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Control of Leaf Spot Disease of *Telfairia occidentalis* Hook f. (Fluted Pumpkin) Using Some Agricultural Effluents in Umudike, Nigeria

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Abstract:

A field trial was conducted in 2017 cropping season at Michael Okpara University of Agriculture, Umudike to test the impact of some agricultural effluent on the control of leaf spot disease of fluted pumpkin. The experiment was laid out in Randomized Complete Block Design (RCBD) and replicated three times. The effluents from rice, cassava, maize, oil palm and a control (sterile water)were applied at 20ml/plant two weeks after germination at plant base and data collected until after 14weeks 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 probability level of 5%. Results obtained from disease incidence and severity showed that rice effluent scored 31.25% and 1.75 respectively, while oil palm scored 32.50% for disease incidence and 1.59 severity which were significantly ($P \leq 0.05$) better than other treatments in reducing incident and severity of leaf spot disease including the control (75% disease incidence and 4.83 severity). Generally all the effluents were observed to enhance growth in all the parameters assessed (vine length, number of leaves and number of branches and stem diameter) when compared with the non-treated plants (control). For instance, rice gave best vine length (247.33cm) followed by corn (199.59cm) and were statistically higher than the control (137.58cm). Pathogenicity test result showed that the organism was able to induce leaf spot on the seedlings and the organism from infected leaves was identified as *Xanthomonas* spp. This study demonstrated the efficacy and potential of some agricultural effluents in reduction of bacterial leaf spot of fluted pumpkin. These agricultural effluents can be used by farmers in order to minimize the risk of leaf spot incidence in the field since they are readily available and affordable.

Keywords: Agricultural effluents, Leaf spot, Disease incidence, Disease severity.

Introduction

Fluted pumpkin, *Telfairia occidentalis* (Hook f.) is one of the basic vegetable crops of major economic importance in the south eastern Nigeria. It occurs mostly in cultivated form in various parts of southern Nigeria for its palatable and nutritious leaves which are mainly used as vegetables in Nigerian foods (Esiaba, 2000; Burkill, 2004; Bassey and Opara, 2016). The seeds of *T. occidentalis* (fluted pumpkin) are high in fat, protein and therefore contribute to a well balanced diet (Okon and Udoffot, 2012). It is drought tolerant, dieocious, perennial that is being trellised (Bosa *et al.,* 1983). 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).

The medicinal importance of *T. occidentalis* has been reported. In Nigeria, the herbal preparation of the plant has been employed in the treatment of such situations like sudden attacks of convulsion, malaria, and anaemia (Gbile, 1985). Its action against hypercholestolaemia has been investigated and reported (Eseyin *et al*., 2010 and Adaramoye *et al*., 2007). It contains some phytochemicals such as saponin, alkaloid, phenol and tannin (Sofowora, 1996) which have curative properties. *T. occidentalis* vegetable and fruit enhance regular bowel movement; prevent constipation, heart diseases, stroke, high blood pressure and accumulation of cholesterol (Etukudo, 2003). A major constraint on *T. occidentalis* in South-Eastern Nigeria is biotic infections while it is growing in the field and during storage, thus reduces the market value at harvest. Disease/disturbance to this highly valued vegetable may be incited by fungi, bacteria and viruses. Leaf spot is one major disease caused by these pathogens which reduces leaf growth of *Telfairia occidentalis* and effort to combat it using chemicals is non-realistic due to high cost of the synthetic pesticides and consequent environmental impact (Fields, 2006; Salem *et al*., 2007; Asawalam *et al*., 2012).

The bacterium gets entrance into the leaves through the water pores or wounds and progresses to the vascular system (Opara and Obani, 2010). Young immature leaves are most susceptible to the pathogen, which expand gradually till the leaf symptoms become visible. Symptoms on foliar parts include legion on leaves, young stem and petioles. The lesions on leaves are observed as water soaked, yellow spots, which may turn brown with age and surrounded by a yellow halo (Willia m, 2005; Dutta *et al*., 2013). The coalescing of lesions gives the appearance more like angular leaf spot caused by *Pseudomonas syringae* pv. *Lachrymans* (Babadoost and Zitter, 2009). A leaf spot disease creates spots on foliage. The spots will vary in size and colour depending on the plant, the organism involved and the stage of the host plant development (Bassey and Opara, 2016). Leaf spots affects size and quality of leaves as well as harvestable fruits number (Neely and Nolte, 1989).

In view of the importance of fluted pumpkin in Nigeria and the devastating effect of leaf spots and pest infestation on yield of this crop, there is need to employ more ecosystem friendly and less expensive methods of controlling the diseases in Nigeria. This study was carried out to test the effectiveness of some agricultural effluents in the management of leaf spot disease of *Telfairia occidentalis* (fluted pumpkin) in the field. Transformation of agricultural effluents offers opportunity of creating marketable value-added products, recovery of energy and elimination of most animal and human health problems associated with contamination (Ubalua, 2007).

