



## EFFECT OF AGRO EFFLUENTS ON THE CONTROL OF BACTERIAL SPOT DISEASE OF FLUTED PUMPKIN (*Telfairia occidentalis* Hook f.) IN SOUTH EAST NIGERIA

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### ABSTRACT

A glass house trial was conducted in the College of Crop and Soil Sciences of Michael Okpara University of Agriculture, Umudike to ascertain the enhancement of some agro effluents on the management of bacterial spot disease of fluted pumpkin (*Telfairia occidentalis* Hook f.) between March-July, 2018. The experiment was laid out in a Complete Randomize Design (CRD) and replicated three times. The effluents employed were from rice, cassava, corn, oil palm and a control (sterile water) which was applied 20ml/plant at two weekly interval with the application focused at plant base. Data were collected on disease incidence, disease severity and growth parameters. Results obtained showed that all effluents used were able to reduce disease incidence and increase yield better than the untreated control irrespective of the concentrations used. It is therefore, recommended that farmers can use these effluents to minimize the risk of leaf spot incidence in the field since they are quiet affordable and readily available.

**Keywords:** *Telfairia occidentalis*, Agro effluent, disease incidence, severity, *Xanthomonas campestris*, and bacterial spot

### Introduction

Fluted pumpkin (*Telfairia occidentalis* Hook f.), is a tropical vine in the family Cucurbitaceae. Common names for the plant include: fluted gourd, fluted pumpkin, Ikongubong and Ugu (Times and Chikezie, 2016). Fluted pumpkin is widely cultivated as garden and farm vegetable (Chukwu *et al.*, 2012) for its edible leaves and seed. It is fast assuming great importance in Nigeria because it is contributing to her dietary needs and other countries in West Africa. Fluted pumpkin grows in the forest zone of West and Central Africa, most common in Benin, Nigeria and Cameroon (Odiaka and Schippers, 2004). For the Igbo tribe of the Eastern Nigeria, it is recognized as the most popular leafy vegetable because it could be used in the preparation of various dishes like soup and sauces (Fasina and Okeowo, 1998). It is also a source of oil used for cooking, soap making, margarine, paints and varnishes. *T. occidentalis* vegetable and fruit enhance human health, prevent constipation, heart diseases, stroke, high blood pressure and accumulation of cholesterol (Etukudo, 2003). The production of pumpkin has been on the increase in Nigeria due to increased awareness on its nutritional values (Odiaka, 2005). Though there are numerous uses and potentials of fluted pumpkin in Nigeria, the average yield of pumpkins in Nigeria remains low due mainly to biotic and abiotic stresses (Times and

Chikezie, 2016). Biotic diseases includes spot diseases induced by various pathogenic groups of fungi and bacteria (Burrows, 2013), Leaf spots which result in defoliation (Nix, 2014), inappropriate farm management and pre and post-harvest losses. This study therefore, was undertaken to isolate and identify the causal organism and determine the efficacy of some selected agro effluents in the control of bacterial leaf spot of fluted pumpkin.

### Materials and Methods

The study was carried out during the 2018 cropping season, under pot trial. Top soil was taken from the eastern farm site of the Michael Okpara University of Agriculture, Umudike. Soil analysis was conducted to determine the physic-chemical contents of the soil. The soil was put into a cut drum, moistened and heated to a temperature of 80°C for 20min for optimum sterilization. It was allowed to cool before being mixed with poultry droppings and fine soil in the ratio of 3:2:1 of top soil, fine sand and poultry droppings respectively. The poultry dropping was allowed to decompose for two weeks before use and later the sterilized soil mixture was dispensed into plastic pots of 15cm in diameter (three quarter filled). The experiment was laid in a complexly randomized design (CRD) consisting of five treatments at three different rates and replicated three times. The agro

effluents used were effluents from palm oil, cassava, corn and rice. Fluted pumpkin seeds were planted in experimental pots at the rate of one seed per pot.

