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CONTACT:
The Editor-in-Chief,
Kebbi Journal of Agriculture and Natural Sciences,
Faculty of Agriculture,
Abdullahi Fodio University of Science and Technology Aliero,
PMB 1144, Birnin kebbi, Nigeria.
Email: kejaanseditor@ksusta.edu.ng,

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The Kebbi Journal of Agriculture and Natural Sciences has the sole aim of providing an intellectual platform and ideas for scholars, by promoting interdisciplinary studies related to agriculture and natural science through publishing the latest scientific research findings that are of direct policy implications and beneficial to the research community. Consequently, the journal covers all aspects of Crop Science, Animal Science, Agricultural Economics, Agricultural Extension and Rural Development, Food Science, Fisheries and Aquaculture, Biotechnology, Soil Science and Agricultural Engineering, Forestry and Environment, Wildlife, Agricultural Education, Agro-allied Industries as well as all Natural Science researches related to Agriculture.

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FIELD ASSESSMENT OF CASSAVA MOSAIC DISEASE AND CASSAVA BEGOMOVIRUSES DETECTION IN KEBBI STATE, NIGERIA

Sanusi Sani Yola, Ibrahim Umar Mohammed, Abdurahman Musa, Aminu Ibrahi Kwaido and Ibrahim Yusuf Jega

Department Crop Science, Faculty of Agriculture, Abdullahi Fodio University of Science and Technology, Aliero

Corresponding author: sanusisaniyola2434@gmail.com, 07064622434

ABSTRACT

Cassava plays an important role in food security of Nigerians and land under its cultivation had significantly increased but its productivity had significantly reduced as a result of Cassava Mosaic Disease (CMD). This research was conducted to assess the current status of CMD in cassava fields and to detect Begomoviruses causing the disease in Kebbi State. Five Local Government Areas (LGAs) were purposely selected which were Argungu, Augie, Birnin-Kebbi, Gwandu and Kalgo LGAs of Kebbi State. In each LGA, five cassava fields were sampled given a total number of 25 cassava fields. In each field, 30 plants were randomly assessed for CMD incidence and severity. Cassava leaf samples were collected and analyzed for the presence of *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) using Polymerase Chain Reaction (PCR). The results revealed that CMD incidence was higher in Augie (59.9%), followed by Argungu (56%) while Birnin-Kebbi had the lowest CMD incidence (46.67%). The disease severity was also higher in Augie (2.85%) while Kalgo had the lowest CMD severity (2.24%). PCR results revealed the presence of ACMV in five LGAs. Argungu had 41.67% incidence of ACMV followed by Birnin-Kebbi (33.33%) and Kalgo had the least (3.33%). EACMV was detected only in Argungu (16.67%), Gwandu (14.29%) and Augie having the least (11.11%). The Findings of this research, revealed the current status of ACMV and EACMV in Kebbi State. The research recommended the use of disease-free cuttings as planting materials, rogueing of the infested cassava plant and use of resistant cassava variety for optimum cassava production in the study.

Keywords: Begomoviruses, Detection, Molecular, Survey

Introduction

Cassava (*Manihot esculenta Crantz*) is a perennial woody shrub plant with an edible root which was first cultivated in South America and introduced to Nigeria in the sixteen Centuries (Jones, 1959; Adeniji *et al.*, 2005). Cassava is one of the world most important root crops and constitutes the staple food of rural and urban house hold in sub-Saharan Africa (SSA) (Spencer *et al*., 2017; Dauda *et al*., 2021). It is a source of carbohydrate for over 800 million peoples across Africa (FAO, 2013). It is also one of the fastest expanding staple food crops in cassava consuming countries and has continued to gain prominence among farmers while in the industrial demand is also raising consistently (FAO, 2018). Globally, cassava experienced consistent growth of above 3% annually (FAO, 2018). Close to 84% of domestic cassava production is available for consumption while the remaining 16% is available for industrial use (Nwokoro *et al*., 2002; Kormawa and Akoroda, 2003). According to FAO (2018), world cassava production

