BEGOMOVIRUSES INFECTING CASSAVA AND WHITEFLY (*Bemisia tabaci* Gennadius) POPULATION IN TARABA STATE, NIGERIA

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SUMMARY

Begomoviruses infecting cassava are transmitted by whitefly and by the use of infected cuttings. They constitute major biotic constraints to cassava productivity in Nigeria. This study reports the occurrence and distribution of African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) infecting cassava in Taraba State, Nigeria during 2015 growing season. Twenty-seven (27) cassava fields were sampled at interval of 10 km from five Local Government Areas (LGAs) of the State. Eighty-one leaf samples from symptomatic and asymptomatic cassava plants were collected and tested for ACMV and EACMV using polymerase chain reaction (PCR). The laboratory analysis revealed the incidence of ACMV and EACMV in the State with significant (p = 0.05) variation in distribution. Gassol had the higher incidence of both ACMV and EACMV (41.7 %) and (16.7 %) respectively followed by Wukari (ACMV 33.3 %) and Ardo-kola (EACMV 14.29 %) while in Zing both ACMV and EACMV were not detected and EACMV was not detected in Wukari. Cassava mosaic disease (CMD) incidence was higher in Wukari (93.3 %) followed by Gassol (47.0 %) while Zing had CMD incidence of 0 %. Symptom severity was statistically mild but Wukari recorded highest mean (2.7) followed by Gassol (2.04) and lowest in Zing (1.0). Adult whitefly populations were generally low, the highest mean (2.0) was recorded in Gassol and Zing and lowest in Wukari (0.0). The CMD source of infection was mainly by the use of infected cuttings than by the whitefly vector transmission. These findings revealed that whitefly, the established insect vector, is not responsible for the spread of CMD in Taraba State but rather by the use of infected cassava cuttings by farmers. The findings of this study also revealed the importance of using virus-free cassava cuttings as planting materials to mitigate the spread of cassava begomoviruses in the State and country at large.

Keywords: Occurrence, Taraba, begomoviruses, vector, incidence, severity

CASSAVA (*Manihot esculenta* Crantz), family Euphorbiaceae, is a perennial woody shrub with an edible root, it was first cultivated in South America and introduced to Nigeria in the sixteenth century (*Adeniji et al*., 2005). In Nigeria, cassava is virtually cultivated in all the states and is also an important root and tuber crop. There has been an overall increase in land area for cassava cultivation and production in the key producing states as a result of the good prices for the crop. There was an increase of 6.03% in production to 58,472.34 metric ton (MT) in 2018 as against 55,147.06 MT in 2017. Land area in 2018 has risen to 9,952,790 Ha compared to 9,194,940 Ha in 2017, indicating an increase of 8.24%. Generally, there was a decrease in national average in 2018 from 6.00 ton/ha in 2017 to 5.87 tons/ha (NAERLS, 2018).

Nigeria currently holds the record as the largest producer of cassava in the world, but the trend in yield performance (production per hectare) remains low. The average cassava yield in Nigeria is 8.76 t ha^{-1}, significantly lower than the global average yield of 11.1 t ha^{-1}, and much lower than the success stories recorded in India (34.2 t ha^{-1}) and Laos (32.1 t ha^{-1}) (FAOSTAT, 2019). This low yield may be linked to begomoviruses infecting cassava in Nigeria. Nine begomoviruses associated with cassava mosaic disease (CMD) have been identified and they include *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Kenya virus*