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 5.28°N and longitude 7.33°E, with an elevation of 122m above sea level.

Field Preparation and Layout:

The land was mechanically cleared using tractor and prepared into ridges. *Telfairia occidentalis* seeds were sown two per hole and later reduced to one per hole at a space of 1m x 1m inter rows and 0.5 x 0.5m intra rows. The experimental design was Randomized Complete Block Design (RCBD) with three replicates. Each replicate measured 25m x 10m while each plot measured 10m x 2m, space between replicates and a plot was 1m and 0.5m respectively. Total area for the experiment was 25m x 33m ($825m^2$). Weeding was done manually when necessary.

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 27°C 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 Amonium acetate saturation method (Roades, 1982).

Sources of experimental materials:

Three pods *T. occidentalis* were procured from a farm in Lodu-Imenyi, Uzuakoli in Abia State. The seeds were removed from the pod and sundried before planting. The treatments consisted of the following agricultural effluents; cassava, oil palm (collected from their respective mills in the rural area), maize from local akamu producers and rice effluent of local rice from kitchen.

Collection and application of organic effluents:

All effluents (cassava, maize, oil palm, rice) collected were kept in their respective gallons for two (2) days to ferment as a means of detoxification (Ubalua, 2007) and improvement of nutrient content (Oboh, 2006) before application. 50% or half dilution of each agricultural effluent were applied at 20ml/plant two weeks after germination at plant base and subsequent application was done until 14weeks after planting (WAP). Similar application was done using sterile water as control.

Data Collection and Disease Assessment

Data on growth and yield parameters were collected every two weeks interval after treatment based on: vine length (cm), stem diameter (cm), number of leaves, number of flowers 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:

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

Severity score was based on the scale of 0-6 (According to scale modified by Opara and Wokocha, 2008) as follows:

1 = leaves without spot

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

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

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

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

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

Pathogenicity Test and inoculation of seedlings

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 (Goszczynska *et al*., 2010).

Young *T. occidentalis* seedlings 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 difference between the means were separated by Least Significant Difference (LSD) at 5% probability level (Steel and Torrie, 1980).

Results

Result of the physicochemical analysis is presented in Table1, the experimental site was found to be sandy loamy, slightly acidic pH5.4 and high organic content.

Table 1: Physicochemical properties of study area

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

Source: National Root Crop Research Institute, Umudike (2017).

Effect of Agro effluents on disease incidence, severity and growth parameters

The effects of different effluents used on the control of leaf spots disease and growth parameters of fluted pumpkin at 14 WAP is shown in Table 2.

Disease incidence and severity

Data on disease incidence and disease severity were collected from six weeks after planting when the symptoms appeared on leaves till fourteenth week after planting. Disease incidence and severity were significantly higher in control than all the treatments. Results obtained showed that disease incidence and severity were; rice effluent scored 31.25% and 1.75 respectively while oil palm had

32.50% and 1.59 for incidence and severity which were significantly($P \leq 0.05$) better than other treatments in reducing incident and severity of leaf spot disease including control (75% and 4.83 respectively).

Growth parameters

Control had the least vine length (137.58cm) but not significantly different with cassava and oil treatments while rice (247.33cm) waste had highest vine length. The same trend was observed for number of leaves. There was no significant difference in the number of branches, stem diameter and number of flowers among the treatments, except between rice (highest) and control (lowest) in all the parameters.

Table 2 Effect of agro waste on growth parameters and disease incidence and severity at 14WAP

Treatment	**DI (%)**	**D.sev**	**V.L(cm)**	**No. Lf**	**No. bra**	**S.dia(cm)**	**No.F**
Rice	31.25	1.75	247.33	182.75	23.08	4.74	60.75
Cassava	36.25	1.70	182.55	140.75	19.42	4.57	40.75
Oil	32.50	1.59	191.14	115.75	18.50	5.12	35.50
Corn	38.75	1.92	199.59	128.75	16.92	3.97	37.00
Control	75.00	4.83	137.58	100.50	13.17	1.50	18.50
LSD($P \leq 0.05$)	14.531**	1.412**	50.665*	56.573*	NS	NS	NS

Legend: NS = Not significant ($p \leq 05$)
*** = Significant**
**** = Highly significant**
V.L = Vine length, No.Lf = Number of leaves, No.bra = Number of branches, S.dia = stem diameter, NO.F = Number of flowers, DI = Disease incidence, D.sev = Diseases severity.

