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Detection and Distribution of *Begomovirus* in Whiteflies (*Bemisia tabaci* Genn.) found Infesting Selected Crops and their Population in Northwest, Nigeria.

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Abstract

Vegetable, root and tuber crops are vital food components of many households in Nigeria. Whiteflies are among the most serious insect pests that transmits many species of *Begomovirus* to these crops. A research was conducted in northwest Nigeria to detect *Begomovirus* in whitefly collected on selected Vegetable, Root and Tuber crops and assess their population. A field survey was conducted during the 2016/2017 dry season in three (LGAs) of each State in Northwest Nigeria. During the survey, Adult whiteflies were counted and collected on Eggplant, Tomato, Cassava and Pepper. Polymerase Chain Reaction (PCR) was used to detect *Begomovirus* in whitefly samples. Results revealed a significant difference ($P<0.05$) in mean whiteflies population among the States. Zamfara State recorded the highest mean whiteflies (57), followed by Katsina (54), while Kano State recorded the lowest (16). Eggplant recorded the highest whiteflies (68), followed by Cassava (50) and Pepper recorded the least (14). Sokoto State recorded the highest *Begomovirus* incidence (88.8 %), Kaduna and Katsina States ranked second (77.7 %) while Zamfara State had the lowest (33.3 %). Cassava recorded the highest incidence (72.7 %), Tomato ranked second (58.3 %) and the lowest was recorded on Eggplant (38.5 %). This research confirmed the occurrence of *Begomovirus* in whiteflies in Northwest Nigeria. The study concluded the use of effective management strategies for the control of whitefly population such as field sanitation, use of resistant varieties and application of appropriate doses of insecticides as it mitigate the spread of the *virus* in the study areas.

Keywords: *Begomovirus*, Northwest, Population, Distribution, Whitefly

Introduction

Vegetable, root, and tuber crops are vital components of Sub-Saharan Africa's (SSA) food systems, particularly in humid and semi-humid zones (Brown *et al*., 2005). These crops provide essential nutrients, income, and employment opportunities for millions of people in SSA. In Nigeria, tomato is one of the most extensively cultivated vegetables in the northwest region, serving as a vital source of micronutrients (vitamins and minerals) and contributing significantly to poverty alleviation through income generation in both rural and urban areas (Brown *et al*., 2005). However, the yields of these crops remain significantly lower compared to

global standards due to biotic and abiotic stresses, including viral diseases (Brown *et al*., 2005).

Begomoviruses, are members of the family *Geminiviridae*, pose a significant threat to vegetable, root, and tuber crop productivity worldwide (Zerbini *et al*., 2017; Varsani *et al*., 2017). These viruses are transmitted exclusively by the whitefly vector (*Bemisia tabaci*), and can cause severe symptoms, including mosaic, yellow mosaic, leaf distortion, and stunting (Harrison *et al*., 1977; Stanley, 1985). The economic impact of Begomovirus diseases is substantial, with significant yield losses reported in various parts of the world. In Africa, Begomoviruses have been identified as a major constraints to crop production, particularly in countries with limited resources for disease management. With over 322 species, *Begomovirus* is the largest genus within the *Geminiviridae* family (Brown *et al*., 2015).

The whitefly vector plays a crucial role in the transmission and spread of Begomoviruses with about 200 species reported worldwide, there has been a rapid increase in whitefly populations, coupled with severe symptoms of viral diseases on vegetable, root, and tuber crops in Nigeria (Perring, 2001). This trend is alarming, given the potentials for Begomoviruses to spread rapidly and cause significant economic losses. To develop effective management strategies, it is essential to determine the population dynamics of whitefly and detect the presence or absence of Begomoviruses in the whiteflies that are the vectors of this virus. This study aimed to assess the whitefly population and presence or absence of Begomoviruses in whiteflies feeding on selected crops in northwest Nigeria.

