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# FIELD SURVEY OF CASSAVA MOSAIC DISEASE AND ITS WHITEFLY (*Bemisia tabaci* Genn.) VECTOR IN KEBBI AND KATSINA STATES, NIGERIA

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## SUMMARY

**Demand for cassava roots and its products by man and industries is rising in Nigeria. However, cassava mosaic disease (CMD) is threatening cassava production in the country. This research was conducted to assess the current status of CMD and its whitefly-vector in Kebbi and Katsina States, Nigeria, during the 2015, 2017 and 2020 growing seasons. Cassava fields were sampled along the major roads at distance of 10 km apart, 30 cassava plants were randomly assessed per field for CMD status and whitefly abundance. Symptomatic and asymptomatic cassava leaf samples were collected from the surveyed fields and indexed against *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and *East African cassava mosaic Cameroon virus* (EACMCV) using polymerase chain reaction. Significant ($P = 0.05$) differences were obtained for CMD incidence for both years and states. Overall, Kebbi State had high CMD incidence (2015: 84.63%, 2017:81.11%, and 2020:21.57%). Disease severity was statistically similar in both year and states. Whitefly abundance significantly increased in 2017 and 2020 compared to 2015 (Kebbi 2015:5, 2017:11, 2020:16) (Katsina 2015:8, 2017:13, 2020:12) per leaf. Overall, ACMV (2015: 92.00%, 2017: 73.00%, and 2020:46.00%) was detected in more samples than EACMV (2015:29.00%, 2017:21.00%, 2020:13.00%) and EACMCV (2015:5.00%, 2017:13.00%, and 2020:7.00%) in Katsina State and it had the highest incidence of ACMV than Kebbi State. EACMV also occurred as mixed infection with ACMV. The findings of this research provide the current status of CMD and its vector in the study areas and that ACMV, EACMV and EACMCV incidences are increasing in states.**

**Keywords:** Cassava, Incidence, Severity, whitefly-vector.

**Cassava** plant, (*Manihot esculenta* Crantz), is a perennial woody shrub plant with an edible storage root. Cassava was first cultivated in South America and it was introduced to Nigeria in the sixteenth century (1). In Nigeria, cassava is one of the most important roots crop. Cassava roots and products are dietary staple food in Sub-Saharan Africa (SSA) and also for rural development, poverty alleviation, economic growth and ultimately, food security (12). Various government initiative and investments on cassava have led to massive production of the crop in northern Nigeria. An area where production of the crop was in the past years seemed as taboo. With the introduction of Central Bank of Nigeria (CBN) Five Stars Cassava Project and Nigerian National Petroleum Corporation (NNPC) Cassava-Based Ethanol Production Project in Kebbi and Katsina States, farmers in Kebbi and Katsina have now appreciated the potential of the crop for food security and income as cash crop. Nigeria still holds the record of the largest producer of cassava in the world

producing over 50 million tons in 2020 (12), but the trend in yield performance (cassava per hectare) remains low compared with what is obtained in Asian countries (12) where cassava production is lower compared with Nigeria but yield per hectare is higher than what is obtainable here in Nigeria.

This low cassava yield per hectare can be attributed to abiotic and biotic factors (19). Among biotic factors, cassava mosaic disease (CMD) is the most devastating disease of cassava and widely distributed in Nigeria. CMD is caused by viruses belonging to the genus *Begomovirus* in the family *Geminiviridae* (11). CMD can be identified on cassava by the symptoms it expresses, which includes: chlorotic mosaic, mottling, leaf distortion and an overall reduction in photosynthetic areas of the diseased leaves. The most noticeable symptom of CMD in infected cassava fields is a mosaic pattern on the leaves, the colouring of which can range from pale green to whitish yellow. At least seven begomoviruses have been reported to cause CMD in Africa (7). CMD is spread through perpetuation of infected planting materials and by whitefly (*Bemisia tabaci*) feeding. Annual tuber yield losses of cassava as a result of CMD have been estimated at 3.6 billion USD (7).

Surveys for CMD incidence, severity, whitefly population, data collection method are needed to provide information on the current CMD status for the management of the disease in these areas. To the best of our knowledge, this work is the first of its kind in Kebbi and Katsina States, since the work of (23). Therefore, the findings of this research will give an updated information on incidence and distribution of *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and *East African cassava mosaic Cameroon virus* (EACMCV) in the two states.