(EACMKV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Zanzibar virus* (EACMZV), *South African cassava mosaic virus* (SACMV), *African cassava mosaic Burkina Faso virus* (ACMBFV), and *Cassava mosaic Madagascar virus* (CMMGV) (Patil and Fauquet, 2009). CMD is caused by viruses belonging to the genus *Begomovirus* in the family *Geminiviridae* (Hong *et al*., 1993). It is the single most important viral disease of cassava in Nigeria. The symptoms characteristic of CMD are well documented. Among the noticeable symptoms usually present in the field is a mosaic pattern on the leaves, the coloring of which can range from pale green to whitish yellow. The extent of chlorosis on the leaf surface varies between <5% to almost 100% (Chikoti *et al*., 2019). Another common feature observed in cassava fields is the extreme narrowing of the leaf near the base of the leaflets. However, symptoms can vary by both season and cultivar (Chikoti *et al*., 2019). Yield losses caused by CMD was estimated between 50% and 70%. In a more recent report (Tembo, 2016), data collected from field experiments indicated even higher yield losses. Clearly, the consequences of CMD are devastating and the disease is one of the major constraints to maintaining sufficient crop yields in Nigeria. Thresh (1997) estimated yield losses in Africa to be 15–28 million tonnes representing 15% to 24% of total cassava production. The estimated annual economic losses in East and Central Africa are between $1.9 and $2.7 billion USD (Patil and Fauquet, 2009).

Cassava is cultivated in all the 19 States of northern Nigeria as well as Federal Capital Territory (FCT) Abuja and it plays an important role in food security of the region. Taraba and Benue States are the leading cassava producing States of the region (Northern Nigeria). Management of plant viruses infecting root and tuber crops requires accurate scientific data on virus strains present in an area, their geographical distribution, or plant genotypes to which they are most susceptible. To this end, a cassava mosaic disease field survey was conducted in cassava producing areas of Taraba State, Nigeria (during 2015 growing season) with the aim of assessing cassava mosaic disease incidence, symptom severity, whitefly population and begomoviruses infecting cassava in the study area. The findings of this research would be of paramount important to cassava farmers, plant virologists, plant breeders, other researchers and policy makers.

MATERIALS AND METHODS

Study Area

The research was conducted in Taraba State (Northeastern Nigeria). The State lies between 60 30’ and 80 30’^{o} north of the equator and between longitude 90 00^{o} and 120 00^{o} east of the Greenwich Meridian. The state shares boundaries with Bauchi and Gombe States in the north, Adamawa State in the east, Plateau State in the north-west, Benue and Nasarawa States in the west, and shared international boundary with the Republic of Cameroon in the south. Taraba state has a tropical wet and dry climate (Adebayo and Orunoye, 2013). Dry season lasts for a minimum of five months (November to March) while the wet season spans from April to October. Mean annual rainfall ranges from 800 mm in the northern part of the state to over 2000 mm in the southern part. Generally, mean annual rainfall is less than 1000 mm in the places above latitude 9° comprising Lau and Karim Lamido areas (Adebayo and Orunoye, 2013). Agriculture is the main occupation of many of the inhabitants of the State. The ecological condition of the state permits cultivation of root crops, cereals and rearing of livestock in large numbers. Tree crops (tea, pears, coffee, and cocoa) are also grown especially on the Mambilla plateau (Adebayo and Orunoye, 2013).

Cassava fields survey and samples collection

Cassava field surveys were conducted along motorable and major roads in Taraba State (Northeastern Nigeria) during 2015 growing season. Twenty-seven (27) cassava fields were sampled from five local government areas of the State (Zing, Ardo-kola, Bali, Gassol, and Wukari LGAs) (Fig. 3). A West African Virus Epidemiology (WAVE) harmonized field sampling protocol was adopted following a previously described method (Sseruwagi *et al*., 2004). Survey routes followed a road map which allowed sampling of cassava fields in various local government areas (LGAs) of the State. Surveyed cassava fields were a minimum of 10 km apart as described by Ogbe *et al*. (2006). The number of cassava fields between sample locations was recorded as a measure of the relative density of cassava fields, and the cassava varieties planted in each surveyed field were also recorded. Geo-location coordinates of fields were obtained using a GPS device (Garmin Inc., Kansas, USA). Data were collected using field survey data sheet. In each field sampled, a total of 30 cassava plants were randomly assessed along two diagonals in the form of an 'X' with 15 plants chosen randomly along each diagonal (15 x 2 = 30 plants per field) and examined for the presence or absence of cassava mosaic disease (CMD) symptoms. CMD incidence and symptom severity were recorded as described by Sseruwagi *et al*. (2004).