Materials and Methods

Materials

The materials used for this research were mainly survey and laboratory materials which include Global Positioning System (GPS) receptor for recording coordinates (Longitude and Latitude), suctioning tube for collecting whitefly samples from the leaves of crops, Eppendorf tube, 70% Ethanol, Paraffin, Centrifuge Machine and Thermo-cycling Machine.

Methods

Field Survey

A field survey was conducted during the dry season of 20016/2017 in seven (7) States of North-west, Nigeria (Kebbi, Sokoto, Zamfara, Kano, Kaduna, Katsina and Jigawa States), three (3) Local Governments Areas (LGAs) were purposively selected in each State, fields were sampled from each LGA and a distance of 10 km apart from one field to another was maintained giving the total number of sixty three (63) fields surveyed. Eggplant (*Solanum melongena*), Tomato (*Solanum lycopersicum*), Cassava (*Manihot esculenta*) and Pepper (*Capsicum annuum*) crops were selected for whitefly population estimation and collection during the survey.

Estimation of Whitefly Population and Collection

Evaluation of whitefly population in a field was achieved by direct visual counting of adult whitefly on five youngest apical leaves of the shoots of each of the 30 plants sampled because the adults feed preferentially on the youngest immature leaves. Due to differences in branching habits of different crops sampled, 3 shoots per plant were chosen and total number of whitefly were taken and calculated to obtained average number of whitefly per leaf (Ndunguru *et al.*, 2009). Whitefly count was done either in the morning or late in the evening when the temperature is low.

Sample Collection and Handling

Adult whiteflies found feeding on the leaves of Eggplant, Tomato, Cassava and Pepper were collected using the Aspirator. Whitefly samples collected from each of the selected crop were preserved separately in an Eppendorf tube containing 70% ethanol which was taken to Molecular Biology Laboratory of Kebbi State University of Science and Technology, Aliero (KSUSTA). The samples were stored in a refrigerator at a temperature of 20^{o}C before the commencement of the Molecular analysis.

Nucleic acid isolation (DNA) from whitefly samples

Isolation of whitefly DNA was achieved using the Chelex Protocol of Musapa *et al.* (2013), with little modification. 10 g of Chelex was weighed and added into a bottle contained 90 ml of sterile distilled water, the mixture was thoroughly shacked until the Chelex was completely dissolved. A single whitefly was placed into fresh 1.5 ml Eppendorf tubes in which 300 ml of 10% Chelex solution was added (10 g Chelex, 90 ml DH_2O). The tubes were vortexed for 10 seconds and incubated in water bath for 5 minutes, the mixture was vortexed again and centrifuged at 13,000 rpm for 5 minutes in non-refrigerate Micro-Centrifuging Machine. The tubes were incubated in heating block at 95^{o}C for 10 minutes and subsequently spun for 10 seconds. The supernatant was decant into new 1.5 ml Eppendorf and stored at -4^{o}C prior to PCR analysis.

Molecular detection of *Begomovirus*

Detection of *Begomovirus* from whitefly samples was achieved using Polymerase Chain Reaction (PCR). Universal Primer for the detection of *Begomoviruses* was used (Table 1) as described by Lodhi *et al.* (1994). The PCR was conducted with the reaction mixture of 25 µl (Table 2) which comprised of 2.5 µl of 10xPCR buffer (Thermo Scientific UK), 0.5mM of dNTPs, $MgCl_2$ 2.5Mm, 1.0µl of forward and reverse Primer, enzyme 0.2µl, 15.3µl of sterilized distilled water (SDH_2O) and 2.0µl DNA template.

Table 1: Primer set

Primer Name	Primer Sequence (5'-3')	Product Size	Reference
MT10/C1-J-2195	TTGATTTTTTGGTCATCCAGAAGT	850bp	Frohlich *et al.*, 1999
MT12/L2-N-3014	TCCAATGCACTAATCTGCCATATTA		

Virus Incidence

Virus Incidence (VI) in percentage was calculated using the formula as described by Chaube and Pundhir (2005). This was achieved after Laboratory analysis of the whitefly samples collected where the number of whitefly samples tested positive of *Begomovirus* and were recorded according to locations and thereafter percentage incidence was calculated using the formula below.