stood at about 278 million tones; Africa total production was about 170 million tones (about 56% of the world production) as of 2018 (FAOSTAT, 2019). In Nigeria, cassava provides more than half of the calorie requirement for people across various ethnic groups (Akinpelu *et al*., 2011). The increasing importance of cassava among other crops grown in Nigeria is not only connected to its increasing demands as food but also for food security (FAO, 2018). The importance of cassava as staple food result mainly from the simplicity of its cultivation; its ability to grown on marginal that are difficult to use for other crops; and its drought tolerance, which justifies it geographical expansion from natural forest areas to Sahelian zones (Nweke, 2004; IFAD, 2008). Cassava is a potentially scalable high biomass producing crop compared to other root crops as well adaptable to land and less favorable climatic conditions (Jolayemi and Opabode, 2018). Nigeria is the world largest cassava producer, with 60 million tons having 21% of the world total production of 278 million tons. Other major growers include Thailand, Democratic Republic of Congo (FAOSTAT, 2019). Although, Nigeria seems to be the world largest cassava producer, the 5 to 10 tons /ha tuber yield obtain in Nigeria is much lower than the average tuber yield of 25 t/ha obtained in other cassava growing region around world (FAO, 2017). One of the major biotic constraints to cassava is its susceptibility to cassava mosaic disease which causes annual tuber yield losses estimated at USD 1.9 to 2.7 billion (Ogwok *et al.*, 2016). It is prevalent in sub-Saharan Africa (Pati and Fauqet, 2009) and it is the major disease of cassava. Cassava mosaic begomoviruses (CMBs), belong to the genus *Begomovirus* in the family *Geminiviride* (Atiri and Winter, 2005). The viruses are transmitted through the use of infected cassava cuttings as planting material and by whitefly vector (*Bemisia tabaci* Genn.) (Legg *et al*., 2015). CMD symptoms vary from mosaic pattern on leaves to leaf distortion, vein clearing and stunted growth (Ndunguru and Thresh, 2004). Several strains of cassava mosaic begomoviruses have been identified to cause CMD in Africa namely: *Africa cassava mosaic virus* (ACMV), *East Africa cassava mosaic virus* (EACMV), *South Africa cassava mosaic virus* (SACMV), *East African cassava mosaic zanzibar virus* (EACMZV), and *East African cassava mosaic Kenya virus* (EACMKV), and *East African cassava mosaic Malawi virus* (EACMMV), and two species which were recently been described *African cassava mosaic Burkina Faso virus* (ACMBV) and *cassava Madagascar virus* (Harmalala *et al*., 2025, Trendrebogo *et al*., 2012).

Cassava production in Kebbi State is increasing day-by-day as a result of various government program in the State. Vegetative propagated crops are prone to virus infection and cassava is no exception to this generalization. Although many researchers been reported on cassava mosaic viruses in the State and its transmission, there is little or limited information on the current status of cassava viruses in the study area. Therefore, the report of this research work will help in understanding how rapid spread of these viruses in the region with aid of molecular techniques (e.g polymerase chain reaction).

Materials and Methods

Study Area

Kebbi State is located at North Western part of Nigeria in the Sudan Savanah agro-ecological zone of Nigeria on latitude 13^0 $08^{'}$ N and longitude 5^0 15’ E on an altitude of about 350 m above sea level, Nigeria metrology (NIMET, 2017). The State shares an extensive boarder with Niger Republic to the North and Benin Republic to the West with many inert-cultural and ethnic linkages (Anonymous 2012b). It is

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internally bordered to the North-East by Sokoto State and to the East by Zamfara State and to the South by Niger State. In terms of rainfall, however, the mean annual rainfall in the State varies significantly from the Northern part (733 mm) to the Southern area (105 mm) (NIMET, 2017).

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) field survey as previously described by Sseruwagi *et al*. (2004). Survey was conducted in Argungu, Augie, Birnin-Kebbi, Gwandu and Kalgo Local Government Areas of Kebbi State, Nigeria. Survey routes followed a road map that allowed the sampling of cassava fields in the five (5) LGAs of Kebbi. Distance between cassava fields surveyed varies depending on the availability of cassava field but the minimum distance was 10 km apart as described by Ogbe *et al*., (2006). Data was recorded using a survey data collection sheet. At each field a total of 30 cassava plants were sampled along 2 diagonals in an X shape (that is 15 plants randomly sampled on each diagonal).

Data Collection

Cassava Incidence and Severity Assessment

Cassava mosaic disease (DMD) 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 disease plant from the 30 plants assessed in the field using the formula of Hidayat *et al.* (2020) below

$$\text{Disease Incidence (\%)} = \frac{Number\ of\ disease\ plants/field}{\text{Total number of plant sample examined/field}} \times 100$$

Where:
n = number of symptomatic plants
N = sum of all plants observed or assessed

CMD severity of each disease plant sampled in a field was scored based on the percentage of the area or proportion of cassava leaf plant that is CMD symptomatic as described by Sseruwagi *et al*. (2004) using the arbitrary scale of 1 to 5 (table 1)

Table 1: Disease severity score

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.