## MATERIALS AND METHODS

### Study area and climatic conditions

This research was conducted in Kebbi and Katsina States and the two states are located in the Sudano-sahelian Savanna ecological belt of Nigeria with latitude $12^0$ 27'00" N and longitude $04^0$ 12'00 E; $12^0$ 98'55" N and $7^0$ 6171'14" E, respectively . The two states encompass wide-ranging tracts of almost flat to slightly rolling landscape . Rainfall in the states as in other parts of Nigeria is dominantly controlled by the movement and pulsation of the Inter-Tropical Discontinuity (ITD) . Like other extreme northern parts of the country, rainfall in the states is very erratic and unpredictable with irregular onsets and cessations which adversely affect the duration of the cropping seasons. The wet season lasts from June to September . Annual rainfall in the areas ranges between 300 and 800 mm while mean annual temperature is 34.5°C with dry seasons temperatures often exceeding 40°C. The grasses with scattered trees which characterized the states have undergone severe modification due to human activities. Agriculture is the mainstay of the people in the states . The zone has tropical weather conditions with two seasons: rainy and dry .

### Field survey, data collection and sampling

A Central and West African Virus Epidemiology's (WAVE) harmonized field sampling protocol was adopted for cassava mosaic disease (CMD) and its whitefly vector field survey as previously described by (27). The survey was conducted in three years (2015, 2017 and 2020) in Kebbi and Katsina States, Nigeria. Survey routes followed a road map which allowed sampling of cassava fields in various local

government areas of the states. Distances between surveyed cassava fields varied depending on the availability of cassava fields but the minimum distance was 10 km apart as described by (22). Local Government Areas (LGAs) of each state that fall within the road map of survey routes were surveyed. Number of cassava fields visited in each state depend on the availability of cassava fields along the survey routes.

In Kebbi State; Argungu, Aliero, Bagudo, Birnin-Kebbi, Bunza, Gwandu, Jega, Kalgo, Koko-Base and Mayama LGAs were surveyed and 18 cassava fields were visited during 2015 growing season. During 2017 growing season, Argungu, Aliero, Birnin-Kebbi, Gwandu and Zuru LGAs were surveyed and 13 cassava fields were visited. While in 2020, Aliero, Gwandu, Argungu, Augie, and Birnin-Kebbi were surveyed and 11 cassava fields were visited (Table 1).

In Katsina State, Batagarawa, Batsari, Safana, and Jibiya LGAs were surveyed and 11 cassava fields were visited during 2015 growing season. During 2017 growing season, Funtua, Charanchi, Kurfi, Batsari, Batagarawa, Danmusa, and Kankara LGAs were surveyed and 13 cassava fields visited. While in 2020, Kurfi, Batsari, Batagarawa, Kankara, Funtua, Safana were surveyed and 9 cassava fields were visited (Table 2).

Data were recorded using a survey data collection sheet in 2015 survey while, in 2017 and 2020 surveys, data were recorded using a tablet with the survey software iForm Zerion (version 9.1.6) developed by the University of Cambridge, UK's Epidemiological Modelling Group. Data collected were CMD symptom types, CMD incidence and severity, source of CMD infection, and whitefly number, date of planting, variety type, field size and cropping type. Geo-location coordinates of fields were recorded using a GPS device (Garmin Inc., KS) in 2015 while, in 2017 and 2020 it was recorded using iForm.

At each field (with average field size of 0.6 ha), a total of 30 cassava plants were sampled along 2 diagonals in an X shape (that is 15 plants randomly sampled on each diagonal). Five apical leaves of each plant were randomly sampled for whitefly count. At least two samples of asymptomatic leaf were collected from a diseased-free cassava field. In a CMD-infected, leaf samples were collected based on the severity of the disease (that is one asymptomatic leaf sample, one mild leaf sample, one moderately severe leaf sample, one severe leaf sample and one extremely severe leaf sample).

A total of 54 asymptomatic and symptomatic cassava leaf samples were collected from Kebbi State during 2015, 39 and 33 samples were collected during 2017 and 2020, respectively. In Katsina State, A total of 33 asymptomatic and symptomatic cassava leaf samples were collected during 2015, 39 and 27 samples were collected during 2017 and 2020, respectively.

**Table 1:** Local Government Areas (LGAs) and Locations Surveyed in Kebbi State during 2015, 2017, and 2020 Cassava Growing Seasons