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

Table 2: PCR master mix

Reagents	Composition 1 X (µl)	Composition 63 X (µl)
10x PCR buffer	2.5	157.5
dNTPs (20 mM)	0.5	31.5
$MgCl_2$ (2.5 Mm)	2.5	157.5
MT10/C1-J-2195	1.0	63
MT12/L2-N-3014	1.0	63
Enzyme	0.2	12.6
DH_2O	15.3	963.9
DNA Template(1:00)	2.0	126
Total	**25.0**	**1575**

Table 3: PCR Conditions

Steps	Temperature (°C)	Time	Number of cycle
Initial denaturation	94	5 min	
Final denaturation	94	30 sec	
Annealing	56	35 sec	x35 cycles
Initial extension	72	30 sec	
Final extension	72	7 min	
	Held at 4°C		

Gel Electrophoresis

PCR products were subjected to Electrophoresis on 1.5 (w/v) Agarose. The gel was prepared by dissolving 1.5 (w/v) of agarose in 100 ml of 0.5 TBE buffer (0.045 M Tris-borate, 0.5 mM EDTA pH 8). The agarose-buffer solution was heated in a Microwave oven for 5 minutes and was cooled down to 40°C before pouring in a Gel Tray that was fitted with a Gel Comb. The solution was allowed to solidify for 30 minutes before loading the samples into the wells after 0.5ug/ml of Ethidium bromide solution was added as a gel staining agent. About 15 µl of samples were mixed with 5µl of 5x blue gel loading dye and loaded into the wells. About 5 µl DNA marker was loaded into first and last wells. Electrophoresis was performed at 80V for 1hour and the bands were visualized under the UV light (Syngene, UK).

Data analysis

Data generated on whitefly population were analyzed using IBMSPSS Statistics software version 20. Descriptive Statistics tools such as means, percentages, and standard deviation were used to determine significance of the results (Gomez and Gomez, 1984).

Results

Mean whitefly population per State

There was a significant difference ($P = 0.05$) in terms of mean whitefly population among

the seven States of North-western Nigeria. The highest mean whitefly population was recorded in Zamfara State which average 57 whiteflies per leaf, this was followed by Katsina State (54) while, Kano State recorded the lowest mean whitefly population with an average of 16 whiteflies per leaf (fig. 1).

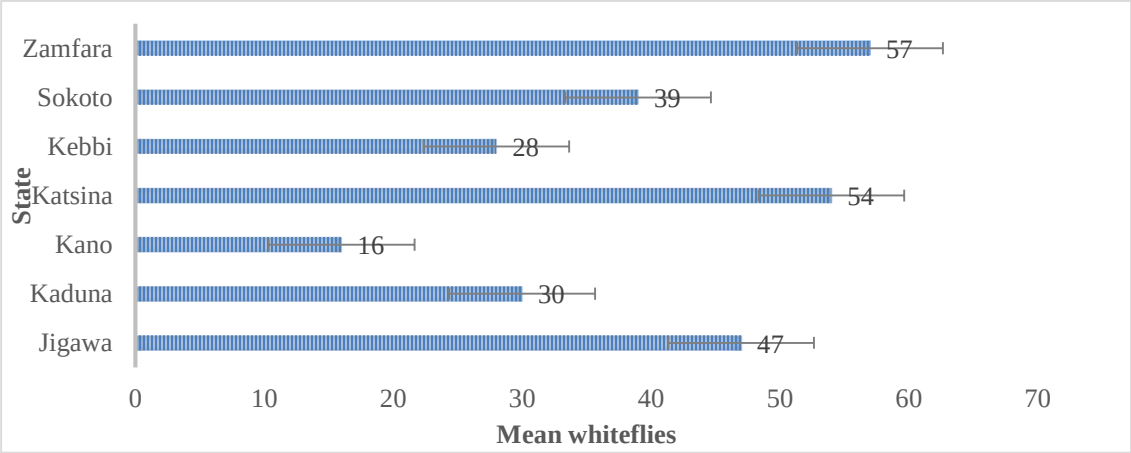

Figure 1: Mean whitefly population per State. Bars indicate Standard Error of means at 5% probability level.

