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CORM YIELD RESPONSE TO DIFFERENT ISOLATES OF BACTERIAL LEAF BLIGHT PATHOGEN (*Xanthomonas axonopodis* pv. dieffenbachia (McCulloch & Pirone) Vauterin) OF COCOYAM (*Colocasia esculenta* (L) Schott)

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ABSTRACT

A study was conducted in 2019 to evaluate cocoyam yield response to four xanthomonad isolates (CE2, CE3, CE4 and CE6) causing bacterial leaf blight in Zaria, Nigeria. Five treatments consisting of corms inoculated with the four bacterial isolates and untreated plants were replicated five times and arranged in a completely randomized design (CRD) in the screen house. Symptoms were observed two weeks after germination (WAG) and records on disease severity were taken 3 WAG and thereafter at weekly interval until 18 WAG. Eight months after planting, corms were harvested, weighed and corm diameter measured. Yield loss was calculated, correlation and regression analyses were done. Records on disease severity showed the highest severity, ranging between 12.8 % and 38 % were recorded on plants inoculated with isolate CE2, followed by CE6 (7.0 % - 29.0 %) and CE4 (6.6 % - 28.6 %). The least severity was observed on plants inoculated with CE3 (6.8 % - 24.8 %). Control plants did not show symptoms of the disease. Corm diameter and yield among the isolates were highest in CE3 (8.4 cm and 48460 kg/ha) and the least in CE2 (5.0 cm and 40120 kg/ha). There were negative and highly significant correlations between disease severity and the yield of corms ($r = -0.87$; $P<0.01$). Regression analysis showed adjusted coefficient of determination (R^2) as 0.760 (76%) with a unit increase in disease severity resulting in a decrease of 257.8 kg/ha in corm yield. In conclusion, bacterial leaf blight significantly reduced the yield of cocoyam, as higher disease severity resulted in lower yield.

Keywords: Bacterial leaf blight; severity; corm yield; screen house; cocoyam

INTRODUCTION

Cocoyam refers to two members of the Araceae family, *Colocasia esculenta* and *Xanthosoma sagittifolium*, commonly grown as staple foods in developing countries of Africa, Asia and the Pacific (Talwana *et al*., 2009). It ranks third in economic importance after cassava and yam among the root and tuber crops cultivated and consumed in Nigeria (Baruwa and Oke, 2012). It is however superior to yam and cassava nutritionally, with higher protein, mineral and vitamin contents and more readily digestible starch (Odebunmi *et al*., 2007). *Colocasia* sp. is grown for its corm and cormels which are consumed after boiling, frying or roasting. The corms can be dried and used to make flour or sliced and fried to make chips. The leaves of the plant are also edible and are usually consumed as a vegetable after cooking in dishes such as stews. *Xanthosoma* sp. produces tubers much like potato and are boiled, baked, steamed or fried prior to consumption. Young leaves are eaten as a vegetable (Azeez and Madukwe, 2010).

Globally, over 10 million tons of cocoyam is produced annually with Africa accounting for at least 60% of the total world production, while most of the remaining 40% comes from Asia and Pacific regions (FAOSTAT, 2017). Nigeria is the world's largest producer of cocoyam, with annual production of 3.18 million metric tons, representing about 26 percent of total world output of cocoyam (FAOSTAT, 2017).

The yield and quality of this crop are threatened by various biotic factors. Diseases caused by fungi and bacteria are among biotic factors militating against the production of cocoyam in Nigeria. The diseases include phytophthora leaf blight caused by *Phytophthora colocasiae* (Bandyopadhyay *et al*., 2011; Zarafi *et al*., 2012), corm rot induced by some species of fungi such as *Aspergillus niger*, *Fusarium solani*, *Fusarium oxysporum* and *Sclerotium rolfsii* (Ugwuoke *et al*., 2008), corm soft rot induced by *Pectobacterium* spp. (Amodu and Akpa, 2012) and the bacterial leaf blight (BLB) of cocoyam induced by *Xanthomonas axonopodis* pv. dieffenbachiae (Opara *et al*., 2013; Abdullahi, 2016).

Bacterial leaf blight was reported as one of the diseases causing devastating yield loss of cocoyam in Northwest Nigeria and the causal agent was identified as *X. axonopodis* pv. dieffenbachiae (Abdullahi, 2016). Crop diseases generally have been reported to cause yield losses and the assessment of these losses is needed for the improvement of production systems that contribute to the incomes of rural families and food security worldwide. However, records of yield loss due to bacterial leaf blight were based mainly on the reports obtained from the cocoyam farmers. There is little or no information on the yield loss assessment of *X. axonopodis* pv. dieffenbachiae infection on cocoyam prior to this study. There is therefore the need to assess the yield loss due to bacterial leaf blight in this region. This is important to enable farmers and researchers take corrective actions to minimize losses due to the pathogen in Zaria.