| Year | State | LGA | Location | Longitude | Latitude |
|---|---|---|---|---|---|
| 2015 | Kebbi | Argungu | Kwartagi | 4.2800 | 12.4834 |
| | | Argungu | Katangar Arawa | 4.2583 | 12.4817 |
| | | Gwandu | Dauduje | 4.2832 | 12.3325 |
| | | Gwandu | Kambaza | 4.34247 | 12.27756 |
| | | Aliero | Kali | 4.2600 | 12.16325 |
| | | Jega | Dudduke | 4.19683 | 12.11813 |
| | | Gwandu | Gittiyal | 4.34054 | 12.21176 |
| | | Gwandu | Gorkomodo | 4.41801 | 12.25835 |
| | | Birnin-Kebbi | Danyaku | 4.0490 | 12.30942 |
| | | Kalgo | Sandare | 4.02246 | 12.2928 |
| | | Kalgo | Galaru | 4.0820 | 12.25094 |
| | | Bunza | Salwai | 3.5924 | 12.10229 |
| | | Mayama | Rafin guzuma | 4.087 | 12.32922 |
| | | Mayama | Sarandosa | 4.08946 | 12.29804 |
| | | Koko-Base | Koko | 4.11946 | 12.25694 |
| | | Bagudo | Bagudo | 4.1182 | 12.29044 |
| 2017 | Kebbi | Aliero | Kuta | 4.477221 | 12.303628 |
| | | Gwandu | Tari | 4.496423 | 12.404009 |
| | | Gwandu | Gwanyal | 4.486092 | 12.564497 |
| | | Argungu | Tasora | 4.475675 | 12.805031 |
| | | Argungu | Tashar kade | 4.405029 | 12.752877 |
| | | Argungu | Danyaku | 4.082007 | 12.51528 |
| | | Birnin-Kebbi | Kuka | 4.008421 | 12.453376 |
| | | Aliero | Kali | 4.433694 | 12.27204 |
| | | Gwandu | Gorkomodo | 4.692019 | 12.42667 |
| | | Zuru | Dikuka | 5.185092 | 11.494148 |
| | | Zuru | Dongo | 5.25672 | 11.354972 |
| 2020 | Kebbi | Argungu | Natsini | 4.485444 | 12.8050251 |
| | | Augie | Augie | 4.5929078 | 12.883869 |
| | | Aliero | Dakala | 4.3977633 | 12.3755437 |
| | | Birnin-Kebbi | Ambursa | 4.355829 | 12.5114077 |
| | | Birnin-Kebbi | Gulumbe | 4.3550326 | 12.4134602 |
| | | Argungu | Argungu | 4.5218802 | 12.7272599 |
| | | Aliero | Kashinzama | 4.4800717 | 12.3771027 |
| | | Gwandu | Malisa | 4.7160281 | 12.4500988 |
| | | Gwandu | Rinaye | 4.6852463 | 12.4168194 |

**Table 2 :** Local Government Areas (LGAs) and Locations Surveyed in Katsina State during 2015, 2017, and 2020 Cassava Growing Seasons

| Year | State | LGA | Location | Longitude | Latitude |
|---|---|---|---|---|---|
| 2015 | Katsina | Batagarawa | Kwarin maikutso | 7.2583 | 12.53521 |
| | | Batsari | Unguwar malamai | 7.14567 | 12.44612 |
| | | Batsari | Kukar samu | 7.12595 | 12.39246 |
| | | Safana | Gimi | 7.17544 | 12.27982 |
| | | Jibiya | Gadare | 7.12494 | 13.05093 |
| | | Safana | Gimin Fulani | 7.12544 | 12.2882 |
| | | Jibiya | Gare | 7.1294 | 13.05493 |
| 2017 | Katsina | Funtua | Mararraba maska | 7.337489 | 11.375261 |
| | | Charanchi | Ganuwa | 7.654223 | 12.660415 |
| | | Kurfi | Yanmarke | 7.527394 | 12.649679 |
| | | Kurfi | Bauji | 7.465258 | 12.676489 |
| | | Batsari | Karare | 7.387461 | 12.718305 |
| | | Batsari | Dadin kowa | 7.308644 | 12.735232 |
| | | Batagarawa | Kwarin maikotso | 7.43054 | 12.891884 |
| | | Batagarawa | Dabaibayawa | 7.582511 | 12.862149 |
| | | Batagarawa | Tafkin almu | 7.544835 | 12.770826 |
| | | Danmusa | Tashar kadanya | 7.48624 | 12.100887 |
| | | Kankara | Tashar samaila | 7.427322 | 11.967742 |
| 2020 | Katsina | Kurfi | Yanmarke | 7.527394 | 12.649679 |
| | | Batagarawa | Dabaibayawa | 7.582511 | 12.862149 |
| | | Batsari | Kukar samu | 7.12595 | 12.39246 |
| | | Kankara | Tashar samaila | 7.427322 | 11.967742 |
| | | Safana | Gimin Fulani | 7.12544 | 12.2882 |
| | | Batsari | Karare | 7.387461 | 12.718305 |
| | | Funtua | Mararraba | 7.337489 | 11.375261 |

CMD incidence and severity assessment

Cassava mosaic disease incidence was determined based on the appearance of the symptoms on each plant sampled. CMD incidence of each field was calculated as the percentage (%) of visually cassava mosaic diseased plants from the thirty (30) plants assessed in the field using the formula (16) of below:

$$\text{Disease Incidence (DI \%)} = \frac{n \times 100}{N}$$

Where:

n = number of symptomatic plants

N = sum of all plants observed or assessed

CMD severity of each diseased plant sampled in a field was scored based on the percentage of the area or proportion of cassava leaf that is CMD symptomatic as described by (27) using the arbitrary scale of 1 to 5 (Table 13).

