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ASSESSMENT OF YIELD LOSS IN FLUTED PUMPKIN INFECTED BY *COLLETOTRICHUM LINDEMUTHIANUM*

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ABSTRACT

Telfairia occidentalis is an important vegetable in the dietary requirements of most Nigerians. In Calabar, the leaves are mainly prepared into soup and the seeds are eaten cooked or as soup thickener when boiled, Yield loss assessment in *Telfairia occidentalis* due to anthracnose disease of *Colletotrichum lindemuthianum* was carried out in a two (2) year study (2007 and 2008) in Akim, Calabar Municipality of Cross River State, Nigeria. The experiment was arranged in a completely randomized design (CRD). Assessment parameters were leaf area, stem length, leaf quantity and seed quantity. Analysis of results using ANOVA showed that at high disease severity, there was reduction in leaf area of the diseased plant ($P > 0.05$) which directly resulted in reduction in the quantity of seeds in the pod (pepo). Stem length and leaf quantity were not affected. When losses for the two (2) study seasons were compared using the Critical Point Model (CPM), higher losses (77.44%) were recorded in 2007 than in 2008 (58.48%). This must have been as a result of high disease incidence (68%) and severity (4.7) in 2007 which must have been favored by the high early (April) rainfall (above 270 mm) in the study area in that year. The results of this research will help Government and local farmers to assess their losses at the instance of disease incidence in this crop in particular and other leafy vegetables in general especially, in disease prone environment.

KEYWORDS: Anthracnose Disease, *Colletotrichum lindemuthianum*, Calabar Municipality, Leaf Area, Pepo

INTRODUCTION

Telfairia occidentalis Hook f. (Fluted pumpkin), belongs to the family Cucurbitaceae. The leaves are an important food vegetable for many people especially in the mid-western and eastern parts of Nigeria. It is a climber with long coiled tendrils and the stem can be as long as 10 metres (Osagie and Eka, 1998). According to Tindall (1983), approximately 0.5kg of leaves and shoot can be obtained from one plant per harvest and up to 15 harvests can be obtained between 3-4 months.

Apart from the leaves and young shoots being used for soup, the seeds are also cooked and eaten by humans and can as well be pounded after cooking and used as soup thickener in some parts of West Africa.

Due to its various uses, this crop is more widely cultivated than any other v egetablein Calabar thus; it is a high income earner for farmers in this part of the State. However, the production of this vegetable is faced with a lot of constraints which include, attack by insects, nematodes and susceptibility to diseases. In Calabar, Cross River State, Colletotrichum anthracnose caused by *Colletotrichum lindemuthianum* is most predominant causing leaf spots which affect growth generally (Udo *et al*., 2008) thereby, reducing market value and nutritive contents. It appears first as a water-soaked spot on the foliage. The spots enlarge, turn brown and shatter. Under severe attack, the entire leaf dies. On fruits, sunken areas appear as black, circular spots that do not penetrate deep but provide entry points to other fungi and bacteria. The fungus survives in infected debris (Yamaguchi, 1983).

According to Nigam *et. al*., (1989), quantitative assessments are necessary to judge the relative importance of diseases so that attention can be directed to the most harmful ones and the various methods needed for control. On this basis, an assessment of yield loss was carried out on the following parameters; leaf count, leaf area, stem length, fruit number, fruit size and seed number.

The objective of this study therefore was to investigate the changes in the yield parameters of *T. occidentalis* infected by the fungus *Colletotrichum lindemuthianum*. This will give a possible index for assessment of economic loss for this crop and thus, provide justification for control measures.

MATERIALS AND METHODS

Seed Collection, Soil Preparation and Seed Planting: Seeds for the experiments were extracted from healthy pods of fluted pumpkin harvested from the Teaching and Research Farm, Department of Agronomy, Cross River University of Technology, Calabar, Nigeria. In the experiment for leaf count, vine length and leaf area assessments, *Telfaira occidentalis* seeds were grown in steam pasteurized (160°C for 3hrs) top soil to eliminate soil-borne pathogens. The soil was put in 50 black polyethylene bags and arranged in two (2) rows of 25 bags each for control an treatment respectively in a screen house. Two (2) seeds were planted per bag at a depth of 2cm. Fruit number, fruit size and seed number assessments were carried out in the field.

