SEROLOGICAL DETECTION AND DISTRIBUTION OF VIRUSES ASSOCIATED WITH LIMA BEANS (*Phaseolus lunatus* L.) IN KADUNA STATE, NIGERIA

ABSTRACT

Leaf samples of *Phaseolus lunatus* L. were collected from Zango-Kataf and Kaura Local Government Areas (LGAs) of Kaduna State. Triple Antibody Sandwich and Double Antibody Sandwich Enzyme Linked Immunosorbent assay (TAS and DAS ELISA) procedures were employed for the detection of *Bean golden mosaic virus, Bean common mosaic virus, Bean yellow mosaic virus* and *Cucumber mosaic virus*, to determine their incidence and distribution. The results of the tests showed that these four viruses were associated with *Phaseolus lunatus* L. in the study area except, *Bean yellow mosaic virus* that was not detected in Kaura LGA. The viral disease with the highest incidence in the LGAs was *Bean golden mosaic virus* (BGMV) with 44.4 and 38.3% for Zango-Kataf and Kaura LGAs, respectively. It is recommended that extensive studies be carried out on the incidence, distribution and economic status of these viruses in other states of the country where the crop is grown.

Keywords: Virus diseases, incidence, ELISA, lima beans.

INTRODUCTION

Beans are among the most cultivated plants and are commonly eaten fresh or dried particularly *Phaseolus,* and it originated from the Americans. Lima beans (*Phaseolus lunatus* L.), is among the five kinds of *Phaseolus* beans that was domesticated by pre-Columbian farmers (Kaplan, 2008). Beans generally are an important source of protein and fibre around the world. They have a short growing season and can adapt to different cropping systems. Bean crops often play a key role in crop rotation and intercropping due to their ability to fix nitrogen (Timko *et al.*, 2007). They also play an important role in human nutrition, animal

feed and the industrial production of citric acid (Todorović *et al*., 2008). Lima bean being edible is a cheaper source of protein compared with the expensive animal protein sources such as egg, meat and fish (Lyman *et al*., 1985).

Reduced yields of this crop may arise as a result of errors in agro techniques, which are even greater when weather conditions are unfavorable for its growth and development (Vasić, 2003). Other possible causes of low yield are the numerous pathogenic microorganisms such as fungi and bacteria, viruses and nematodes. The damages caused by phytopathogenic virus attack are manifested primarily in reduced yield of infected plants, premature decline, extinction of infected plants, and poor quality products from those plants (Babovic, 2003).

Throughout the world, the most important causal agents of viral diseases reported on beans are *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Cucumber mosaic virus* (CMV) and *Alfalfa mosaic virus* (AMV) (Petrović, 2008). All these viruses can cause great damage to plant production, especially when vector population on the bean plants is high (Spence and Walkey, 1995).

Despite the great potential of lima beans, it is highly underutilized in Nigeria. More so, from the basis of symptoms observed on bean plants which are mainly attributed to virus diseases, range from various forms of mosaic to distortions (Thottappilly and Rossel, 1997). Some viral diseases are being suspected to be associated with lima beans in the southern region of Kaduna State, Nigeria. They include *Bean golden mosaic virus* (BGMV), *Bean yellow mosaic virus* (BYMV), *Bean common mosaic virus* (BCMV) and *Cucumber mosaic virus* (CMV).

The occurrence of these viruses can lead to major drawbacks in its cultivation and unfortunately their incidence and distribution had not been determined through deliberate studies in Nigeria. Lima beans has not also received the benefits of intensive research

programme as devoted to cowpea and soyabean where early detection of viral infection can guarantee the initiation of appropriate management measures become difficult. Thus, there is need to unravel the prevalence of these viral diseases in the area that will help in their management and lead to increase in yield of the crop. Therefore, this study was carried out to identify the viruses associated with *Phaseolus lunatus* in Zango-kataf and Kaura Local Government Areas of Kaduna State, Nigeria.

MATERIALS AND METHODS

Collection of Samples

The survey sites were visited in November 2012. Leaf samples were collected from Zango-Kataf and Kaura Local Government Areas of Kaduna State (Fig. 1). Three locations in each of the LGAs were visited for sample collection; Zonkwa, Mabushi and Samaru in Zango-Kataf and Mallagum, Manchok and Kagoro in Kaura LGAs respectively.

Figure 1: Kaduna State map showing the Local Government Areas where samples were collected.