Cassava mosaic disease incidence

Cassava mosaic disease incidence of each field was calculated as the percentage (%) of visually assessed diseased cassava plants over the total plants examined in the two diagonal method using the following formula as suggested by Sseruwagi *et al*. (2004) and the percentage of CMD incidence of each field was used to calculate percentage CMD incidence of each local government using the relationship below.

$$\text{Disease incidence (\%)} = \frac{number\ of\ diseased\ plants}{total\ number\ of\ plants\ examined} \times 100$$

Cassava mosaic disease symptom severity

CMD symptom severity of each cassava plant sampled was scored using a scale of 1 to 5 as described by Sseruwagi *et al*. (2004) where: 1 = asymptomatic plants, 2 = plants with 25% of leaves showing mild chlorotic pattern or mild distortion, 3 = infected plants with 50% exhibiting moderate mosaic pattern, narrowing and distortion at base of the leaves, 4 = infected plants with 75% exhibiting severe mosaic symptom, leaf distortion and general reduction of leaf size, and 5 = infected plants with 100% of plants exhibiting severe mosaic, leaf distortion, reduced leaf size, vein clearing and in most cases stunted growth. At each field, a minimum of one and a maximum of five-leaf samples were collected from asymptomatic and symptomatic cassava plants of varying disease severity. Leaf samples were collected, labelled, and stored in herbarium presses prior to laboratory analysis.

Estimation of Whitefly population

In each field, the thirty (30) cassava plants sampled were also assessed for whitefly population which involved direct counting of adults on five (5) youngest immature apical leaves of the shoots because the adults feed preferentially on the youngest immature leaves. Each leaf was held by the petiole and gently inverted, the adults present on the lower surface were then counted and recorded (Sseruwagi *et al*., 2004).

Sources of Cassava mosaic disease infection

Source of CMD infection were categorized as "C" (cassava stem cutting as source of infection) and "W" (whitefly as source of infection) infections. The possible source of the observed CMD infection in each 30 cassava plants sampled was determined based on the location of the leaf symptoms. Cassava plants that showed symptoms either only on the lower leaves or on all leaves were assumed to have been infected through the use of infected cassava cuttings. Cassava plants that showed symptoms only on their upper leaves but not on any lower leaves were assumed to have been infected by the whitefly vector (Sseruwagi *et al*., 2004).

Virus incidence

Virus incidence (VI in %) was calculated using the formula of Chaube and Pundhir (2005):

$$VI = \frac{Number\ of\ positive\ samples/state}{Total\ number\ of\ samples\ tested/state} \times 100$$

Total nucleic acid extraction

Total nucleic acids (DNA) were extracted from cassava leaf samples collected using the cetyl trimethyl ammonium bromide (CTAB) method described originally by Porebski *et al*. (1997) and later modified by Maruthi *et al*. (2002). The CTAB extraction buffer [2% (w/v) CTAB, 1.4 M NaCl, 0.2% (v/v) 2-mercaptoethanol, 20 Mm EDTA, 100 Mm Tris-HCl, pH 8.0] was preheated to 60^0C for 10 min. Mercaptoethanol was added fresh. 33 mg of dry leaf sample was measured and placed into a thick gauged plastic bag. The tissue was ground using a roller and with 10 volumes (1 ml) of CTAB extraction buffer. 750 µl of the sample was poured into a 1.5 ml Eppendorf tube and the samples were heated at 60^0C for 30 min. The samples were mixed with an equal volume (750 µl) of phenol: chloroform: isoamyl alcohol (25:24:1) and centrifuge at 13000 rpm for 10 min. Repeat No. 5 without phenol for extra purity. The top aqueous phase was transferred into a new Eppendorf tube. The DNA was precipitated by adding 0.6 volumes (600 µl) of cold (-20^0C) isopropanol and incubated at -20^0C for at least 1 hour. The samples were centrifuged at 13000 rpm at 4^0C for 10 min and supernatant was discarded. The pellet was washed in 500 µl of 70% ethanol by vortexing and then centrifuge for 5min. at 13000rpm. The ethanol was removed, and pellet was vacuum dried for 5 min. the dried pellet was suspended in 100 µl of 1x TE buffer and stored at -20^0C. Extractions were diluted 1:10 fold SDW before being used in PCR amplifications.