MATERIALS AND METHODS

Study Area

This study was conducted in the screen house of the Department of Crop Protection, Ahmadu Bello University, Samaru, Zaria in 2019. Samaru is located on latitude 11˚11'19.3"N and longitude 7˚37'02"E, with an altitude of 686 m above sea level in the Northern Guinea Savanna ecology of Nigeria. The mean annual rainfall of the study area is 986.5 mm, concentrated between May and October with a peak in August. The mean daily air temperature of the area is 24˚C (IAR, 2018).

Preparation of Inoculum and Inoculation of Pathogen

Cultures of four bacterial isolates CE2, CE3, CE4 and CE6 with GenBank (www.ncbi.nlm.nih.gov) accession numbers KT758338, KT758339, KT758340 and KT758341 respectively, were obtained from Bacteriology laboratory, Department of Crop Protection, Ahmadu Bello University, Zaria. They were revived on nutrient agar (NA) medium and adjusted to 10^8 CFU/ml. Two millilitres of each of the isolates were taken with a hypodermal syringe and infiltrated into Cocoyam corms (*Colocasia esculenta*) and left at room temperature (e.g. 25 - 27°C) on the laboratory bench for one week.

Planting of Inoculated Corms

Inoculated cocoyam corms were planted into 30 cm diameter pots filled with heat sterilized soil at the rate of one corm per pot on the 2nd of February, 2019. Pots containing uninoculated corms served as control. Each treatment (CE2, CE3, CE4, CE6 and Control) were replicated five times and pots were arranged on the screen house slab in a completely randomized design (CRD).

Assessment of Severity of Bacterial Leaf Blight of Cocoyam

Symptoms appearance was observed two weeks after germination (WAG) and records were taken at 3 WAG and thereafter at weekly interval for 16 weeks. Number of lesions developed was recorded and the disease severity assessed on 10 leaves selected at random and tagged from each pot by using disease scale of 0-5 below (Abdullahi *et al*., 2019):
0 = no disease symptoms visible on the leaves;
1= less than 10 % of leaves affected;
2 = 10-30 % of leaves affected;
3 = 31-50 % of leaves affected;
4 = 51-70 % of leaves affected;
5 = above 70 % of leaves affected.

Disease severity (DS) was evaluated using the formula below:

$$DS = \frac{\text{Sum of individual disease scores}}{Total\ number\ of\ leaves\ assessed\ x\ maximum\ score} x\ 100$$

Assessment of Yield Loss

Corms were harvested at eight months after planting and soils attached to the corms were removed. The corms were weighed using a weighing balance and corm diameter measured using a vernier caliper. Yield loss was assessed using the following formula (Mousanejad *et al*., 2010):

$$Yield\ loss\ (\%) = \frac{Yield\ in\ control\ pots - yield\ in\ treated\ pots}{Yield\ in\ control\ pots} x\ 100$$

Data on yield were correlated with that of severity at 18 weeks after germination and a regression equation on the effect of severity on yield was drawn.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) with the aid of statistical analysis software (SAS), version 9.0 (SAS, 2002). Where significant differences ($p<0.05$) exist, means were separated using least significance difference (LSD).

RESULTS

Severity of Bacterial Leaf Blight

Symptoms of the disease on the inoculated plants with all the isolates were observed two weeks after germination (WAG) on cocoyam leaves. There were no symptoms on the stem and the petioles. There were no seedling deaths on the inoculated plants. The symptoms were characteristic of bacterial leaf blight, consisting of small, water-soaked lesions, surrounded by prominent chlorotic halo (Plate I). These symptoms began from the underside of the leaves, extended to the upper leaf surface and increased progressively. Pronounced water-soaking continued to develop on the abaxial leaf surface. Leaf spots were delimited by veins. Some lesions coalesced, resulting in large dead areas. Table 1 show the severity of bacterial leaf blight on cocoyam for each of the four isolates (CE2, CE3, CE4 and CE6) recorded at 3, 6, 9, 12, 15 and 18 WAG. At 3 WAG, there were no significant differences ($P>0.05$) in the disease severity among inoculated cocoyam plants, but their disease severity was significantly higher than the untreated plants, which had no symptoms of the disease (Plate II). At 6 WAG to 18 WAG, plants inoculated with CE2 had significantly higher ($P<0.05$) severity of the disease than those of other isolates. These were closely followed by those inoculated with CE4, which were statistically at par with plants inoculated with CE6. CE3 inoculated plants had the least disease severity but was significantly higher than the control ($P<0.05$), which showed no symptoms.

