calyx, plantain as well as superGro and super10 can be used by farmers in the control of bacterial spot of fluted pumpkin. It was apparent that the use of these organic products, notably SuperGro and super 10 and plantain fluid has the potential to control bacterial spot disease at a low cost without any negative effects in the environments and therefore recommended to help reduce the incidence of bacterial spot disease on this vegetable while in the field. Growth of fluted pumpkin and leafy yield at harvest were enhanced by the application of the organic products, especially SuperGro and roselle extract and also recommended to farmers for the propagation of fluted pumpkin. Further research should be done proximate and biochemical analysis of these organic products.

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Table 1: Physicochemical properties of the study area

Physical properties	
Sand	70.80%
Silt	11.00%
Clay	18.20%
Texture	Sandy loam
Chemical properties	
pH	5.10
Nitrogen	0.154mgkg^{-1}
Organic matter	2.451mgkg^{-1}
Organic carbon	1.425mgkg^{-1}
Exchangeable bases	
Calcium	5.20cmolkg^{-1}
Magnesium	1.20cmolkg^{-1}
Potassium	0.135cmolkg^{-1}
Sodium	0.209cmolkg^{-1}
Exchangeable acidity	0.88cmolkg^{-1}

Source: National Root Crop Research Institute, Umudike (2020)

Table 2: Effect of Organic Product on Disease Incidence, Disease Severity and on growth parameters at 20 Weeks after Planting

TRT	V.L (cm)	No.Lf	No.Br	DI (%)	D. SEV
Ros ext	120.08	21.83	8.00	26.87	1.58
Plantain F.	154.83	22.75	7.08	21.47	1.53
SuperGro/10	135.33	18.33	7.42	16.31	1.44
Control	109.92	17.00	6.67	50.74	4.21
LSD(P≤0.05)	**25.37***	**3.23****	**1.93***	**11.59****	**0.63***

Legend: * = Significant (p≤0.05), **= Highly significant, **Ros ext = Roselle calyx extract, plantain F= plantain fluid, superGro/10= Supergro and super 10, V.L = Vine length, No.Lf = Number of leaves, No.br = Number of branches, DI = Disease incidence, D.sev = Disease severity. Trt = Treatment**

Table 3: Effect of organic products on disease incidence, severity and on growth parameters at 12weeks after planting (12WAP).

TRT	V.L (cm)	No.Lf	No.Br	DI (%)	D. SEV
Ros ext	174.25	20.92	11.50*	11.61	1.56
Plantain F.	148.58	18.33	11.50	18.22	1.14
SuperGro/10	176.17*	20.75	10.50	15.45	1.02
Control	135.25	18.83	9.33	36.80**	2.82**
LSD(P≤0.05)	**38.75**	**NS**	**NS**	**70.14**	**0.40**

Legend: NS = Not significant (p≤05), * = Significant, = Highly significant, V.L = Vine length, No.Lf = Number of leaves, No.br = Number of branches, DI = Disease incidence, D.sev = Disease severity. Trt = Treatment, plantain F = plantain fluid, SuperGro/10 = SuperGro and super 10, Ros ext = Roselle extract, LSD = Least Significant difference at 5% probability level.**

Table 4: Effect of organic products on growth parameters at 10weeks after planting

Trt	V.L (cm)	No.L	NoBr.	D.I	D.Sev.
Ros Ext.	96.58*	13.67	11.17	19.92	0.86
Plantain F.	85.58	13.42	9.75	19.08	0.89
SuperGro/10	89.33	13.33	10.75	12.83	0.95
Control	73.92	12.42	9.58	28.41*	2.18**
LSD (p<0.05)	**18.93**	**NS**	**NS**	**11.47**	**0.34**

Legend: NS = Not significant (p<05), * = Significant, V.L = Vine length, No.Lf = Number of leaves, No.br = Number of branches, DI = Disease incidence, D.sev = Disease severity. Trt = Treatment, plantain F = plantain fluid, SuperGro/10 = SuperGro and super 10, Ros ext = Roselle extract, LSD = Least Significant difference at 5% probability level.

Table 5: Effect of organic product on leaf yield at 8, 12, and 16 weeks after planting

TRT	Yield at 8WAP	Yield at 12WAP	Yield at 16WAP
Ros ext	152.25	242.50	132.92
Plantain F.	130.08	212.50	148.00b
SuperGro/10	155.42*	254.58*	156.58*
Control	130.03	208.08	141.25b
LSD(P<0.05)	**51.48**	**60.14**	**37.02**

Legend: NS = Not significant (p<05),* = Significant, Trt = Treatment, plantain F = plantain fluid, SuperGro/10 = SuperGro and super 10, Ros ext = Roselle extract, LSD = Least Significant difference at 5% probability level.

Table 6: Cultural charaecteristics of the pathogen

Tests	Chracteristics
Colour oand texture of colonies	Mucoid, yellow and creamy
Gram staining Test	Gram negative rods
Microscopic examination	Motile, without spores or capsules
Colony characteristics and shape	Mucoid and convex

Table 7: Biochemical Characteristics

Tests	Results
Gram reaction	-
Starch hydrolysis	+
Catalase activity	+
Glacin hydrolysis	+
Oxidase activity	-