Samples collection and Preservation

At each field sampled and surveyed, a minimum of two asymptomatic leaf samples and a maximum of five (5) 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 analysis.

Source of CMD infection

Source of CMD infection was categorized as "C" (cassava stem cutting as source of infection) and "W" (whitefly as a source of infections). The possible source of the observed CMD infections in each 15bcassava plants sample 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 was 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 was assumed to have been infected by the whitefly vector (Sseruwagi *et al.*, 2004).

Total DNA Isolation

DNA isolation was performed following CTAB (cetyltrimethylammonium bromide) protocol as initially described by Dellaporta *et al*. (1983). 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 ≥ 1.8 was choose for downstream analysis.

Polymerase Chain Reaction (PCR)

PCR was performed using specific primers (Table 2) for detection of ACMV and EACMV in cassava leaf samples collected from the field surveyed. DNA template from previously characterized isolates of the two virus species was used as positive control while, 2% CTAB buffer was used as negative control. A ready-made PCR master mix 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 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 was separated by electrophoresis alongside a 1 kb plus DNA ladder (Thermo Fisher Science, USA) 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 2: Primer pairs to be used for the amplification of *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV)

Primer name	Primer sequences (5' to 3')	Target region	Size	Reference
JSP 001 JSP 002	ATGTCGAAGCGACCAGGAGAT TGTTTATTAATTGCCAATACT	ACMV DNA-A (CP)		Pita *et al*., 2001
JSP 001 JSP 003	ATGTCGAAGCGACCAGGAGAT CCTTTATTAATTTGTCACTGC	EACMV DNA-A (CP)	780 bp	Pita *et al*., 2001

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Virus Incidence

Virus incidence (VI) in percentage was calculated using the formula of Chaube and Pundhir (2005). This was achieved after laboratory analysis of the leaf samples collected.

$$\text{Virus Incidence (\%)} = \frac{Number\ of\ positive\ samples/field}{\text{Total number of sample examined/field}} \times 100$$

Data analysis

Data collected on disease incidence, disease severity, virus incidence, and source of infection in the five (5) LGAs of the Kebbi State were analyzed using IBMSPSS statistics software version 20. Descriptive statistics tools as means, percentage, and standard error were used to determine significance of the results (Gomez and Gomez, 1984).

Results

CMD symptoms observed

Mosaic patterns on leaves and leaf distortion were the two major cassava diseases symptoms observed across all the survey locations.

Cassava Mosaic Disease Incidence

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From the survey conducted, the results showed that cassava mosaic diseases naturally occurred infecting cassava plants in all the five Local Government Area (LGAs), Argungu, Augie, Birnin-Kebbi, Gwandu and Kalgo of Kebbi State but with significant (P = 0.05) variations in percentage incidence. Figure 1 revealed the incidence (%) of cassava mosaic diseases in Five LGAs of Kebbi State. The highest CMD incidence (59.9%) was recorded in Augie followed by Argungu (56%) which was not significantly different at (P = 0.05) from the incidence recorded in Kalgo (54.67%) and Gwandu (52%) while Birnin Kebbi had the lowest incidence (46.67 %) of cassava mosaic diseases among the surveyed areas

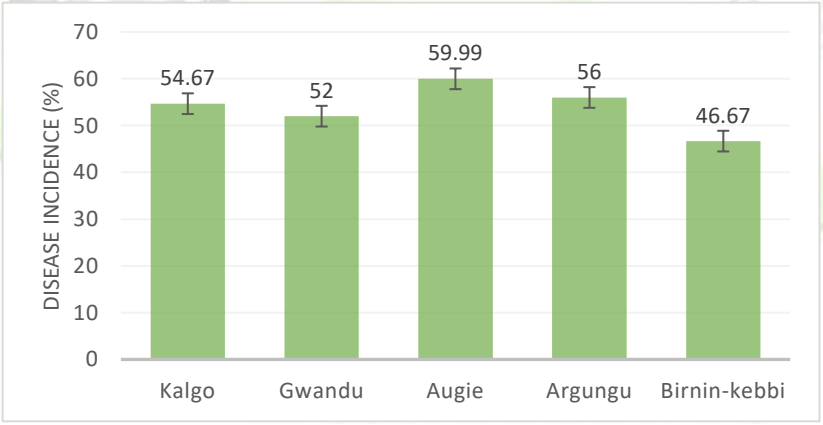

Figure 1: Mean Cassava mosaic disease incidence in the five LGAs of Kebbi State. Bars indicate standard error at 5% level of probability

Cassava Mosaic Symptom Severity (%)

Similarly, the results on the symptoms severity shows that there was significant (P = 0.05) difference on the CMD severity recorded among the five surveyed LGAs of Kebbi State. Same trend was observed as Augie had the high symptoms severity (2.85%) over Gwandu (2.64%) which was statistically similar, Argungu LGA (2.49%) was ranked third. The lower severity was recorded in Birnin-Kebbi (2.29%) and Kalgo (2.24%) (Figure 2).