Plant Infection: Artificial infection of the test plants was carried out 1-month after seeds germination. Leaves of fluted pumpkin infected by *C. lindemuthianum* were collected from farms located in Akim, Calabar Municipality of Cross River State, Nigeria in 2007 and 2008 cropping seasons. Pathogenicity was ascertained through Koch's postulate. Inoculation was done by spore infiltration with *C. lindemutianum* spore suspension (Mao and Newman, 1998). 25ml of sterile distilled water was poured on a 7-day old culture growing on potato dextrose agar (PDA) in culture plates. The spores were carefully brushed off the sporophores with a camel hair brush. The water and spore mixture was decanted into centrifuge tubes and centrifuged at 4,000 x g for 5 min. The supernatant was poured out and, 3 ml of sterile distilled water added. Spore concentration was then estimated for every 1ml of spore mixture with a haemocytometer (Neubauer-improved 0630010 model) under a binocular microscope.

With a sterile hypodermic syringe equipped with a 20 gauge needle, 2ml of spore suspension containing approximately 5×10^5 spores were inoculated on the abaxial surface of wet and healthy host leaves by spraying to run-off level. The leaves were covered with transparent polyethylene bags and allowed to stay for 24 hours. Plants responses were recorded daily for 2 weeks from 3 days post inoculation (Heist *et al*., 2001). Control plants were sprayed with sterile distilled water only.

Experimental Design: The experiment was laid in a completely randomized design (CRD) and data analysis was carried out with a one-way analysis of variance (ANOVA). Means comparison was carried out with least significance difference (LSD) test.

Yield Loss: For assessment of yield loss, stands showing signs of infection (symptoms) were considered to be diseased. Inoculation of the test plants was done 3 weeks after germination (3 WAG) and data were taken 1 month post inoculation (1 MPI) to tally with farmers' conventional time of harvest for the market. Assessment indicators for the experiment were disease incidence and severity. One hundred (100) plants were sampled for every experiment.

The Critical Point Model (CPM) by Joshi (1980) was used for the assessment of losses in the two experimental years. The formula is as below:

$Y = 35.33 + 27.17\log x$

Where; *Y* is the % yield loss and *x* is the severity of the disease.

For leaf count, leaf weight and leaf area assessments, 0.5m-long stems (diseased and healthy) of the test plant were cut slantingly with a sterilized kitchen knife and analyzed destructively at intervals of 1 week after symptoms development. Samples were taken for four (4) weeks. Data on fruit number, fruit size and seed number were taken from the field experiment in November of the study years.

Incidence of Disease: Disease incidence (I) was recorded as the proportion of diseased stands to total number of plants in the study plot. The percentage incidence was calculated using the formula below;

$$\% \text{ incidence} = \frac{\text{No. of stands infected}}{\text{Total stands sampled}} \times \frac{100}{1}$$

Disease Severity: Disease severity (S) was recorded as the sum of the proportion of infected leaves (within a point scale) per stand divided by the total number of stands sampled including those with zero incidence rates (Groth *et. al*., 1999). The disease score was measured on a 1-to-4 point scale (1 = no disease, 2 = few spots on lower leaf, 3 = spots present on lower and upper leaf surfaces and 4 = plants severely affected with necrotic patches) rating scale. The formula below was used:

$$\frac{(X_1x1) + (X_2x2) + (X_3x3) + (X_4x4)}{(X_1 + X_2 + X_3 + X_4)}$$

Where X is the stand severity multiplied by the different rating class scales (1, 2. …..n).

Leaf Count: Leaf count was taken at 1-month post inoculation (1mpi) by taking a macroscopic count of the deeply divided (digitate) leaves from 0.5m-long diseased and healthy samples for comparison. Four (4) samples were taken at weekly intervals.