#### **Source of Experimental Materials**

The fluted pumpkin pods were procured from a farm in Lodu-Imenyi, Uzuakoli in Abia State. The seeds were removed from the pods and sundried before planting. The treatment consists of the following agro effluents; cassava, oil palm, corn and rice effluents sourced for from the local environment including milling stations in Umueze village, Umuahia, Abia State.

#### **Source of Agro Effluents**

Fresh cassava and palm oil effluents were collected from their respective milling stations in Agbo-Umueze, Umuahia North of Abia State. Fresh Corn effluent was collected from local *akamu* (pap) producers within the same location and rice effluent from local rice parboiled in the kitchen. All effluents were collected directly into sterile 4litres plastic gallons and kept in their respective gallons for 3days to ferment as a means of detoxification and nutrient improvement (Ubuala, 2017; Oboh, 2006) before taken to the farm for application. Sterile water was sourced from the lab.

#### **Preparation of Bacterial Inoculum**

At the university farm, infected leaf samples were collected and brought to the laboratory, thoroughly washed with sterile water and then surface sterilized for 10seconds in 0.5% aqueous solution of sodium hypochlorate, the leaf samples were cut (4mm<sup>2</sup>) between the junction of disease and healthy leaf tissue (Opara *et al.*, 2013). Each sample was then placed on a sterile microscopic slide, covered with a drop of sterile water and observed under a dissecting microscope (x25). A sterilized small piece of infected portion was also placed in a drop of water inside a petri dish, teased apart and left to stand for about half an hour to allow the multiplication of the bacterium before inoculating into the culture media.

#### **Preparation of medium and inoculation of the pathogen**

Nutrient agar (NA) was used in the bacteria culture. The culture medium was prepared according to manufacturers' instructions by weighing out 7g of ready-made nutrient agar powder into a conical flask and dissolving in 250ml of sterile water (Fahy and Hayward, 1983). The mixture was thoroughly shaken and autoclaved at 120°C for 30 minutes. This was followed by pouring 15ml of the medium into 9cm diameter petri-dishes after it has cooled to 45°C. The medium (nutrient agar) was kept in an incubator to enhance drying of the surface agar in the petri-dish for about 8hours at 28°C before use. Before inoculation of bacterial suspension into the medium, the inoculation chamber was mopped with 70% absolute alcohol using

sterile cotton wool to avoid contamination. The bacterial suspension was streaked onto the nutrient agar in petri-dishes using a flamed rod and cooled wire loop after which the culture was placed in the incubator at 30°C for 48hours. After this period, the culture colonies obtained was sub-cultured severally to get pure bacterial colonies.

#### **Pathogenicity Test and Inoculation of *T. occidentalis* seedlings**

The 45 pots containing fluted pumpkin seedlings were arranged in completely randomised design (CRD) with three replicates. Prior to the application of the agricultural effluents, the pumpkin seedlings were pre-inoculated using the bacterial inoculums of a concentration of 10<sup>8</sup>cful/ml. The seedlings were inoculated by spraying the bacterial inoculums on the leaves using hand atomizer in the evening (6pm). The younger leaves and emerging shoots were also sprayed until there was a run-off. The inoculated seedling were later covered with transparent polyethene bags to create a high condition and allowed for 48hours at 25-27°C for the bacterial pathogen to incubate (Jones *et al.*, 2000).

#### **Application of Agricultural Effluents**

The agricultural effluents used were obtained from Cassava (*Manihot* Spp.), Rice (*Oryza sativum*), Oil palm (*Elaeis guinensis*) and Corn (*Zea mays*) while sterile water was use as untreated control. All the effluents collected were kept in sterilized 4liters galloons and left for two days to ferment as a means of detoxification and improvement of nutrient content (Oboh, 2006; Ubuala, 2017). A 100% (non-diluted), 50% (half dilution) and 25% (one quarter dilution) of each agricultural effluents were applied at 20ml/plant two weeks after germination at plant base and subsequent application was done at two weeks interval till 16 weeks after planting (WAP). A similar application was done using sterile water as control.