Sampling within each field was done by picking leaf samples from quadrants of 4 x 4 m^2. The samples were placed in a properly labeled polyethylene bag and taken to the Virology Laboratory in the Department of Crop Protection, Ahmadu Bello University, Zaria for analyses.

Preparation of Samples

Thirty eight leaf samples were tested for the presence of *Bean golden mosaic virus* (BGMV), *Bean yellow mosaic virus* (BYMV), *Bean common mosaic virus* (BCMV) and *Cucumber mosaic virus* (CMV). One gramme each of leaf samples was ground into 10 ml of sample extraction buffer in a sterile mortar and pestle until a uniform consistency was obtained.

Laboratory analyses of samples

Triple Antibody Sandwich Enzyme-linked immunosorbent assay (TAS-ELISA) was carried out for the serological detection of *Bean golden mosaic virus* (BGMV) and *Bean common mosaic virus* (BCMV) while, Double Antibody Sandwich Enzyme-linked immunosorbent assay (DAS-ELISA) was also used for the detection of *Cucumber mosaic virus* (CMV) and *Bean yellow mosaic virus* (BYMV).

Serological procedure for DAS-ELISA

Polyclonal antibody (PSA 44501/0096) was diluted in a coating buffer containing 1.59 g of Sodium carbonate, 2.93 g of Sodium bicarbonate, and 0.20 g of Sodium azide in one litre of sterile water with pH 9.6 at a dilution ratio of 1:100. One hundred microlitres of the mixture was introduced into the wells of the ELISA plate leaving out the border wells.

The coated plate was incubated at 37℃ for 2 hr and washed 3 times using phosphate buffered saline-Tween 20 (PBS-T) afterwards. The plates were tap dried using paper towel, and 100 µl of antigen of each sample ground 1;10 (w/v) in sample extraction buffer made up of PBS-Tween 20 and 2 % PVP at pH 8.5 was pipetted into the test wells and incubated at 4℃ overnight.

The next day, the content of the test wells was poured out and washed 3 times using PBS-T as earlier stated. The conjugate antibody (PSA 44501/0096) was diluted in a conjugate buffer containing 0.2 % egg albumin in sample extraction buffer at 1:100 and used as trapping antibody. One hundred microlitres of the mixture was introduced into each coated well and incubated at 37℃ for 2 hr. The contents of the test wells were then poured out and washed 3 times using PBS-T afterwards. The substrate p-Nitro-phenyl phosphate (Sigma 104-105) tablet was dissolved in 1:1 w/v substrate buffer made of 0.2 g Sodium azide, 1 litre of sterile water and 97 ml diethanolamine at pH 9.8 and 100 µl of the mixture was introduced into the test wells.

After incubation at room temperature in the dark, the plate was read using an ELISA reader to obtain the absorbance values at A_{405nm} wavelength. Absorbance values that were twice the value of the negative control were considered positive as described by Kumar (2009).

Serological procedure for TAS-ELISA

Polyclonal antibody (AS-0616) was diluted in a coating buffer containing 1.59 g of Sodium carbonate, 2.93 g of Sodium bicarbonate, and 0.20 g of Sodium azide in one litre of sterile water at pH 9.6 at a dilution ratio of 1:1000. One hundred microlitres of the mixture were introduced into the wells of the ELISA plate leaving out the border wells.

Each of the coated plate was incubated at 37℃ for 2 hr and washed 3 times using phosphate buffered saline-Tween 20 (PBS-T) afterwards. The plates were tap dried using paper towel, and 100 µl of antigen sample ground in 1:10 (w/v) in the sample extraction buffer containing PBS-Tween 20 and 2 % PVP at pH 8.5 was pipetted into the test wells and incubated at 4℃ overnight.

The next day, the content of the test wells was poured out and washed 3 times using PBS-T as earlier stated. The monoclonal antibody (AS-616/2) was diluted in a conjugate buffer at a dilution ratio 1:500. One hundred microlitres of the mixture was introduced into the test wells

and incubated at 37^{o}C for 2 hr; the content of the test wells were poured out and washed 3 times using PBS-T. The conjugate antibody (RAM-AP) was diluted in a conjugate buffer containing 0.2 % egg albumin in sample extraction buffer at dilution ratio of 1:1000 and used as trapping antibody. One hundred microlitres of the mixture was introduced into each test well and incubated at 37℃ for 2 hr. The contents of the test wells were then poured out and washed 3 times using PBS-T afterwards. The substrate p-Nitro-phenyl phosphate (Sigma 104-105) tablet was dissolved in 1:1 w/v substrate buffer containing 0.2 g Sodium azide. One litre of sterile water and 97 ml diethanolamine at pH 9.8 and 100 µl of mixture was introduced into the test wells. After incubation at room temperature in the dark for 1 hr, the plate was read using an ELISA reader to obtain the absorbance values at A_{405nm} wavelength. Absorbance values that were twice the value of the negative control were considered positive as described by Kumar (2009).