Results from this study showed that disease incidence and severity of leaf spot of fluted pumpkin was significantly reduced by the application of different agricultural effluents. All the effluents significantly ($P \leq 0.05$) reduced severity and infection on leaves. However, percentage of leaves infected and severity was particularly lower with effluent of oil palm and rice. This is in line with earlier reports that many plant effluents and plant parts contain anti-bacterial and fungitoxic constituents that have the potentials to control plant diseases (Hadar and Mandelbaum, 1986; Ditter *et al*., 1990; Stindt, 1990; Obi *et al*., 2006; Bassey and Opara, 2016). Ng *et al*., (2017) reported on the suppressiveness of *Fussarium oxysporium* using agro compost. 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 in the cultivation of cocoyam greatly improved its production in humid agroecological zone of South-eastern Nigeria. The good performance of cassava effluent is in line with the work of Barana (2000) who showed that cassava liquid contains minerals, which after anaerobic biodigestion, can still be used for fertilization, since the digestion process does not substantially decrease the mineral content. Palm oil effluent performed better than corn and control; this is in line with the finding of Vidyana and Yahya (1993) who reported that oil palm sludge/soil mixture promoted good growth of palm oil seedlings due to its beneficial effect on physical properties and nutritional aspects of the crop

Effect of agro effluents on growth parameters of *Telfairia occidentalis* at six weeks after planting (6WAP).

Table 3 shows the vine length, number of leaves, number of branches and stem diameter of fluted pumpkin with different agricultural effluents. The result of the analysis shows that plants treated with rice (71.84cm) and cassava (68.00cm) effluents were significantly different with the control (32.08cm) at 6WAP in their vine lengths. Plants treated with rice had highest number of leaves (57.75), followed by cassava waste treatment (39.75) and value was significantly different from those treated with oil palm effluent, corn effluent and control. The control had the least number of leaves (19.25).

The result obtained indicated that the utilization agricultural effluents enhanced crop performance and growth. This is in agreement with the previous findings (Uwah *et al*., 2011, Onwudike*et al*., 2015; Ubalua, 2007; Al-Qhatani, 2011; Sylvester and Soh-fong, 2018). Utilization of agro effluents will be a good substitute to synthetic chemicals for poor rural farmers due to high cost and hazardous effect of the later. Soil amended with organic matter of rice, maize, grape seed, oil palm husk had been found to alter the soil physical and chemical conditions and have important consequences on the growth of plants and the occurrence of soil born diseases (Northover and Schnider, 1993).

Table 3: Effect of agro waste on growth parameters at 6weeks after planting

Trt.	V.L(cm)	No. Lf	No. bra	S.dia (cm)
Rice	71.84	57.75	5.46	3.65
Cassava	68.00	39.75	5.05	2.98
Oil	59.75	30.25	4.31	2.96
Corn	45.58	30.50	4.58	2.57
Control	32.08	19.25	2.96	1.88
LSD ($p<0.05$)	22.45**	15.901*	1.698*	NS

Legend: NS = Not significant at 5% probability level
*** = Significant**
**** = Highly significant**
Trt. = Treatment, V.L = vine length, No.Lf = Number of leaves, No.bra =Number of branches, S.dia = stem diameter.

Effect of agro effluents on yield of *T. occidentalis* at 14weeks after planting

In table 4, the effect of the effluent on harvest yield were compared with the control of the experiment and results obtained on yield of *T. occidentalis* was significant ($p<0.05$). It was observed that rice waste displayed superiority over all other treatment in terms of plant yield (3.7kg), followed by cassava waste (2.9kg), oil palm waste (2.7kg), corn waste (1.7kg). The least weight was recorded by the untreated control (1.0kg).

Table 4: **Effect of agro effluents on yield of *T. occidentalis* at 14weeks after planting**

Treatment	Yield weight(kg)
Rice	3.7
Cassava	2.9
Oil	2.7
Corn	1.7
Control	1.0
LSD ($p<0.05$)	0.7

The significant increase in yield of fluted pumpkin at the application of agro effluents compared with the control is commendable and agrees to previous reports that incorporation of organic matter increases soil nutrient which in turn enhance crop yield (Eze *et al*, 2013). Iwuagwu *et al*., (2017) reported on the effectiveness of organic wastes from organic effluents in improving growth and yield of cocoyam. Throughout the experiment, it was observed that addition of agricultural effluents, enhanced growth of fluted pumpkin. This agrees with the report of Eze *et al.* (2013) who showed that incorporation of organic waste to soils increases plant growth, because they contain considerable amounts of plant nutrients including micronutrients which are beneficial to plant growth.

Conclusion

The research examined the efficacy of agricultural effluents on the control of leaf spot disease of *Telfairia occidentalis* (fluted pumpkin) in Umudike. This work showed that effluents of rice, cassava, oil palm and corn can be used by poor farmers in the control of leaf spot of fluted pumpkin. They contain antibacterial compound that can be utilized to prepare a potential phyto-bactericide for the control of leaf spot disease of cucurbits. It was apparent that use of these effluents has the potential to control leaf spot disease at less or no cost.

This safe and low cost approach is economically viable and ecosystem friendly. The effluents are also accessible to farmers as there are milling stations around the farmers especially in the rural settings. This research recommends the use of these agro effluents to avoid inhibition of crop growth and intoxication of crop via use of synthetic pesticides. There is need for further research work on the constituents in these agricultural effluents.

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