Corm Yield Response of Cocoyam Inoculated with Different Bacterial Isolates

The differences among bacterial isolates with regards to cocoyam corm diameter and corm weight were highly significant ($P<0.01$) (Table 2). Cocoyam corms inoculated with CE3 isolate had the highest corm diameter (8.4 cm). This was was significantly higher ($P<0.05$) than those inoculated with CE4 and CE6, but lower than the untreated corms. The lowest corm diameter was recorded with corms inoculated with CE2 isolate. Similarly, records of corm weight per stand showed that the highest (0.458 kg) corm weight was obtained from the untreated plants, which was significantly higher ($P<0.05$) than corm weights obtained from all the treated plants. The corm weight recorded from CE3 treated plants was significantly higher ($P<0.05$) than those of CE4 and CE6 which were statistically similar ($P>0.05$). The least corm weight was recorded from plants inoculated with CE2.

Plate I: Inoculated cocoyam plants showing symptoms of bacterial leaf blight

Plate II: Healthy cocoyam plants (Control)

Table 1: Severity of bacterial leaf blight of cocoyam weeks after germination

Isolate	3WAG	6WAG	9WAG	12WAG	15WAG	18WAG
CE2	6.8^{a}	12.8^{a}	22.8^{a}	26.8^{a}	30.8^{a}	38.0^{a}
CE3	6.8^{a}	8.8^{c}	14.8^{c}	16.8^{c}	20.8^{c}	24.8^{c}
CE4	6.6^{a}	10.6^{b}	16.6^{b}	20.6^{b}	24.6^{b}	28.6^{b}
CE6	7.0^{a}	11.0^{b}	17.0^{b}	21.0^{b}	25.0^{b}	29.0^{b}
Control	0.0^{b}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}
LSD	1.1	1.2	1.2	1.4	1.5	1.3

WAG = Weeks after germination; CE = *Colocasia esculenta* bacterial isolate; Means followed by the same superscripts along a column are not significantly different (P>0.05) using least significant difference (LSD).

Table 2: Corm yield parameters of cocoyam inoculated with different bacterial isolates

Bacterial Isolate	Average Corm Diameter (cm)	Corm Weight (kg/per stand)
CE2	5.0^{d}	0.361^{d}
CE3	8.4^{b}	0.436^{b}
CE4	6.3^{c}	0.401^{c}
CE6	6.3^{c}	0.398^{c}
Control	10.6^{a}	0.458^{a}
LSD	0.8	0.010

CE = *Colocasia esculenta* bacterial isolate; Means followed by the same superscripts along a column are not significantly different (P>0.05) using least significant difference (LSD).

The yield (kg/ha) of corms obtained from treated plants and the control also varied significantly (P<0.05) with the highest yield (50880 kg/ha) recorded from the untreated plants (Table 3). This was followed by CE3 treated plants whose yield was significantly higher than those of CE4 and CE6, both of which were the same statistically (P>0.05). CE2 treated plants had the least yield. Bacterial isolates significantly (P<0.05) reduced the yield of cocoyam corms (Table 4). The highest yield reduction (21.1 %) was recorded from plants inoculated with CE2 isolate. This was significantly higher (P<0.05) than yield reductions recorded from plants inoculated with other isolates. Reduction in yield among CE4 and CE6 isolates were similar (P>0.05), but higher than lowest yield reduction recorded from CE3 treated plants.

Table 4 showed the correlation coefficients between disease severity and yield parameters. The correlations between Disease Severity and Corm Diameter (r = -0.79; P<0.01), disease severity and Corm Weight (r = -0.87; P<0.01) and Disease Severity and Corm Yield (r = -0.87; P<0.01) were negative and highly significant.

Table 3: Corm yield and percentage yield reduction of cocoyam inoculated with different bacterial isolates

Bacterial Isolate	Corm Yield (kg/ha)	Yield reduction (%)
CE2	40120^{d}	21.1^{a}
CE3	48460^{b}	4.7^{c}
CE4	44540^{c}	12.4^{b}
CE6	44200^{c}	13.1^{b}
Control	50880^{a}	0.0^{d}
LSD	1109	1.9

CE = *Colocasia esculenta* bacterial isolate; Means followed by the same superscripts along a column are not significantly different (P>0.05) using least significant difference (LSD)

Table 4: Correlation coefficients of disease severity and yield parameters

	Disease Severity (%)	Corm Diameter (cm)	Corm Weight (kg/plant)	Corm Yield (kg/ha)
Disease Severity (%)	1.00			
Corm Diameter (cm)	-0.79**	1.00		
Corm Weight (kg/plant)	-0.87**	0.63**	1.00	
Corm Yield (kg/ha)	-0.87**	0.63**	1.00	1.00

**Significant at 1 %.

The effect of disease severity on corm yield of cocoyam is presented on Table 5. Corm yield had a linear and negative significant relationship with disease severity, adjusted coefficient of determination was R^2= 0.735 which revealed that the variation in disease severity explained about 75 % of the variation of corm yield and this rate is represented by the nearest dots to the linear line (Figure 1). The linear regression equation was formed as follows:

$$CY = 51849 - 257.8DS$$

Where CY represents corm yield, DS stands for disease severity, 51849 is the intercept and -257.8 is the regression coefficient, indicating that increase of one unit (%) in disease severity will reduce corm yield in the rate 257.8 kg per hectare.