Data analysis

The results from the tests were analyzed using Analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2008) at P=0.05. Means were separated using Least Significance Difference (LSD).

RESULTS

Disease incidence

Disease incidence of *Bean goden mosaic virus* (BGMV) in all the locations where leaf samples were collected varied significantly (p=0.05) (Fig. 2). The highest incidence of 75 % elicited by BGMV was recorded at Kagoro followed by Samaru with 62.5 %. This was significantly different from the incidence values from the other study locations at $p< 0.05$. However, there were no significant differences in BGMV incidence among the other locations and the virus was not detected in Manchok.

For Bean common mosaic virus, Mallagum had the highest incidence caused by BCMV of 20 %, which was significantly different p=0.05 from the incidence at Samaru of 12.5 % and the virus was not detected in the other locations (Fig. 3).

The incidence of *Bean yellow mosaic virus* (BYMV) was highest at Zonkwa with 33.3 % followed by that at Samaru with 23.8 %. However, the differences in incidence of BYMV between the two locations were not significantly different (p<0.05) and the virus was not detected from the other locations (Fig. 4).

From the ELISA test, *Cucumber mosaic virus* (CMV) was detected in the leaf samples collected from all the survey locations (Fig. 5). Zonkwa location had the highest incidence of 66.7 % incited by CMV, followed by that at Manchok of 33.3 %. There were significant differences in CMV incidence between Zonkwa, Manchok (p=0.05) respectively, while its incidence in the other locations was not significantly different (Fig. 5).

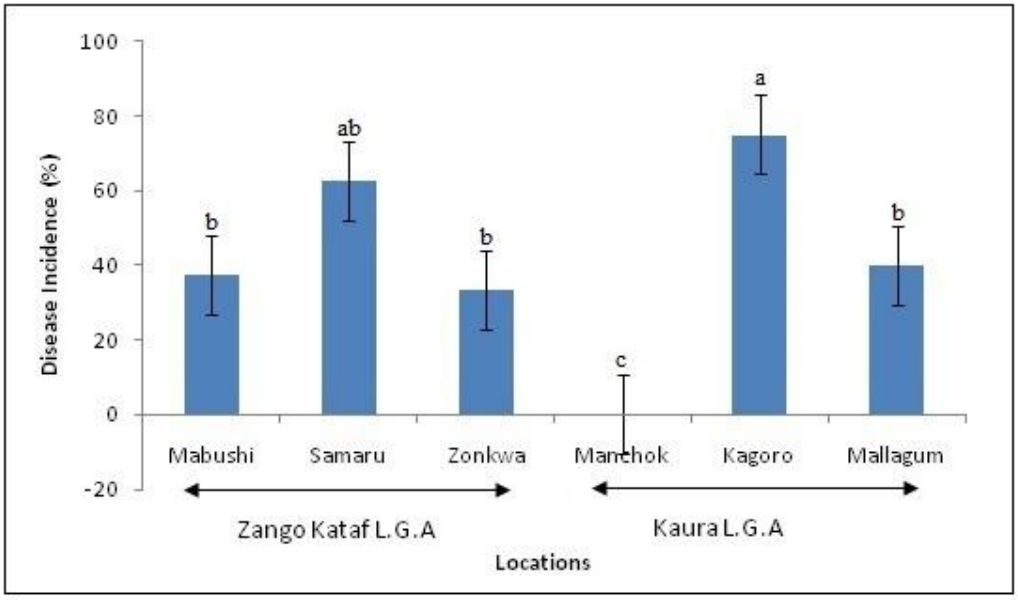

Figure 2: Incidence of *Bean golden mosaic virus* in Zango-Kataf and Kaura Local Government Areas in Kaduna State, 2012