**Table 3 :** Disease severity scores

| Score | Description |
|---|---|
| 1 | Asymptomatic plant. |
| 2 | Plant with 25% of leaves showing mild chlorotic pattern or mild distortion. |
| 3 | Infected plant with 50% of leaves exhibiting moderate mosaic pattern, narrowing and distortion at base of the leaves. |
| 4 | Infected plant with 75% of leaves exhibiting severe mosaic symptom, leaf distortion and general reduction of leaf size. |
| 5 | Infected plant with more than 75% of leaves exhibiting very severe mosaic, leaf distortion, reduced leaf size, vein clearing and, in most cases, stunted growth. |

Source: (11)

**Samples collection and preservation**

At each field sampled and surveyed, a minimum of two asymptomatic leaf samples and a maximum of five-leaf samples from both asymptomatic and symptomatic were collected from cassava plants of varying CMD severity. Leaf samples were preserved in herbarium presses prior to laboratory analysis. Whitefly samples were also collected using aspirator and preserved in a tube containing 70% ethanol.

**Estimation of whitefly abundance**

At 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 as described by .

**Source of CMD 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 on the cassava plant. 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. 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 (27).

**Total DNA isolation**

Nucleic acid (DNA) isolation was performed following CTAB (cetyltrimethyl ammonium bromide) protocol as initially described by (9). The DNA was dried in a vacuum dryer for 10 minutes before dissolving the pellet in 100 µl of TE buffer and stored at -20$^0$C for downstream analysis. The purity (A260/A280) and the concentration (ng/µl) of the samples were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and recorded. Only samples that had A260/A280 $\geq$ 1.8 were chosen for downstream analysis.

**Polymerase chain reaction (PCR)**

**Polymerase chain reaction** (PCR) was performed using specific primers (Table 4) for detection of ACMV, EACMV, and EACMCV in cassava leaf samples collected from the field survey. DNA template from previously characterized isolates of the three virus species was used as positive control while, 2% CTAB buffer was used as negative control. A ready-made PCR master mix (SOLIS BIODYNE , Estonia) was used and each reaction mixture consist 2.5 µl of PCR master mix, 7.0 µl of molecular grade water, 0.5 µl of forward primer, 0.5 µl reverse primer and 2 µl of DNA template. The amplification conditions were: an initial denaturation at 94$^0$C for 2 minutes, followed by 30 cycles of denaturation at 94$^0$C for 1 minute, annealing at 55$^0$C for 1 minute, extension at 72$^0$C for 1 minute and a final extension of 72$^0$C for 10 minutes. Following amplification, PCR products were separated by electrophoresis alongside a 1 kb plus DNA ladder (SOLIS BIODYNE, Estonia) on 1.5% agarose gel stained with ethidium bromide (10 mg mL$^{-1}$). The gel was viewed under UV light using a Bench top UV transilluminator (UVP, USA).

**Table 4 :** Primer pairs used for the amplification of *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and *East African cassava mosaic Cameroon virus* (EACMCV)

| Primer name | Primer sequences (5' to 3') | Target region | Size | Reference |
|---|---|---|---|---|
| JSP 001 | ATGTCGAAGCGACCAGGAGAT | | | |
| JSP 002 | TGTTTATTAATTGCCAATACT | ACMV DNA-A (CP) | | (26) |
| ACMVBF | TCGGGAGTGATACATGCGAAGGC | | | |
| ACMVBR | GGCTACACCAGCTACCTGAACT | ACMV DNA-B (BV1/BC1) | 628 bp | (18) |
| JSP 001 | ATGTCGAAGCGACCAGGAGAT | | | |
| JSP 003 | CCTTTATTAATTTGTCACTGC | EACMV DNA-A (CP) | 780 bp | (6) |
| EACMV1 | GTTCGGCTATCACCTTCTAGAACA | | | |
| EACMV2 | CAAGGCTTACATTGAAAAGGGA | EACMV-BC1 (DNA - A) | 375 bp | (18) |

## RESULTS

### CMD symptoms observed in the fields

Visual assessment of CMD symptom types was employed in all cassava fields sampled and surveyed in the two states during 2015, 2017 and 2020 filed survey respectively. Mosaic pattern (patches of pale green and dark green) and leaf distortion were the major CMD symptoms observed in almost all infected cassava fields surveyed (Figure 1). Other symptoms observed in the course of field survey were filiform, stunted growth of diseased cassava plants, vein clearing, and dieback.