Leaf Area: Non-destructive estimate was carried out here by ascertaining a correlation between the leaf area and the linear dimensions of the leaf width and length (Ting, 1982). In this method, the leaf of assay plant was placed on a $1cm^2$ graph paper. The leaf size was traced on the paper and the total area calculated based on the number of squares covered within the traced region. The formula is as below:-

$$A = KLB$$

Where A=Leaf Area, B=Leaf Width, L= Leaf Length and K=Correlation coefficient (constant).

Vine Length

The stem measurement was taken acropetally from the soil surface to the stem tip (apical meristem) for both diseased and healthy samples. A 150cm measuring tape was used for the measurement. Data were taken 1 month post inoculation (1mpi).

Seed Count

At harvest, the fruits from the various stands were incised along two (2) furrows with a sterile kitchen knife. The seeds were carefully separated from the pulp, washed and selected to separate the filled seeds from the empty ones. Their numbers were noted.

RESULTS

Results of the research are shown in Tables 1, 2 and 3. Early rainfall was higher in 2007 with a corresponding

increase in disease incidence and severity as against the low rainfall, disease incidence and severity recorded for 2008. This shows a significant difference ($P<0.05$) between disease incidence and severity in the years and a positive correlation between the amount of rainfall, incidence and severity.

There was no significant difference ($P>0.05$) in leaf number produced between the healthy and diseased in the two seasons. For leaf area, a significant difference was obtained between the treatments. The healthy samples had larger leaf area than the diseased ($p<0.05$). The difference in vine length between the healthy and diseased samples in the 2 study years were found to be significant ($P<0.05$) with the diseased having longer vines than healthy samples. The same trend was observed in the 2 study years.

Seed count was higher in the healthy than diseased samples. This was observed in the 2 years of study (Table 2).

Disease Incidence

Table 1 shows the percentage incidence of the anthracnose disease for the study periods. It was recorded that the year 2007, had 68% incidence as against 46% in 2008.

Early rain was above 250mm in 2007 with high disease incidence and below 150mm in 2008 and incidence for this year was low (<50%).

Disease Severity

When a 1-4 points severity index was worked out to test the severity level, the highest disease severity index of 4.7 was obtained in 2007 Table 1. In this year, the leaves that were infected had spots that coalesced within 1 month to produce large dead zones on the leaves' lamina. This is an indication of high severity. For the year 2008, there was a downward trend of 3.4 in the result.

Table 1: Influence of Rainfall on Disease Incidence and Severity for the 2007 and 2008 Study Seasons

Rainfall and Disease Index			
	Rainfall (mm)	**Incidence (%)**	**Severity**
Year			
2007	258	68	4.7
2008	145	46	3.4

Effect on Yield Parameters

Table 2 shows the results of the anthracnose disease on yield parameters (Leaf count, Vine length and Leaf area) of the assay plant for the 2 study years. Results show that there was little or no difference in leaf count between the healthy (14.8) and diseased (14.5) samples for the 2007 cropping season. The same trend was observed in 2008 cropping season. This implies that the disease incidence and severity do not have any effect on the quantity of leaves produced by this leafy green vegetable. However, it was observed that leaves on stems of the infected stems were sparsely distributed compared to the control (healthy). Their mosaic in phylotaxy (pattern of arrangement on stem) was different from that in the uninfected.

Vine length from infected *T. occidentalis* stands, showed increase in length than healthy stands. Comparatively, the healthy plant vines were larger and more succulent than those of the diseased stands. Towards maturity of the plants, the diseased stem was observed to have aged faster than the healthy stems. A corresponding ageing of the leaves along the stem, was also noted.

The leaf area of the diseased leaf sample was recorded as being smaller than that of the healthy sample. Larger leaf area was obtained from 2008 samples than 2007. The leaf surface of the healthy sample was more glabrous than that from the diseased. It was also observed that the leaf lamina from the infected sample showed some symptoms of cupping-over. The leaves from the infected plants also showed some signs of flaking. Depending on these disease symptoms, the infected leaves are always selected against by farmers during harvesting and in the market place by buyers.