#### **Disease Severity Index and Disease Incidence**

Disease severity was recorded bi-weekly based on the scale of 0-6 modified by Opara and Wokocho (2008) as follows;

- 1 = no disease symptom visible on the leaf surface
- 2 = a few lesions covering about 5% of leaf surface
- 3 = about 25% of the leaf surface affected by the lesions
- 4 = spots enlarge and extends o leaf margin, about 50% surface covered
- 5 = 75% of the leaf surface affected by the legion
- 6 = Leaf collapse/completely rotten, turn apart and may fall, lesion covering 100% of leaf surface.

### **Disease Incidence**

The diseases incidence was determined as follows:

$$\text{Percentage (\% ) disease incidence} = \frac{\text{Number of leaves}}{\text{Total number of leaves sampled}} \times 100$$

Data on growth and yield parameters were collected fortnightly, starting from two weeks after treatment (effluent) application or four weeks after planting based on; vine length (cm), number of leaves, stem diameter (cm), and leaf yield weight (g). The scores based on first 5 leaves from the youngest open foliage were used to assess the disease severity whereas; disease incidence was obtained by the percentage of affected number of leaves per plant and per replication.

### **Data Analysis**

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

### **Results and Discussion**

#### **Soil Analysis and Characterization**

The result of soil sample analysed is shown in Table 1.

#### **Pathogenicity Test**

Results of pathogenicity test conducted showed that bacterium from fluted pumpkin induced symptoms in the leaves, which was seen 7-8days after inoculation and at 14days, lesions had drastically increased, coalesced and prominent chlorotic halos developed around the lesions similar to those observed in the field. These symptoms were however not observed in the control experiment. After another one week, spots and lesions on the upper surface turned yellowish with the chlorotic halos becoming enlarged in the seedling inoculated with the pathogen.

#### **Biochemical and Cultural Analysis of the pathogen**

Result of some biochemical and cultural test conducted is shown on Tables 2 and 3. Following the results of physiological and biochemical analysis including pathogenicity tests conducted, it was concluded that the bacterium isolated from leaf spot of fluted pumpkin in Umudike, South East Nigeria for *X. campestris* pv. *curcubitae* was similar to the report made in Urbana and the strain identified as *X. campestris* pv. *curcubitae* (Babadoost, 2002; Thapa, 2014).

#### **Effect of Agro Effluents on Growth parameters of *T. occidentalis* in Pot trial at Six weeks after planting**

The results obtained at six weeks after planting on the effect of agro effluents on growth parameters are

shown on Table 4. Rice Effluent treatment gave the highest vine length (94.85cm), followed by palm oil (88.18cm) and corn (78.19cm) which differ significantly from untreated control at  $P < 0.05$ . Also, all the treated plants had higher number of leaves than the control which had the least value (17.00). For number of branches: plants treated with corn effluent had the highest number of branches (4.46) which was not significantly different from rice (3.44) and cassava (3.33) but higher than palm oil (1.76) and control with the least number of leaves (1.55), whereas there was no significant difference among the agro Effluents and the control with regards to stem diameter.

#### **Effect of Agro Effluents on Disease Incidence and Severity**

The effect of agro effluents on disease incidence and severity are shown on Table 5. Disease incidence and severity were significantly higher in the control than all the treated plants. Rice effluent had the least disease incidence (7.81%), followed by palm oil effluent (11.44%) while control had the highest disease incidence (33.33%). Likewise, rice effluents had the least disease severity (0.89), followed by corn effluent (0.99) and the control had the highest severity (2.51).

#### **Effect of Agro Effluents on Growth parameters at 12Weeks after planting**

On the effect of effluents on growth parameters (Table 5); Rice effluent gave the best vine length (152.67cm), and control with the least vine length (83.78cm). Corn effluent had the highest number of leaves (73.22), which was significantly different from cassava effluent (55.93) and control with the least number of leaves (32.56). Corn gave the highest number of branches (9.06) while control had least number of branches (4.11). There was no significant difference between the treatments and control with regards to stem diameter of the plants.