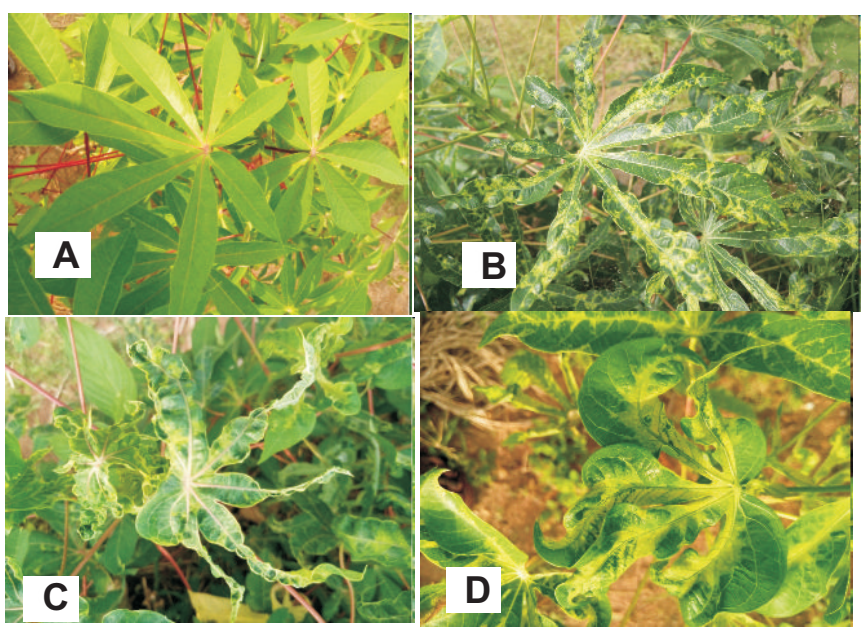


Figure 1: A = symptomless cassava leaf, B = mosaic symptom on cassava leaf, C = leaf distortion and D = filiform and distortion.

**CMD incidence, CMD severity and whitefly population**

Cassava mosaic disease incidence was statistically ($P$ = 0.05) higher in 2015 than in 2017 and 2020 in both states. Kebbi State had the highest mean CMD incidence of 84.63%, 81.11% and 21.57% in 2015, 2017 and 2020, respectively. While, Katsina State had the lowest mean CMD incidence in both years (Table 5 and 6). There was no significant ($P$ = 0.05) different in term of mean CMD severity in all the three years and statistically similar in the two states. CMD severity was slightly high in Kebbi State than in Katsina State in all the three years (Table 5 and 6).

Whitefly population significantly ($P$ = 0.05) differed between 2015 and 2017 but statistically similar between 2017 and 2020 in the two states. Katsina State had the highest mean whitefly population of 8 and 13 adults per leaf in 2015 and 2017, respectively. While, in 2020 Kebbi State had the highest mean whitefly population of 16 adults per leaf (Table 5 and 6).

**Table 5:** Mean CMD incidence, seve rity and whitefly population during 2015, 2017 and 2020 growing seasons in Kebbi State

| Year | State | LGA | Mean CMD incidence (%) | Mean CMD severity | Mean whitefly population |
|---|---|---|---|---|---|
| 2015 | Kebbi | Argungu | 95.00 | 2.7 | 4.5 |
| | | Gwandu | 78.32 | 2.25 | 1.9 |
| | | Jega | 10 | 1.2 | 0.2 |
| | | Aliero | 90.0 | 2.1 | 5 |
| | | Kalgo | 96.67 | 2.7 | 7.85 |
| | | Birnin-Kebbi | 96.67 | 2.7 | 7.2 |
| | | Mayama | 95.0 | 2.65 | 6.4 |
| | | Bunza | 100 | 3.03 | 7.2 |
| | | Bagudo | 100 | 2.8 | 7.9 |
| **Mean** | | | **84.63** | **2.5** | **5** |
| 2017 | Kebbi | | | | |
| | | Aliero | 80.0 | 2.4 | 9.4 |
| | | Argungu | 73.32 | 2.27 | 29.83 |
| | | Gwandu | 80 | 2.4 | 9.4 |
| | | Birnin-Kebbi | 83.33 | 2.2 | 5.4 |
| | | Zuru | 100 | 2.65 | 1.25 |
| **Mean** | | | **81.11** | **2.33** | **11** |
| 2020 | Kebbi | Argungu | 25.42 | 2.4 | 19.34 |
| | | Augie | 21.05 | 2.3 | 16.67 |
| | | Aliero | 22.23 | 2.1 | 11.23 |
| | | Gwandu | 20.67 | 2.3 | 15.6 |
| | | Birnin-Kebbi | 18.56 | 2.7 | 18.36 |
| **Mean** | | | **21.57** | **2.36** | **16** |