Table 2: Effect of Anthracnose on Yield Parameters of *T. occidentalis*

Plot	Leaf Count		Vine Length (cm)		Leaf Area ($cm^{2)}$	
	2007	2008	2007	2008	2007	2008
1	12 11	11 11	116 123	148 145	58.51 56.80	63.54 81.32
2	14 14	13 14	95 131	124 160	69.08 73.43	69.06 72.70
3	15 16	16 17	120 180	150 121	68.13 78.61	67.51 65.86
4	17 18	17 17	119 102	161 152	71.12 69.87	70.44 73.08
Mean	**14.5 14.8**	**14.3 14.8**	**112.5 109.0**	**145.8 144.5**	**66.69 69.93**	**67.64 73.24**

Di = Diseased (infected) plant
Hi = Healthy plant

Seed Count: The number of seeds in the 2 treatments was different for both filled and empty seeds. More seeds were extracted from the pod harvested from the healthy *T. occidentalis* stand than the infected stand. When the empty seeds were separated from the filled seeds and their count taken, more empty seeds were as well, recorded for the infected test plant (Table 3).

Table 3: Result of Seed Count for *T. occidentalis* (Diseased and Healthy) Infected with Anthracnose Disease of *C. lindemuthianum* for the Two Study Years

Plot					Experimental Year/ Yield			
2007					2008			
	FS		ES		FS		ES	
	Di	Hi	Di	Hi	Di	Hi	Di	Hi
1.	8	14	13	6	15	23	7	2
2.	13	21	7	9	20	29	11	5
3.	16	18	2	3	9	14	9	2
4.	11	27	9	1	12	28	5	4
Mean	**12**	**20**	**7.8**	**4.8**	**14**	**23.5**	**8**	**3.3**

FS = Filled seed
ES = Empty seed
Di = Diseased (infected) plant
Hi = Healthy plant

Yield Loss: Analysis of overall yield loss using the Critical Point Model **(Y)** showed that there were high yield losses in the infected test plant for the two (2) farming seasons. However, the percentage yield loss in 2007 was higher (77.44%) than that in 2008 (58.48%).

DISCUSSIONS

According to Nigam *et. al.,* (1989), infection on leaves of Opium poppy infected by *Peronospora arborescens* increased with age of leaves and amount of rainfall. Their results showed that the diseased plants were smaller in size and bore smaller capsules. Their seeds were lighter and less in number as compared to healthy plants. They concluded that the yield in terms of raw opium was also reduced. This is evident in the reduction in seed yield of the infected plants in this research. This may be due to the fact that during infection, photosynthates that are meant for the sinks are misdirected to the diseased portions hence, the reduction in yield of the plant and excessive respiration in the diseased areas (Bedbrook and Matthews, 1973). According to Ting, (1982), a reduction in the relative growth rate (RGR) of a particular

plant, leads to a concordant reduction in the yield of the plant so affected thus, the yield of cucurbits infected by *C. lindemuthiianum* is bound to reduce.

According to Madunagu *et. al*. (2008), there is correlation between amount of rainfall and rate of incidence of this disease. The reason here may be that rainfall incites incidence through the dispersal of spores from the soil debris to the abaxial surfaces of leaves through rain splash impaction because, an inoculum can only be infectious if it is viable and brought in contact with the host by an agent under favorable environmental conditions like ambient humidity (Tu, 1981).

Increase in the severity must have been due to the high amount of rainfall for that year. This was suspected to have led to increased evapo-transpiration resulting to frequent and excessive opening of the host stomata which are the points of ingress for the pathogen (Merhotra and Aggarwal, 2006, Udo *et. al*., 2008).

According to Madunagu *et. al*., (2008) during severe infection by this pathogen, there is reduction in photosynthetic efficiency leading to reduction in growth parameters of the host plant. This implies that during infection, as the photosynthetic efficiency reduces respiration is increased.

These physiological responses therefore lead to a general reduction in yield. This agrees with the works of Sharma and Sharma, (1990) on excessive respiration and yield reduction in hypertrophied peach leaves infected by *Taphrina deformans.*

In conclusion, this quantitative assessment has shown the relative importance of the disease so that attention can be drawn to the harmful effects of this disease. Also, control measures can be sorted out against the disease or the effect of environment on it.

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