Molecular test

Molecular test was conducted for the detection of cassava mosaic begomoviruses in the collected cassava leaf samples using polymerase chain reaction (PCR). PCR amplification was done using specific primers JSP001/F ATGTCGAAGCGACCAGGAGAT and JSP002/R TGTTTATTAATTGCCAATACT for ACMV, JSP001 ATGTCGAAGCGACCAGGAGAT and JSP003 CCTTTATTAATTTGTCACTGC for EACMV. The PCR program for JSP001/JSP0012 and JSP001/JSP003 were 94^0C for 2 min. as initial denaturation, 94^0C for 1 min. as denaturation, while annealing was achieved at 45^0C for 1 min. then extension and final extension at 72^0C for 1- and 10-min. respectively, number of cycles was 30 cycles and reaction volume was 25 µl. PCR products were subjected to (1% w/v) agarose gel electrophoresis for analysis according to standard protocol (Green and Sambrook, 2019).

Data Analysis

Data collected on disease incidence, virus incidence and symptom severity of begomoviruses infecting cassava in Taraba State of Nigeria were subjected to analysis of variance. Variation of means were considered significant at 5 % level of probability by plotting standard error of means as described by Gomez and Gomez (1984).

RESULTS

Based on visual assessment of cassava mosaic disease (CMD) symptoms on cassava plants in fields sampled, distinctive leaf mosaic and leaf distortion were the two major symptoms observed on cassava plants (Figs. 1 and 2) with dieback in very few fields sampled. The result from the visual assessment of CMD incidence showed that CMD occurred in four of the five local government areas surveyed but with significant (p = 0.05) variation in distribution. In Wukari LGA, CMD incidence was significantly higher (p = 0.05) (93.3%) followed by Gassol (47%) while Zing LGA recorded the lowest CMD incidence (0%) (Fig. 4) indicating all the cassava fields sampled in Zing LGA were CMD free based on visual assessment of symptoms. CMD symptom severity was generally mild in all the LGAs surveyed. The mean symptom severity was higher in Wukari LGA (2.7) followed by Gassol LGA (2.04) while Zing had lowest mean symptom severity (1.0) (Fig. 5) indicating all the cassava plants sampled were symptomless. Average whitefly population was also generally low in all the LGAs surveyed, Zing and Gassol LGAs recorded the same average (2) whitefly population while Wukari LGA recorded the least average whitefly population (0) (Fig. 6). It was also observed that average whitefly population did not correlate with CMD incidence in all the LGAs surveyed.

A total of 81 cassava leaf samples collected during 2015 field survey were analyzed for the presence of two major begomoviruses, (ACMV) and (EACMV), infecting cassava in Africa using polymerase chain reaction (PCR). The PCR results obtained showed that ACMV and EACMV occurred in the state but with significant (p = 0.05) variation in distribution. ACMV was detected in four LGAs out of five LGAs surveyed while EACMV was detected only in three LGAs out of five LGAs surveyed. In Gassol LGA, ACMV incidence was significantly higher (p = 0.05) having been found in 41.67% (10/24) of all cassava leaf samples collected in 2015 followed by Wukari LGA 33.33% (4/12) while in Zing ACMV was not detected in all cassava leaf samples collected in 2015. EACMV was detected only in three LGAs out of five LGAs surveyed with Gassol LGA having the highest EACMV incidence of 16.67% (4/24) followed by Ardo-kola LGA having 14.29% (3/21) while in Zing and Wukari LGAs EACMV was not detected in all the cassava leaf samples collected. Based on the location of symptoms on sampled plants, the source of CMD infection was mainly by cassava cuttings with Wukari LGA having the highest percent incidence due to use of infected cuttings as planting material (80%) followed by Gassol (73.33%) and Zing recorded the lowest (0%) (Figs
. 7 and 8).