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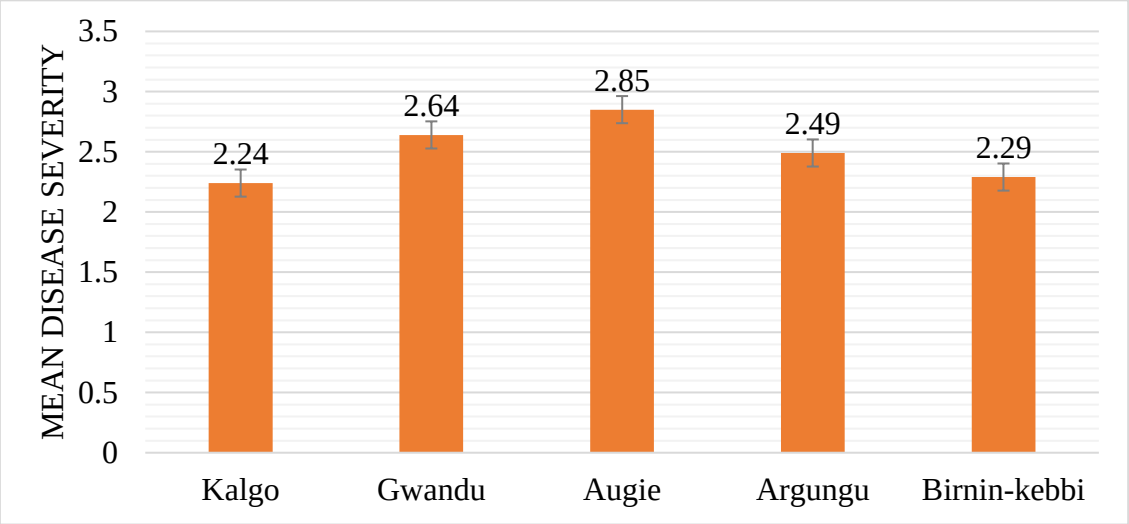

Figure 2**:** Mean Cassava mosaic disease severity in the five LGAs of Kebbi State. Bars indicate standard error at 5% level of probability

Begomoviruses Detected

Cassava leaf samples collected during the survey in 2023 were analyzed to detect the presence and distribution of the two major begomoviruses, *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) infecting cassava in five (5) LGAs of Kebbi State using polymerase Chains Reaction (PCR). The PCR results obtained showed that ACMV and EACMV occurred within the State but with significant (*P* = 0.05) variation in distribution.

Similarly, ACMV was detected in all the 5 LGAs where the sample were collected. The results revealed that, ACMV was significantly higher (*P* = 0.05) in Argungu (14.67%), followed by Birnin-Kebbi with (33.33%) which was statistically similar to Gwandu (23.81%) while Augie (22.22%) had the lower virus incidence. Kalgo LGAs recorded the least ACMV of (3.33%).

However, the cases is reversed in terms of EACMV, EACMV was only detected in Argungu, Gwandu and Augie LGA, but similar trends was also occurred as Argungu recorded the highest EACMV (16.76%) followed by Gwandu (14.29%) and Augie (11.11%) which was recorded third.