Mean whitefly population per crop

Figure 2 shows mean whitefly population per leaf of the four (4) crops sampled in the seven States of North-western States of Nigeria. Eggplant recorded the highest mean whitefly population which average 68 whiteflies per leaf, this is followed by cassava (50) while, pepper recorded the lowest mean whitefly population which average 14 whiteflies per leaf.

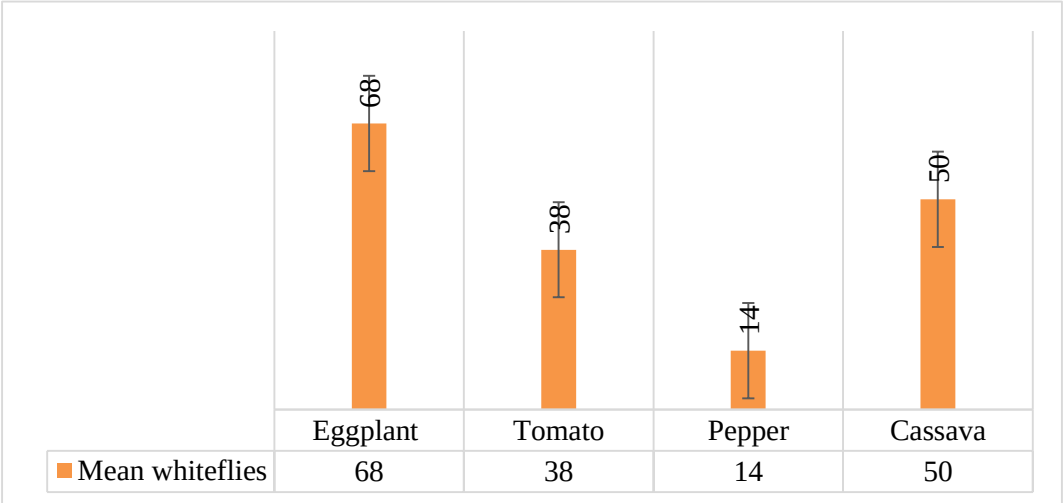

Figure 2: Mean whitefly population per crop. Bars indicate standard error of means at 5% probability level.

Mean *Begomovirus* incidence per State

There was significant (P = 0.05) difference in terms of *Begomovirus* incidence detected from whitefly found infesting selected crops among the seven (7) states of North-western Nigeria. Sokoto State recorded the highest

Begomovirus incidence (88.8%), followed by Kaduna and Katsina States that recorded 77.7%; while, Zamfara State recorded the lowest *Begomovirus* incidence (33.3%) (Fig.3).