Figure 2: Leaf distortion symptom of Cassava due to Cassava mosaic disease

Figure 2: Mosaic symptoms on leaves Cassava plant due to Cassava mosaic disease

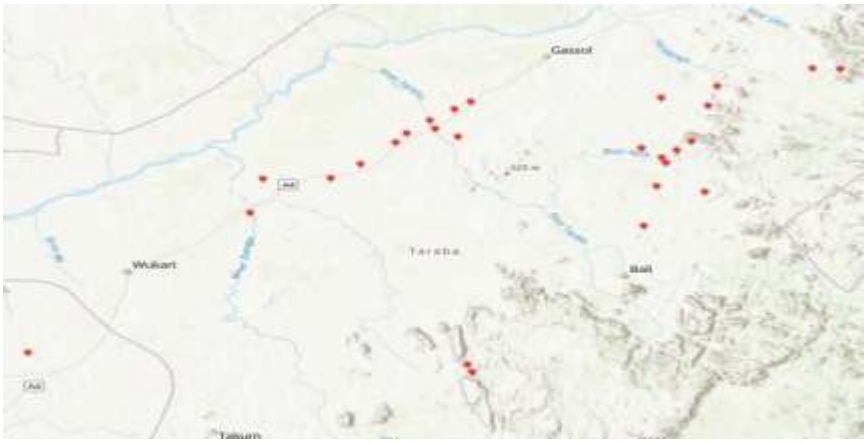

Figure 3: Cassava fields surveyed during 2015 in Taraba State, Nigeria (Red dot)

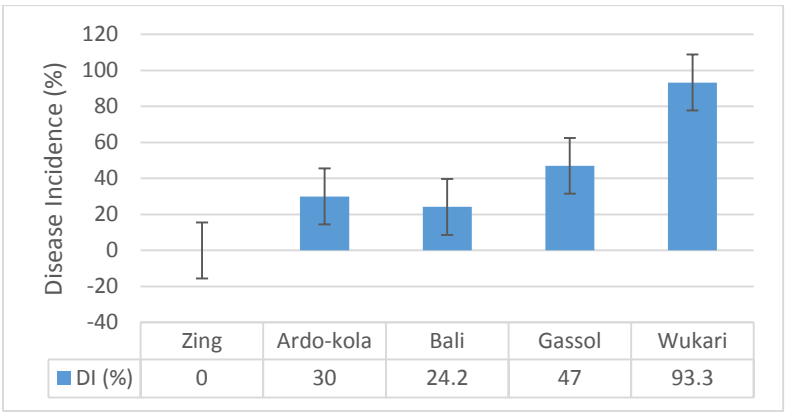

Figure 4: Mean disease incidence of Cassava mosaic disease in five Local Government Areas of Taraba State during the 2015 field survey. Bars indicate standard error of means at 5 % probability level.

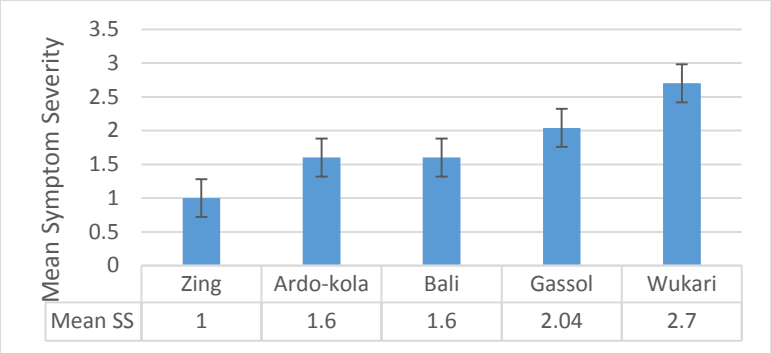

Figure 5: Mean Cassava mosaic disease symptom severity in five Local Government Areas of Taraba State during the 2015 field survey. Bars indicate standard error of means at 5 % probability level.