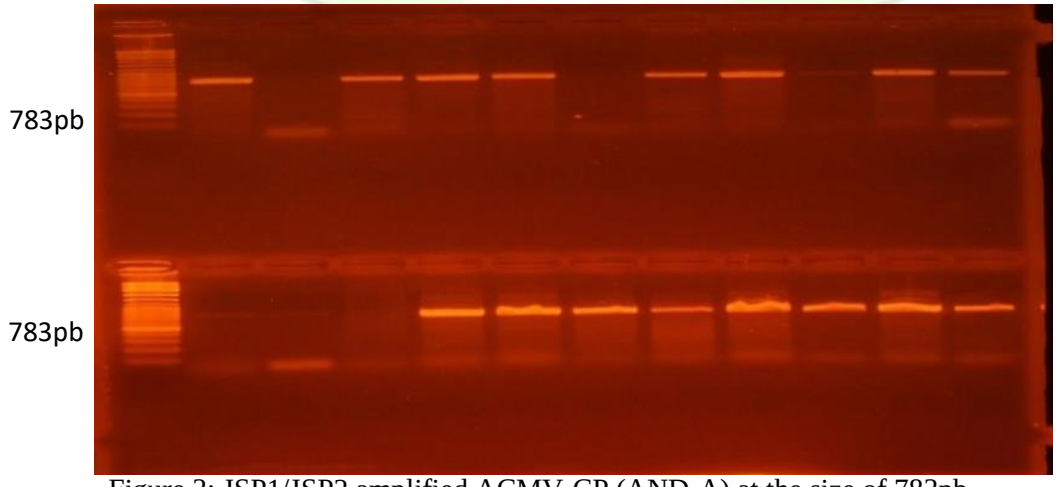

Figure 3: JSP1/JSP2 amplified ACMV-CP (AND-A) at the size of 783pb.

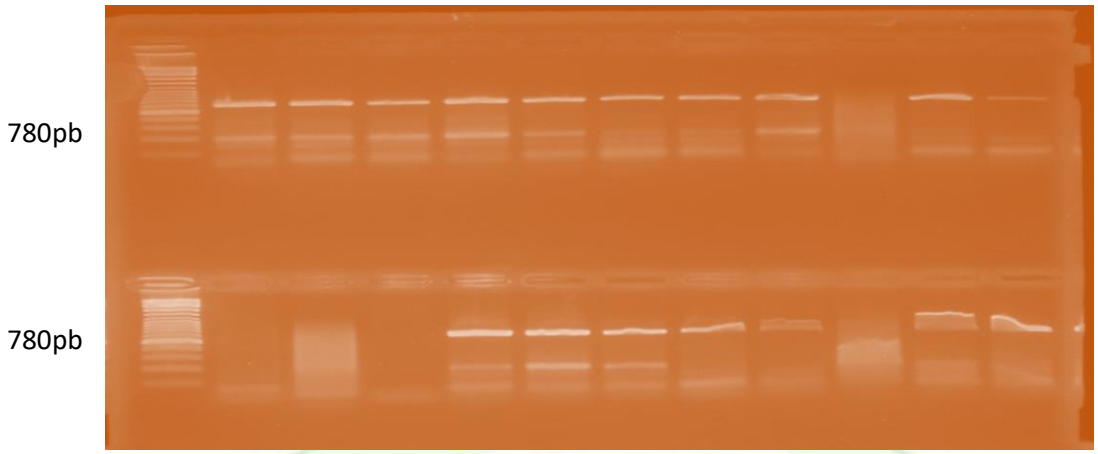

Figure 4: JSP1/JSP3 amplified EACMV-CP (AND-A) at the size of 780

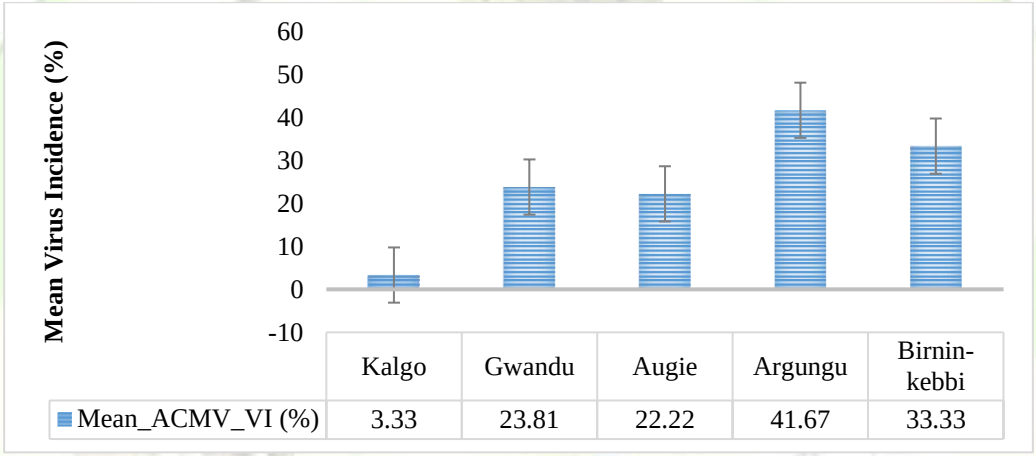

Figure 5: ACMV incidence in five Local Government Areas of Kebbi State during the 2023 field survey. Bars indicate standard error of means at 5 % probability level.