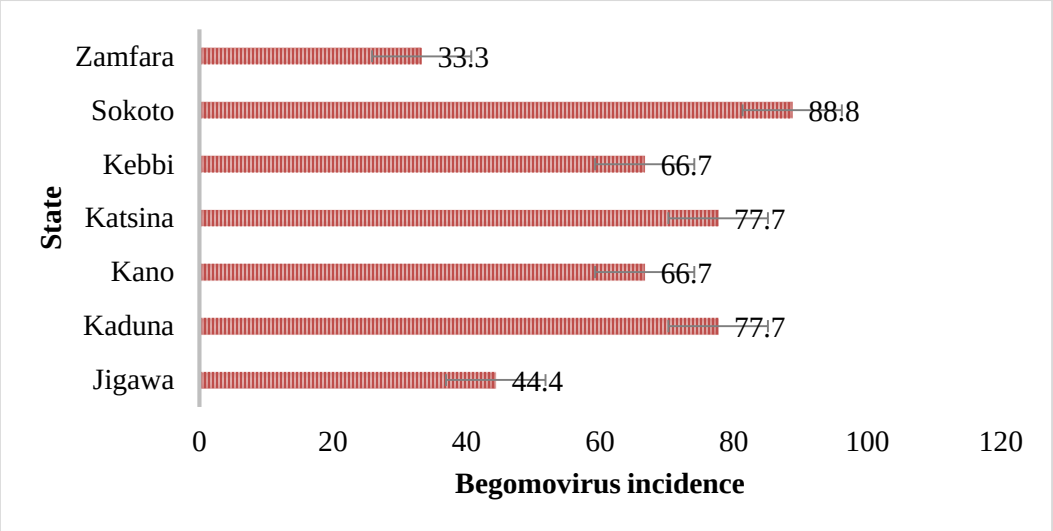

Figure 3: Mean *Begomovirus* incidence per state. Bars indicate standard error of means at 5% probability level.

Mean *Begomovirus* incidence among whiteflies collected from selected crops

Figure 4 shows mean *Begomovirus* incidence of whitefly samples collected from eggplant, cassava, tomato and pepper. Whitefly samples collected from cassava recorded the highest *Begomovirus* incidence (72.7%) as the percentage of whitefly samples tested positive to *Begomovirus* that were collected from cassava plants; this is followed by whitefly samples collected from tomato plants (58.3%), while, samples collected from eggplant recorded the lowest *Begomovirus* incidence (38.5%) (Fig. 4).

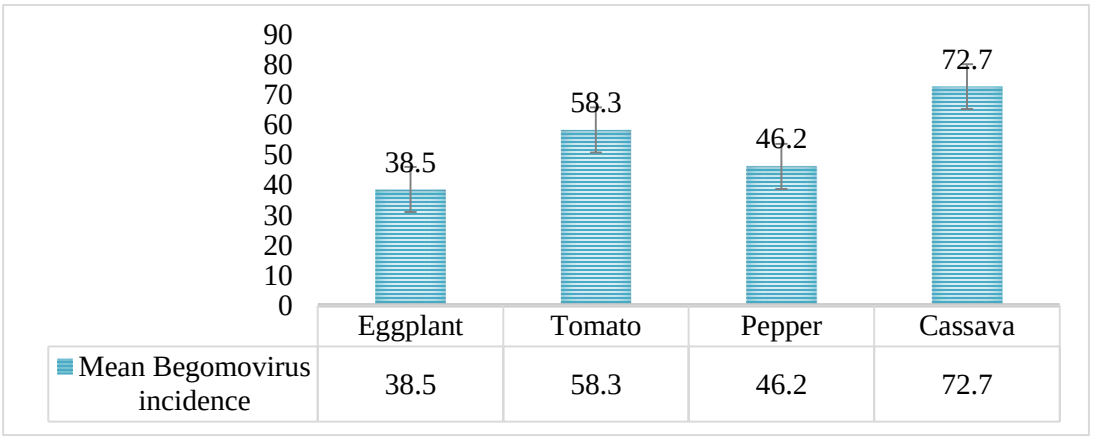

Figure 4: Mean *Begomovirus* incidence among whiteflies collected from selected crops. Bars indicate Standard Error of Means at 5% probability level.