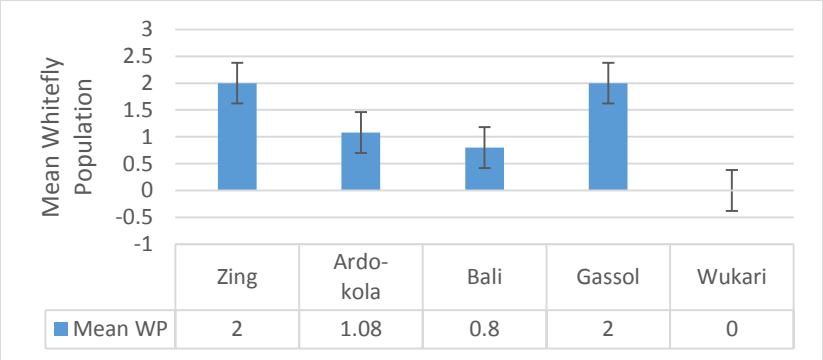

Figure 6: Mean whitefly population in five Local Government Areas of Taraba State during the 2015 field survey. Bars indicate standard error of means at 5 % probability level.

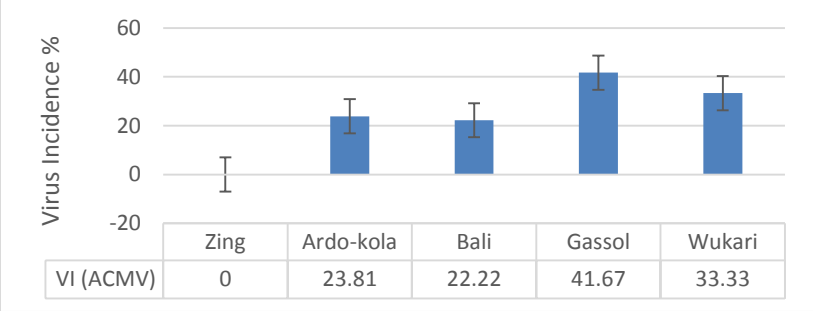

Figure 7: *African cassava mosaic virus* incidence in five Local Government Areas of Taraba State during the 2015 field survey. Bars indicate standard error of means at 5 % probability level.

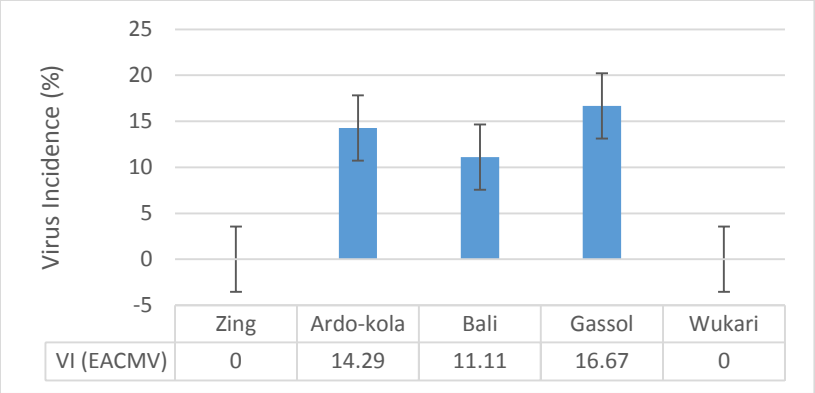

Figure 8: *East African cassava mosaic virus* incidence in five Local Government Areas of Taraba State during the 2015 field survey. Bars indicate standard error of means at 5 % probability level.