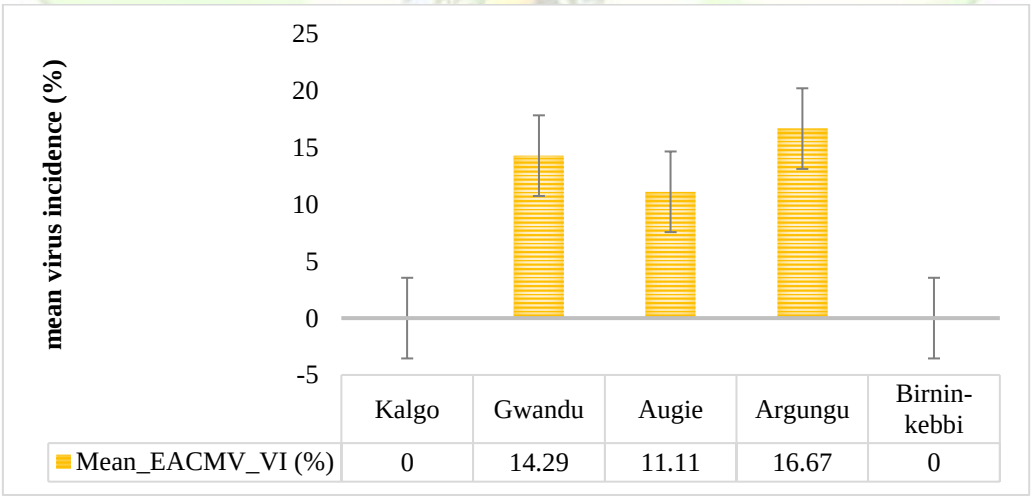

Figure 6: EACMV incidence in five local government Area of Kebbi State during the 2023 field surveyed. Bars indicate standard error of means at 5% probability level.

Discussion

cassava is one of the important staple root crops grown in many countries of Sub-Saharan Africa including Nigeria, the crop serves as a source of food, feed, and income and in various industrial applications (FAOSTAT, 2020), Africa account for >80% of the world's cassava-cultivated land while Nigeria takes the leads in Africa. CMD is one of the most devastating viral diseases infecting cassava in most of the growing regions in Africa. Africa account for about 50-80% of cassava fresh storage root yield losses due to *Cassava Mosaic Virus Disease* (Wor *et al*., 2004). (According to Legg *et al*., 2006), CMD account for more than 30 million tons of fresh storage root yields losses of cassava annually. *African cassava mosaic virus* and *East African cassava mosaic virus* (ACM-EACMV) co-infection can cause up to 82% crop loss in Africa (Winter *et al*., 2020) in Sub-Saharan Africa.

This study revealed the occurrence of incidence and symptoms severity and presence of ACMV and EACMV infecting cassava in five cassava growing regions of Kebbi State, Nigeria.

Diagnostically leaf mosaic pattern, chlorotic pattern and narrowing and distortions of leaf were the symptoms observed on cassava plants in each surveyed cassava field during the conduct of survey in 2023 from which ACMV and EACMV was detected. This is in agreement with the finding of Eni *et al*. (2020) and Badamasi *et al*. (2020) whose reported similar disease symptoms to be incited by ACMV and EACMV in North Central, Nigeria. This study showed the presence of CMD in Cassava field survey in the study area. However, higher incidence and symptoms severity of ACMV and EACMV recorded in Augie (59.99%), Argungu (56%) and Kalgo (54.67%) could be due to the attributed to the susceptible cassava varieties used by the farmers during planting (Musa *et al*., 2021), this was in contradicted with finding of (Mohammed *et al*., 2017) who reported that, cassava mosaic disease incidence increases through the used of infected cassava stem cuttings shared among the farmers. Therefore, this research recommended that, to mitigate the production constraint by the viral diseases, the use of only diseases free cutting as planting material should be use by the farmers, as well as management of the vector transmitting the diseases which will help minimize and mitigate the spread of the disease.

Conclusion

African cassava virus (ACMV) and East African cassava mosaic virus (EACMV) was detected in the LGAs of Kebbi survey during the study. The major factors responsible for CMD spread in the study area was as a result of the susceptibility of the cultivars used, using diseased cassava stem cuttings as a planting material by the farmers which when kept unchecked will results to CMD outbreak in the study area, State and country in general.

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