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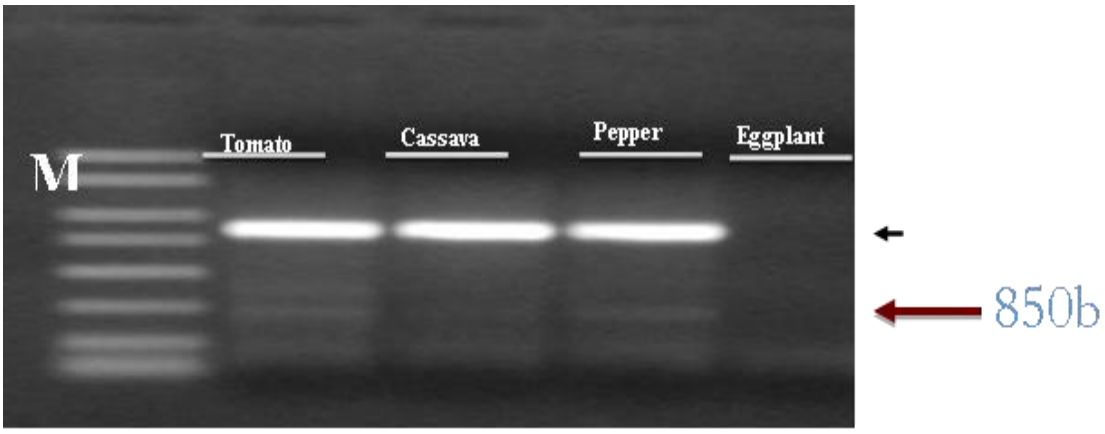

Figure 5: A gel picture showing detected *Begomovirus* in whitefly found infesting Tomato, Cassava, Pepper and Eggplant using Universal Primer MT10/C1-J-2195 and MT12/L2-N-3014, the size of the nucleotides was 850 Base Pair (bp) and the Molecular Weight Marker (M) at the left-hand side of the gel = 1kb.

Distribution of *Begomovirus* in the Study Areas

Figure 6 shows the distribution of *Begomovirus* in the study areas. *Begomovirus* occurred in all the study areas with variation in its distribution. *Begomovirus* was distributed in almost all the locations surveyed in Sokoto State when compared to Jigawa State (Fig. 6).