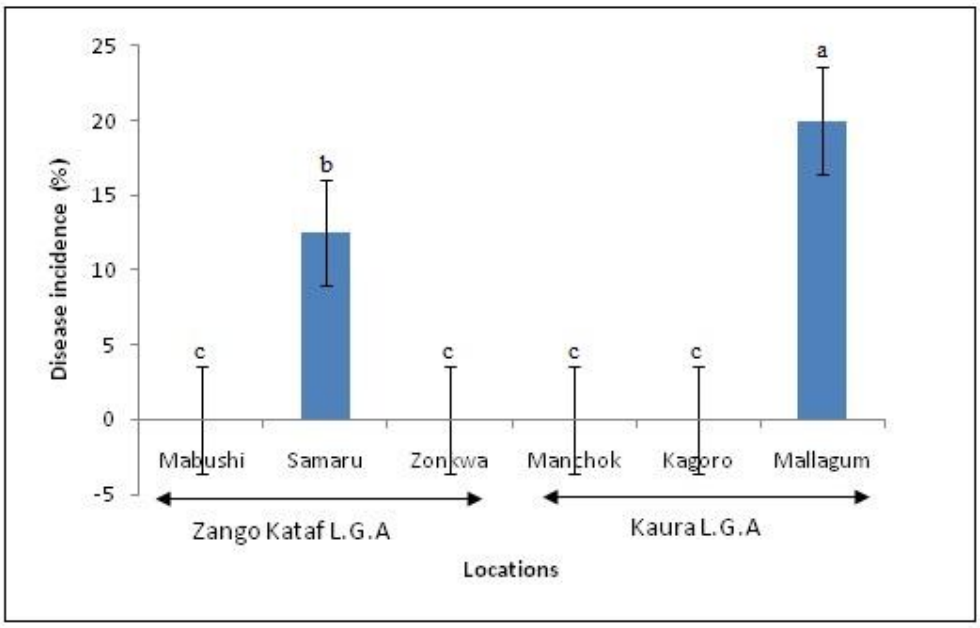

Figure 3: Incidence of *Bean common mosaic virus* in Zango-Kataf and Kaura Local Government Areas of Kaduna State, 2012.

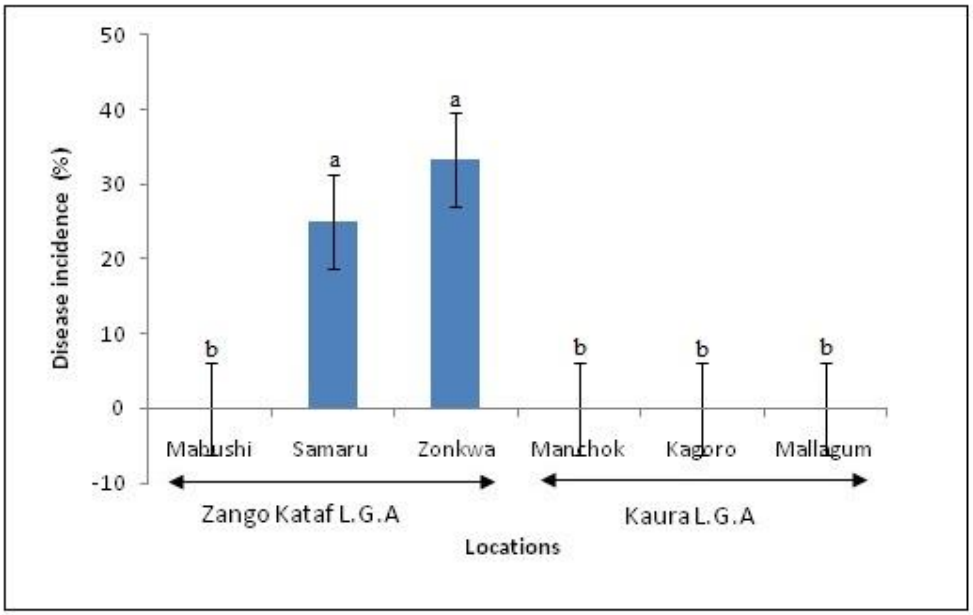

Figure 4: Incidence of *Bean yellow mosaic virus* in Zango-Kataf and Kaura Local Government Areas of Kaduna State, 2012.