**Table 6:** Mean CMD incidence, severity and whitefly population during 2015, 2017 and 2020 growing seasons in Katsina State

| Year | State | LGA | Mean CMD incidence (%) | Mean CMD severity | Mean whitefly population |
|---|---|---|---|---|---|
| 2015 | Katsina | Batagarawa | 96.67 | 2.5 | 1.1 |
| | | Batsari | 23.34 | 1.65 | 2.15 |
| | | Safana | 96.67 | 2.6 | 25.35 |
| | | Jibiya | 38.34 | 1.6 | 4.85 |
| **Mean** | | | **63.76** | **2.09** | **8** |
| 2017 | Katsina | Funtua | 0.0 | 1.0 | 1.7 |
| | | Charanchi | 0.0 | 1.0 | 12.2 |
| | | Kurfi | 6.67 | 1.1 | 18.4 |
| | | Batsari | 6.67 | 1.1 | 20.75 |
| | | Batagarawa | 16.67 | 1.23 | 25.63 |
| | | Danmusa | 83.33 | 2.0 | 10.3 |
| | | Kankara | 13.33 | 1.2 | 4 |
| **Mean** | | | **18.09** | **1.23** | **13** |
| 2020 | Katsina | Kurfi | 0.0 | 1.0 | 8.6 |
| | | Batagarawa | 56.45 | 2.3 | 15.67 |
| | | Batsari | 16.67 | 1.5 | 11.3 |
| | | Kankara | 13.33 | 1.7 | 10.0 |
| | | Safana | 11.67 | 1.3 | 8.9 |
| | | Funtua | 13.33 | 2.4 | 18.4 |
| **Mean** | | | **18.57** | **1.7** | **12** |

**Source of CMD infection**

Based on the location of CMD symptoms on sampled cassava plants, there was statistical ($p$ = 0.05) difference between mean cutting source of infection and mean whitefly source of infection in Katsina State in 2015 but, in Kebbi State, CMD transmission in most of the surveyed fields was mostly through use of infected cuttings (2015 = 57.04%, 2017 = 57.62%) than by whitefly vector (2015 = 42.92%, 2017 = 42.38%). In 2020, CMD transmission by whitefly vector had the highest percentage in both states (Figure 2).

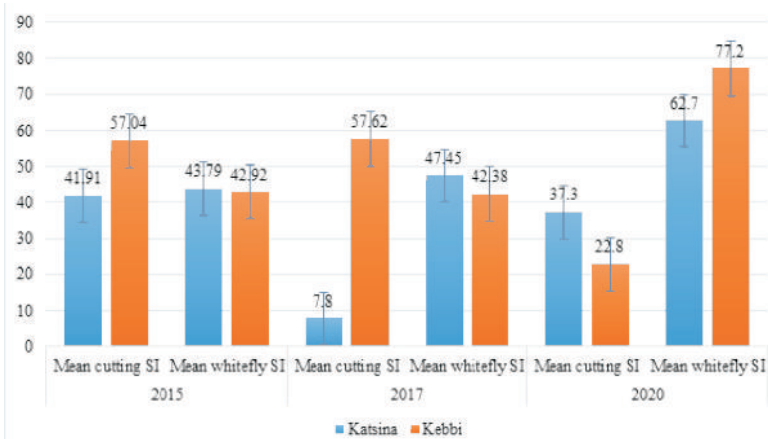


Figure 2: Mean cutting and whitefly source of infection in Kebbi and Katsina States in the three years' survey. Bars indicate standard error of means at 5% probability level. SI = source of infection.

**ACMV, EACMV and EACMCV incidence in cassava leaf samples**

A total of 80, 109 and 146 cassava leaf samples were tested for ACMV, EACMV and EACMCV in 2015, 2017 and 2020, respectively from both states. ACMV had the highest mean virus incidence in all the three years of our field surveys in the two states. There was significant ($p$ = 0.05) difference in term of ACMV, EACMV and EACMCV incidence in the two states. Katsina State had the highest mean ACMV incidence of 92%, 73% and 46% in 2015, 2017 and 2020, respectively. Kebbi State had the highest mean EACMV incidence of 31% and 23% in 2015 and 2017, respectively. However, there was no statistically significant ($p$ = 0.05) between the two states in term of EACMCV incidence in all the three years of field surveys (Table 7). EACMV also occurred as mixed infection with ACMV. Mixed infection of ACMV + EACMV was higher in Katsina State during the 2015 and 2020 field survey. While, in 2017 both states recorded the same percentage mixed infection (Table 7).