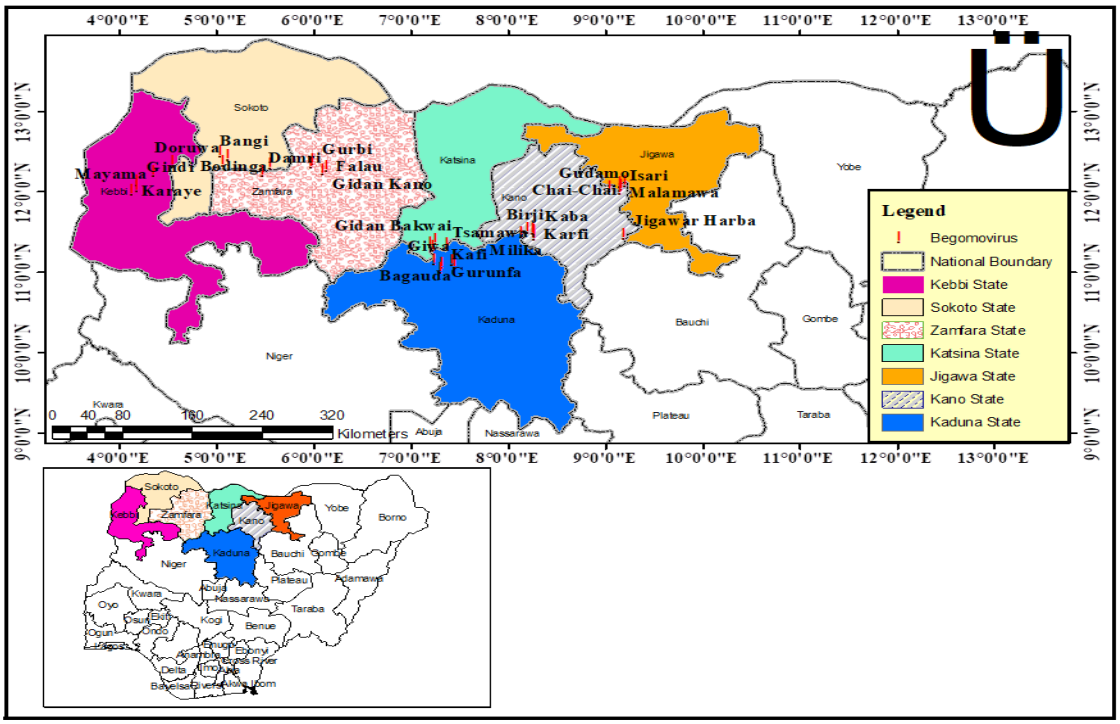

Figure 6: A map showing distribution of *Begomovirus* in the study areas

Discussion

Population of whitefly infesting Cassava, Tomato, Eggplant and Pepper; *Begomovirus* incidence in seven States of North-western Nigeria and among the whitefly samples collected from the selected crops were investigated in this study. The results showed that there is whitefly infestations in each of the three Local Government Areas surveyed in all the seven States of North-western Nigeria. Population of whitefly differs significantly from one State to another and from one crop to another. Higher population of whitefly recorded in Zamfara State, likely due to the favourable environmental conditions as observed during the field survey might be the factor responsible for high population of whitefly in Zamfara State as most of the farmers' fields surveyed were under irrigation farming during the study periods. Sseruwagi *et al.* (2004) supported this results that environmental conditions significantly influenced population of whitefly. Banjo *et al.* (2003) also reported that agro-climatological factors greatly influenced population of whitefly. Among the vegetable, root and tuber crops selected for this study, eggplant recorded the highest whitefly population and infestation. The high whitefly infestation of eggplant observed during the field survey can be attributed to preference of whitefly to eggplant. This is supported by Legg *et al.* (2013), who reported that whitefly have preference on the type of crop plant it infests. Banjo and Banjo, (2003), reported that population and spread of whitefly can be greatly influenced by human traffics, farmers activities, rainfall and flush of new leaves. This work also agrees with the works of (Asiwe *et al.*, 2002) who also reported that population of whitefly was influenced by climatic conditions and whitefly preference of the crops. Musa *et al*. (2021), also found out that whitefly was found infesting cassava in Taraba State, a Northern part of Nigeria.

Findings in this research revealed that whitefly vectored *Begomoviruses* and therefore spread these viruses from the infected crop to the healthy ones and from infected farm to healthy one. High incidence of *Begomovirus* was recorded in Sokoto State while the lowest incidence of *Begomovirus* was recorded in Zamfara State. This indicated that there is no correlation between population of whitefly and incidence of *Begomovirus*. The high incidence of *Begomovirus* recorded in Sokoto State might be attributed to the presence of many infected crops in the area at the time of this field survey was similar to the research conducted by Maruthi *et al*. (2002) with a similar works by Fauquet and Stanley (2004). High incidence of *Begomovirus* was recorded from whitefly samples collected from cassava plant which can be attributed to high incidence of Cassava Mosaic Diseases (CMD) in the study areas which caused by *Begomovirus*. Musa *et al*. (2021), reported that CMD is common in anywhere cassava is grown.

From the findings of this research, it can be concluded that there is whitefly infestations in the study areas and that whitefly was found infesting eggplant, cassava, pepper and tomato vectored *Begomovirus* infecting these crops in the study areas.

Conclusion

Based on the findings of this research, it can be concluded that, adult whiteflies infestation on cassava, tomato, eggplant and pepper occurred in the study area and such insects vectored *Begomovirus* responsible for the viral disease. Furthermore, the PCR results showed the presence of *Begomovirus*

in all the states where the survey was conducted.

Therefore, the rapid potential of *Begomovirus* and whitefly population coupled with their polyphagous feeding behavior can lead to the emergence of damage of such crops. This occurrence of *Begomovirus* and whitefly population may quickly explain the rate at which the diseases can be spread in the farmer's fields, which may eventually lead to a reasonable yield loss or total crop failure.

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