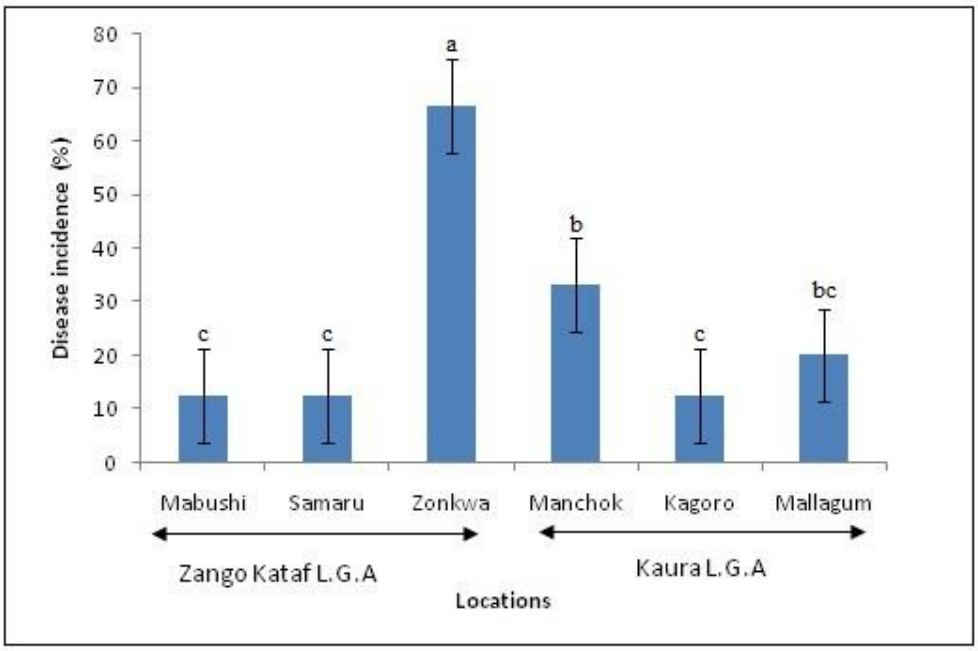

Figure 5: Incidence of *Cucumber mosaic virus* in Zango Kataf and Kaura Local Government Areas of Kaduna State, 2012.

The combined incidence of the four virus diseases, BGMV, BCMV, BYMV and CMV tested for in the leaf samples collected from Zango-Kataf and Kaura Local Government Areas is depicted in Fig. 6. In Zango-Kataf LGA, BGMV recorded incidence of 37.5 %, while 21.1 % incidence was recorded for CMV and 15.8 % and 5.3 % incidences were recorded for BYMV and BCMV respectively. In Kaura LGA, BGMV had 50 % incidence, while 18.8 % and 6.3 % incidences were recorded for CMV and BCMV respectively and there was no incidence of BYMV in the local government (Fig. 6).

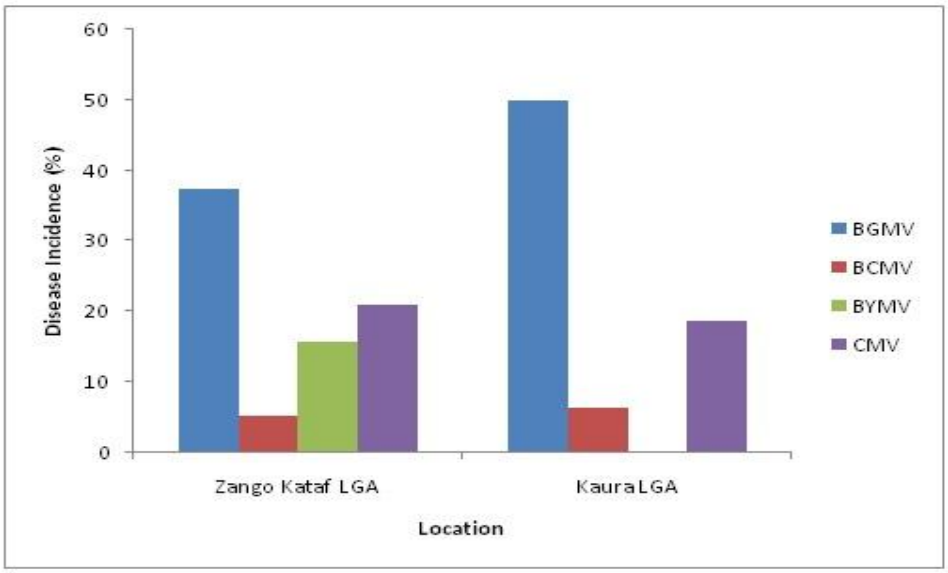

Figure 6: Incidence of the four virus diseases in Zango-Kataf and Kaura Local Government Areas of Kaduna State, 2012