**TABLE 7:** Percentage incidence of ACMV, EACMV and EACMCV in Kebbi and Katsina States for 2015, 2017 and 2020

| Year | State | ACMV % | EACMV % | EACMCV % | ACMV + EACMV % |
|---|---|---|---|---|---|
| 2015 | Kebbi | 86 | 31 | 2 | 20 |
| | Katsina | 92 | 29 | 5 | 25 |
| 2017 | Kebbi | 68 | 23 | 11 | 18.19 |
| | Katsina | 73 | 21 | 13 | 18.19 |
| 2020 | Kebbi | 43 | 13 | 5 | 13.33 |
| | Katsina | 46 | 13 | 7 | 16.67 |

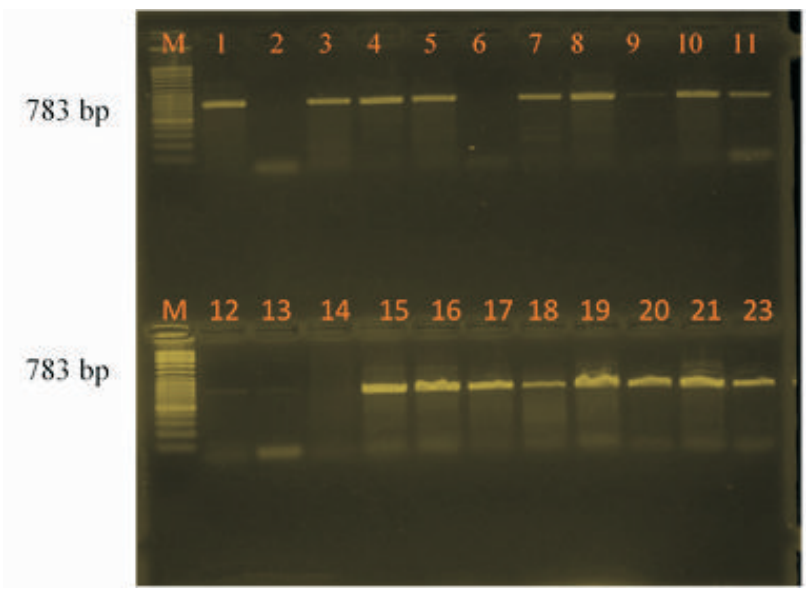


Figure 3: Detection of ACMV using JSP01/JSP02 from cassava leaf samples of both Kebbi and Katsina States

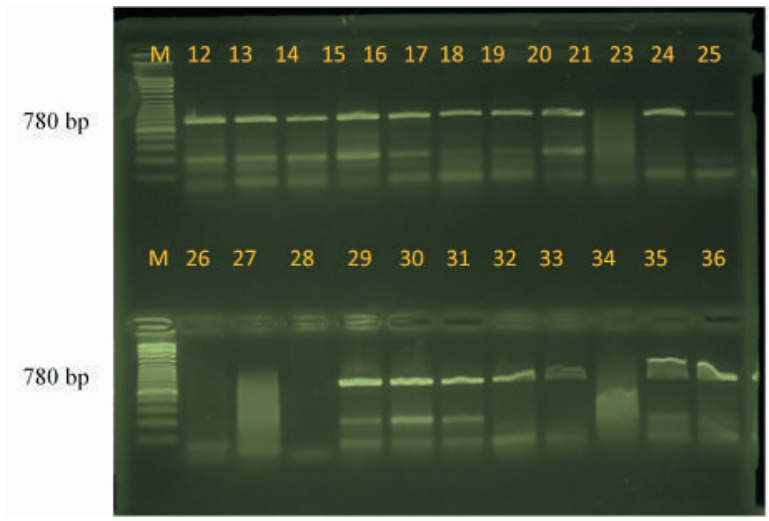


Figure 4: Detection of ACMV using JSP01/JSP03 from cassava leaf samples of both Kebbi and Katsina States