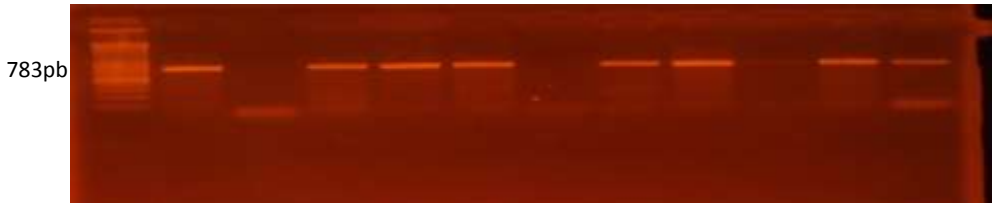

Figure 9: Electrograph of 783pb *Africa cassava mosaic virus* coat protein amplicon amplified in a polymerase chain reaction using JSP1/JSP2 primer pair.

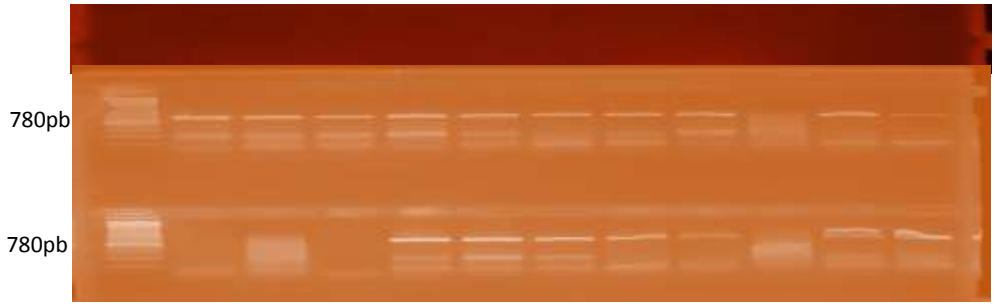

Figure 10: Electrograph of 780pb *East Africa cassava mosaic virus* coat protein amplicon amplified in a polymerase chain reaction using JSP1/JSP3 primer pair.

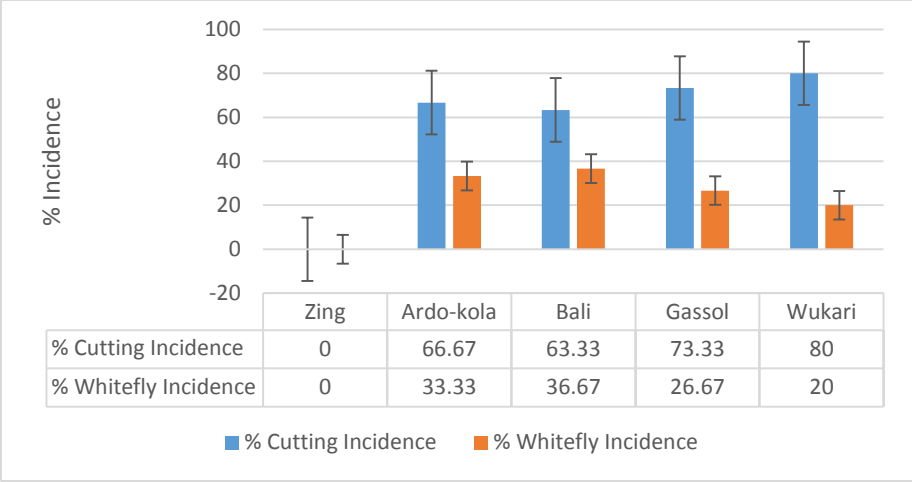

Figure 11: Percentage incidence of Cassava mosaic disease in five Local Government Areas of Taraba State during the 2015 field survey. Bars indicate standard error of means at 5 % probability level.