DISCUSSION

Lima bean (*Phaseolus lunatus*) is relatively free of important diseases and pests, although it is susceptible to many of the same maladies that cause devastating losses to beans (*Phaseolus vulgaris*) in Africa (Rachie and Rockwood, 1972). Based on the symptoms observed during the present study and which are similar to the symptoms described by Hadegorn *and* Ingilis (1986), the following four viruses: BGMV, BCMV, BYMV and CMV were suspected to be associated with Lima beans. In order to prove the presence of the four viruses, serological tests with DAS-ELISA and TAS-ELISA were employed in the laboratory analyses of the Lima beans leaf samples collected from the locations in Zango-Kataf and Kaura LGAs which showed that the viruses affected *Phaseolus lunatus* L in the two LGAs.

The four viruses encountered in the present work had been reported on beans generally. Thus, BGMV was reported as a viral disease of *P. lunatus* by Schwartz *et al* (2005). Similarly, CMV is a naturally occurring virus of *P. lunatus* in Nigeria as stated by Taiwo and Shoyinka (1988). The results of the current study confirm earlier reports of *P. lunatus* being host to BGMV and CMV and also being present in the two LGAs of Kaduna State, in North western

Nigeria. The implication of this finding as reported by Babovic (2003), is that damages caused by phytopathogenic virus attack are manifested primarily in reduced yield of infected plants, premature decline, extinction of infected plants, and poor quality products from those plants, lima bean production in the surveyed areas can be limited if adequate management tools are not initiated quickly.

Similarly, BYMV, CMV and BCMV are reported to be among the naturally occurring and most common viruses of the *Phaseolus* spp. in Africa (Brunt *et al*., 1996). This study recorded BCMV and BYMV as viral diseases of *P. lunatus* in Zango-Kataf and Kaura LGAs of Kaduna State, Nigeria. Thus, lima bean production in the two LGAs by farmers can further be hampered if proactive preventive measures are not taken by appropriate extension agents.

Also, from the results showing the distribution of these viruses, BGMV, BYMV, BCMV and CMV were found in the two LGAs except, in Kaura LGA where BYMV was not observed and recorded. This brings to the fore the importance of these viruses in *P. lunatus* production by farmers. It means that when vector populations and epiphytotic levels of these viruses occur, farmers of these areas can hardly harvest their lima bean crops as the result of severe damage and subsequent total crop loss as earlier reported by Babovic (2003).

In Nigeria, unfortunately, Lima bean has not received the same attention in research and focus as other members of the bean family. However, considering the nutritional significance, as well as its market intended prospects at local and international levels, there is an urgent need for more research in the improvement, cultivation and the upgrading of the present status of the crop. The present study has also revealed the necessity for increased research on virus studies on lima bean in the surveyed areas of Kaduna state. Such as restriction enzyme

studies to determine the type of viruses, either DNA or RNA viruses and the determination of the detected viruses at genomic levels.

Although, the present study was limited in scope, it can be extended into other areas where the crop is grown or is to be promoted. Similarly, an understanding of variety responses of the crop to the viruses detected, as well as the interaction among viruses at the host-pathogen levels can prove to be instrumental in their management in the lima bean pathosystem.

CONCLUSION AND RECOMMENDATIONS

Four viruses namely *Bean common mosaic virus, Bean yellow mosaic virus, Bean golden mosaic virus* and *Cucumber mosaic virus* infect lima beans as revealed by the results of the serological tests conducted on leaf samples collected from different locations in the Zango-Kataf and Kaura LGAs of Kaduna State.

The results from this study can serve as ~~a~~ background information for in-depth research on the distribution of these viruses in other lima bean-producing areas of Nigeria.

The present information on the distribution of these viruses in the surveyed areas can also lead to studies on their incidence, economic importance, and to the development of adequate management options that will inhibit their further spread. Such information will bring about increased and profitable cultivation of lima beans not only in Kaduna State, but the whole of Nigeria.

The present study has revealed the necessity for increased research on virus studies on lima bean in the surveyed areas of Kaduna state. Such studies like restriction enzyme assay to determine the type of viruses, either DNA or RNA viruses and the determination of the detected viruses at genomic levels are to be carried out.

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