#### **Effect of different Rates of Effluents on Growth parameters of *T. occidentalis* in Pot experiment after Weeks of planting**

Table 6 shows that there was no significant difference in the different rates of the agro effluents used in this experiment on growth parameters of *T. occidentalis* all through the period of experiment. The different rates of effluent used were 100%, 50% and 25%.

#### **Effect of Effluents on Leaf yield of *T. occidentalis* in pot trial**

Results obtained at 9 and 19 weeks after planting on the leaf yield data of *T. occidentalis* (Table 7) shows that all agro effluents used in this experiment gave higher leaf yield than the control irrespective of their concentrations. At 9weeks after planting, Oil palm effluent gave the highest yield weight (123.27g), followed by corn effluent (97.69g) which were

significantly higher than the control (60.83g) at 5%. At 19WAP, corn effluent had the highest yield weight (912.5g), followed by rice effluent (748.3g), which differed significantly from that of the control, sterile water (332.8g) at  $P < 0.05$ .

This study reveals the bactericidal potential and efficacy of the four agro effluents used (effluents from Rice, Corn, Cassava, Oil Palm) in comparison with the control reduced both disease severity and incidence and enhanced the growth and yield of fluted pumpkin which is an important vegetable in South East Nigeria. This study is similar to those reported by previous studies (Ditter *et al.*, 1990; Bassey and Opara, 2016) who reported that many plant parts and effluents contain antibacterial properties. Ng *et al.*, (2017) also reported the inhibition of *F. oxysporium* using organic effluents. The consistent best performance of rice effluent in this experiment is in line with the findings of Iwuagwu (2017), who reported that application of rice waste for the cultivation of cocoyam greatly improved its production in humid agro-ecological zone of South-East Nigeria. It was also observed that the addition of agro effluents in this study enhanced growth of fluted pumpkin. This agrees with the report of Eze *et al.*, (2013) who showed that incorporation of organic waste to soils increased plant growth, because they contain considerable amount of plant nutrient including micro nutrients which are beneficial for plant growth. Result of the laboratory experiment showed that the bacterial isolate from the leaves of fluted pumpkin was gram negative, motile with a single polar flagellum, catalase positive and oxidase negative and produced yellow colonies on nutrient agar. The above characteristics suit the description for *Xanthomonas cucurbitae* (Schaad *et al.*, 2001; Asuquo and Opara, 2016). Different authors have implicated this organism as the causal agent of bacterial leaf spot on cucurbits (Babadoost and Zitter, 2009; Pruvost *et al.*, 2009; Dutta *et al.*, 2013; Thapa, 2014).

### Conclusion

This study showed the efficacy of agro effluents in the control of leaf spot disease of fluted pumpkin and hence effluent of Rice, Cassava, Corn and Oil Palm can be used by resource poor farmers in the management of leaf spots of fluted pumpkin. This low cost biological approach would be economically viable and ecosystem friendly. The effluents are also accessible to farmers especially in the rural communities where there are milling stations. The significance reduction in disease severity and incidence shows that formulations of agro effluents could have important role in biologically based strategies for the control of diseases caused by *X. campestris* pv. *cucurbitae*.

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**Table 1: properties of the soil sample collected at 0-20 cm depth**

<b>Physical properties</b>	
Sand	72.79%
Silt	10.40%
Clay	16.81%
Texture	sandy loam
<b>Chemical properties</b>	
pH	5.40
Nitrogen	0.042mg kg <sup>-1</sup>
Organic matter	1.31mg kg <sup>-1</sup>
Organic carbon	1.20mg kg <sup>-1</sup>
<b>Exchangeable bases</b>	
Calcium	2.38cmol kg <sup>-1</sup>
Magnesium	1.22cmol kg <sup>-1</sup>
Potassium	0.085cmol kg <sup>-1</sup>
Sodium	2.51cmol kg <sup>-1</sup>
Exchangeable acidity	1.32cmol kg <sup>-1</sup>

**Table 2: Cultural Characteristics of the pathogen**

<b>Tests</b>	<b>Characteristics</b>
Colour and 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 3: Biochemical characteristics**