Table 5: Regression analysis between Cocoyam corm yield and severity of bacterial leaf blight

Source	df	Sum of squares	Mean squares	F	Significance F	R	R^2	Adjusted R^2
Regression	1	252665891.6	252665891.6	64.78	5.33547E-08	0.864	0.746	0.760
Residual	22	85803691.74	3900167.806					
Total	23	338469583.3						

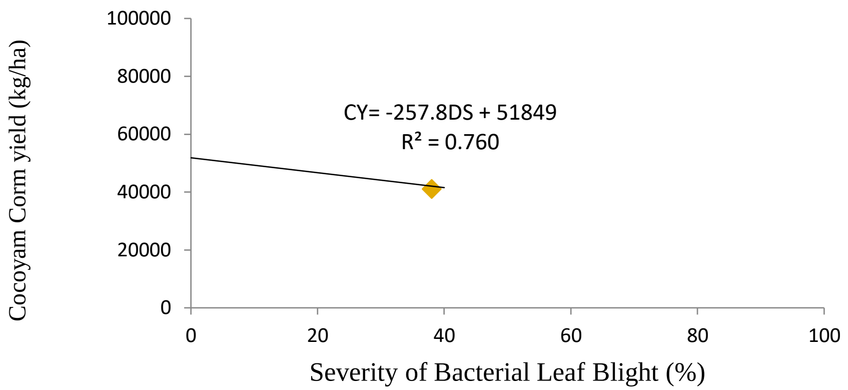

Figure 1: Effect of disease severity on cocoyam corm yield

DISCUSSION

Bacterial leaf blight is an important disease of cocoyam in Nigeria, particularly in the Northwest. A survey of cocoyam fields in 2016 led to the first report of the disease in this region, where high incidence and severity were reported and the causal organism (*Xanthomonas axonopodis* pv. dieffenbachiae) identified and characterized (Abdullahi, 2016). Significant difference in the severity of the disease following inoculation of the four xanthomonad isolates showed pathogenic variability among the isolates. The highest severity on plants inoculated with isolate CE2 indicated that the isolate was the most virulent This isolate was reported to cause severe disease problems on cocoyam in Tudunsarki area of Kaduna state (Abdullahi, 2016). Pathogenic variability among bacterial isolates was reported to cause difference in disease severity of bacterial spot of tomato (AL-Saleh *et al*., 2011).

The disease severity recorded among the four isolates used in this study differs from those recorded with the same isolates in 2016. Abdullahi (2016) reported 48.0 %, 28.0 %, 32.7 % and 35.3 % disease severity for CE2, CE3, CE4 and CE6 isolates respectively, as compared to 38.0 %, 24.8 %, 28.6 % and 29.0 % reported in this study, respectively. This difference in severity means decrease in pathogenicity of the bacterial isolates. Factors such as fluctuation in storage temperature and repeated subculturing have been reported to affect virulence and pathogenicity of bacteria (Molina-Torres *et al*., 2010). Repeated subculturing or long-term storage can cause variations in the genetic profiles of bacterial strains (Iguchi *et al*., 2002).

For effective disease management strategies, there is the need to evaluate the impact of disease severity on crop yield. These investigations have demonstrated a significant relationship between bacterial leaf blight severity and cocoyam corm yield. This association was evident for not only corm yield but also for corm diameter. High disease severity resulted in more yield loss by different bacterial isolates used in this study. As more leaf area is covered with symptoms, photosynthesis is reduced not only through a reduction in green leaf area, but also through an effect on the photosynthesis of green leaf tissue surrounding the lesions (Bastiaans, 1993). This would further result in the reduction of the photosynthetic products to the roots, which contribute to cocoyam corm production. Consequently, smaller and fewer corms would be produced under severe disease problems.

The analysis of crop losses versus disease severity by regression offers an opportunity to view variety tolerance to disease. Tolerance to disease can be quantitatively measured by the coefficient of regression of the crop loss to disease severity equations (Whelan, 1992) This information will be helpful in developing management strategies for bacterial leaf blight and for developing research priorities for control of cocoyam diseases in order to minimize losses.

CONCLUSION

The study revealed pathogenic variability among the bacterial isolates tested, resulting in significant differences in disease severity and corm yield. Bacterial leaf blight severity had effect on the yield of cocoyam, as high severity resulted in significant reduction in the yield.

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