## DISCUSSION

This research gives an update on the current CMD status and its whitefly vector population in Kebbi and Katsina States, north-western Nigeria. Findings of this research shows the presence of CMD and its whitefly vector in farmers' cassava fields in all the three years of the survey in Kebbi and Katsina States with variation in incidence of CMD and whitefly population among the three years and between the two states. Mosaic pattern (patches of pale green and dark green) and leaf distortion were the major CMD symptoms observed in farmers' cassava fields surveyed. Similar symptoms have been reported in Nigeria and other African countries (10, 20, 11, 4, 7). The Polymerase Chain Reaction (PCR) conducted confirmed that the symptoms observed on cassava plants were induced by cassava mosaic begomoviruses (CMBs) because most of the symptomatic leaf samples were tested positive of at least one of the three cassava mosaic begomoviruses (CMBs) detected in this research (that is ACMV, EACMV and EACMCV). Other symptomatic leaf samples that were tested negative against ACMV, EACMV and EACMCV might be due other species/strain of cassava mosaic begomoviruses (CMBs) that were not tested in this work. Reports by (13) and (23) stated that CMD symptoms observed on cassava plants depend on virus species, strain and mixed infections.

Findings from this research showed that CMD occurred in Kebbi and Katsina States of Nigeria, however, it showed that there was significant reduction of CMD incidence in cassava fields in the second and third years (2017 and 2020) of the field survey when compared with first year (2015) of the field survey in the two states. This research also showed CMD severity varied from one cassava field to another ranging from mild to very severe as was previously reported in other parts of Nigeria (22, 11, and 20). The high CMD incidence recorded in the first-year survey in the two states can be attributed to common practices of using cassava cuttings from previous season for next season planting and

purchase of cassava cuttings from unknown source by the farmers in the study areas that might be CMD infected cuttings. Use of CMD infected cuttings have been reported as the major factor of wide spread of CMD among the farmers' fields (8). The reduction in CMD incidence recorded in second and third years of our field survey can be attributed to the awareness created during the first-year survey by the survey team of the important of using only disease-free cuttings for next season planting. It can also be attributed to the awareness and sensitization campaign on CMD symptoms identification and selection of disease-free cutting for next season planting conducted by Central and West African Virus Epidemiology (WAVE) Program, Kebbi State University of Science and Technology, Aliero hub. Use of disease-free cassava cuttings as planting materials for next season planting have been recommended as a CMD management practice (11). It is therefore important for extension officers in the two states to sensitize cassava farmers on the important of using only cassava mosaic disease-free cuttings for next season planting.

This research showed the presence of whitefly that vectored *Cassava mosaic begomoviruses* (CMBs) in farmers' cassava fields in the study areas in all the three years of the field survey. However, there was no correlation between whitefly population and CMD incidence; between whitefly population and virus incidence. Similar results have been reported in previous studies (5, 11). This research recorded a high percentage of mean whitefly transmitted CMD infection as an average of the three years field survey when compared with CMD cutting transmitted infection. This is contrary to previous studies conducted in Nigeria and other African countries (22, 8, 21, 11).

The PCR results of this research shows the presence of ACMV, EACMV and EACMCV in Kebbi and Katsina States of Nigeria with variation in their incidence and distribution in the three years of the survey and in the two states. Mixed infections of ACMV and EACMV were also detected from some field during both years and in the two states. Previous studies also reported ACMV and EACMV mixed infection (4, 11). Findings of this research indicate ACMV as the most predominant CMBs species found in both Kebbi and Katsina States of Nigeria in all the three years of field survey. This agreed with the saying of (27) that ACMV occur in most of the cassava producing areas of Africa. This is also similar to findings of previous field survey conducted in Nigeria (2, 22, 4, 11, 20) and to other findings in West Africa (26, 28). From these findings, the incidence of EACMV and EACMCV was low in all the three years of our field survey in both states but EACMV incidence was high than EACMCV in both years and states. EACMV have been reported to be more predominant in East African countries than in West African countries (8, 7, 30). Some CMD symptomatic cassava leaf samples tested against ACMV, EACMV and EACMCV were tested negative using all the primer pairs utilized in this research. However, some of the asymptomatic samples were tested positive of one or more of the three CMBs species detected in this research. This is in agreement with work of (8) and (15). A research that will detect other species of CMBs that are responsible for the CMD symptoms observed on cassava leaf samples which were tested negative of ACMV, EACMV and EACMCV in this study is needed.

## CONCLUSION

This research provides the current status of

CMD and its whitefly vector in Kebbi and Katsina States of Nigeria. The research established that ACMV, EACMV, EACMCV and mixed infection of ACMV and EACMV occurred in the two states and that ACMV is the predominant species of CMBs in the two states. The use of disease-free cuttings as planting materials significantly reduced the incidence and spread of CMD. The detection of CMBs in asymptomatic cassava leaf samples in this research highlight the need for a functional clean seed certification system in Nigeria.

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