<b>Test</b>	<b>Results</b>
Gram reaction	-
Starch hydrolysis	+
Catalase activity	+
Glatin hydrolysis	+
Oxidase activity	-

**Table 4: Effect of Agro Effluents on Growth parameters of *T. occidentalis* in Pot trial at 6WAP**

<b>Treatment</b>	<b>V.L</b>	<b>No.Lf.</b>	<b>NO. BRA</b>	<b>S. DIA.</b>
Rice	94.85	37.39	3.44	3.13
Cassava	66.28	31.59	3.33	3.83
Palm oil	88.18	33.22	1.56	3.41
Corn	78.19	32.67	4.56	3.09
Ctrl.	49.94	17.00	1.55	2.14
<b>LSD (P&lt;0.05)</b>	<b>19.27</b>	<b>13.33</b>	<b>1.99</b>	<b>1.19</b>

**Legend:** V.L = Vine length, No.Lf = Number of leaves, NO.BRA = Number of branches, S.DIA = stem diameter, WAP = weeks after planting

**Table 5: Effect of Agro Effluents on Disease Incidence, Severity and Growth parameters of *T. occidentalis* in pots at 12WAP**

<b>TRT.(Effluents)</b>	<b>D.I (%)</b>	<b>D.SEV</b>	<b>V.L (cm)</b>	<b>NO.LF</b>	<b>NO.BRA</b>	<b>S.DIA(cm)</b>
Rice	7.81	0.89	152.67	66.11	8.78	4.94
Cassava	18.52	1.30	134.22	55.93	7.17	5.37
Palm oil	11.44	1.53	154.00	57.44	6.72	4.31
Corn	23.26	0.99	141.28	73.22	9.06	4.70
Ctrl.	33.33	2.51	83.78	32.56	3.11	5.56
<b>LSD (P&lt;0.05)</b>	<b>12.04**</b>	<b>0.61*</b>	<b>35.20**</b>	<b>17.07*</b>	<b>2.80*</b>	<b>1.29*</b>

\* = significant at 5% alpha level, \*\* = highly significant at 5% alpha level

Trt = Treatment, V.L = Vine length, No.Lf = Number of leaves, NO.BRA = Number of branches, S.DIA = stem diameter, NO.F = Number of pods, DI = Disease incidence, DSEV = Diseases severity.

**Table 6: Effect of different Rates of effluents used on Growth parameters of *T. occidentalis* at 16WAP**

Rates %	8WAP			10WAP			12WAP			14WAP			16WAP		
	V.L	NO.Lf	N.Br	V.L(cm)	NO.Lf	N.Br	V.L(cm)	NO.Lf	N.Br	V.L(cm)	NO.Lf	N.Br	V.L	NO.Lf	N.Br
100	73.77	31.22	3.07	81.92	30.80	5.53	133.77	53.20	7.03	160.79	79.12	7.47	174.63	70.07	8.20
50	76.28	29.31	2.80	86.09	37.73	5.58	130.10	55.43	6.80	149.90	62.23	7.99	172.81	70.73	8.33
25	78.23	30.80	2.67	85.13	37.27	4.38	135.50	62.53	7.07	164.47	68.00	8.03	177.51	77.33	8.51
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

(P&lt;0.05)

Legend: V.L = Vine length, No.Lf = Number of leaves, NO.br = Number of branches

NS = Not significant, WAP = Weeks after planting

**Table 7: Effect of Effluents on yield of *T. occidentalis* in pot trial at 9 and 19WAP**

Treatment (Effluents)	9WAP Weight (g)	19WAP Weight (g)
Rice	65.50	748.3
Cassava	72.87	464.4
Palm oil	123.27	516.7
Corn	97.60	912.5
Control	60.83	332.8
<b>LSD (P&lt;0.05)</b>	<b>30.32*</b>	<b>222.65*</b>

Legend:\* = significant at 5% alpha levelLSD = Least Significant difference

WAP = weeks after planting