DISCUSSION

Cassava mosaic disease incidence, symptom severity, whitefly population and two major begomoviruses infecting cassava, ACMV and EACMV, were investigated in this study. Distinctive leaf mosaic, leaf distortion and dieback were the symptoms observed on cassava plants in cassava fields surveyed during 2015 from which ACMV and EACMV detected. Similar symptoms were reported to be incited by ACMV and EACMV in other parts of Nigeria (Eni *et al*., 2020; Badamasi *et al*., 2020). All the asymptomatic cassava leaf samples tested negative against ACMV and EACMV. This is in contrary to the work of (Eni *et al*., 2020) who detected ACMV and EACMV from asymptomatic leaves. This study showed the presence of CMD in cassava fields surveyed during 2015 in four LGAs out of five LGAs surveyed in Taraba State, Nigeria. The higher CMD incidences observed in Wukari LGA (93.3%) and Gassol LGA (47%) may be due to use of different cultivar from the one used by cassava farmers in Zing LGA (0%) that planted (Farin rogo) cultivar. The cultivar 'Farin rogo' so called by cassava farmers in Taraba State appeared to be resistant to CMD because almost all cassava fields surveyed where this cultivar was planted, showed mild symptom or no symptom of CMD. The use of this cultivar should be encouraged among cassava farmers in the state as a management strategy for CMD. Mean symptom severity correlated with CMD incidence in the study area as higher mean symptom severity were observed in Wukari (2.7) and Gassol LGAs (2.04).

Mean whitefly population from this study showed no correlation between whitefly population and CMD incidence as higher mean whitefly population was recorded in Zing and Gassol LGAs (2) while Wukari LGA that had higher CMD incidence had lowest mean whitefly population. Similar results were reported (Toualy *et al*., 2014; Manani *et al*., 2017; Boykin *et al*., 2018; Badamasi *et al*., 2020). This indicate that the source of CMD infection in Wukari LGA was mainly from infected cassava stem cuttings that were used by the farmers as planting materials. The use of infected cassava stem cuttings as planting material should be discouraged among cassava farmers in Taraba State and Nigeria at large.

The PCR results of this study revealed the presence of ACMV and EACMV in Taraba State, Nigeria. ACMV was predominant begomovirus species infecting cassava in four of the five LGAs surveyed. This study agrees with the work of Sseruwagi *et al*. (2004) that ACMV has been known to occur in most of cassava producing areas of Africa. This results further validate previous country-wide survey conducted in Nigeria (Ariyo *et al*., 2005; Ogbe *et al*., 2006; Alabi *et al*., 2008) and to other studies in West Africa (Pita *et al*., 2001; Torkpo *et al*., 2017). Gassol LGA recorded the higher incidence of both ACMV and EACMV (41.67%). Age of the cassava plants at the time of leaf samples collection could be a factor for higher incidence of ACMV and EACMV in Gassol LGA because most of the cassava plants sampled were between the ages of three to four months during 2015 survey.

The source of CMD infection in the study area was mainly due to the use of infected cassava cuttings as planting materials than by transmission by whitefly vectors. Percentage incidence due to cutting infection was generally higher in all fields surveyed with Wukari LGA recorded highest incidence due to cutting infection (80%). Similar results have been reported in Nigeria (Badamasi *et al*., 2020; Eni *et al*., 2020)

CONCLUSION

African cassava mosaic virus (ACMV), and *East African cassava mosaic virus* (EACMV) were detected in Taraba State Nigeria during 2015 growing season survey with varying level of distribution in the five LGAs surveyed. The study also confirmed CMD incidence, symptom severity and presence of whitefly population in the study area. The use of diseased cassava stem cuttings as planting materials by the farmers in the area is a major factor responsible for CMD spread in the area and could result to CMD outbreak in the region and country at large if not checked. Although the whitefly population was statistically low in the study during 2015 growing season, management of the vector and use of only disease free cuttings planting material is recommended in the study area to minimize the spread of the disease. Awareness program on the important of planting only disease-free cassava cuttings need to organized for cassava farmers in the country. Effort should be made to screen the cultivar "Farin rogo" against resistant to CMD as the cultivar shown mild symptom and most of the time it appeared symptomless during the survey.

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