Serological Detection and Distribution of Viruses Associated With Lima Beans (*Phaseolus lunatus* L.) in Kaduna State, Nigeria

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Original Research Article

ABSTRACT

The survey sites used in the present work were visited in November 2012. Leaf samples were collected from Zango-Kataf and Kaura Local Government Areas of Kaduna State. Three locations each were visited for sample collection for serological test for viruses in Zonkwa, Mabushi and Samaru in Zango-Kataf and Mallagum, Manchok and Kagoro in Kaura LGAs respectively.
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. Results of the tests showed that four viruses were associated with *Phaseolus lunatus* L. in the study area namely *Bean golden mosaic virus, Bean common mosaic virus,* 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

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Zango-Kataf and Kaura LGAs, respectively. It is suggested 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 because of its importance among farmers.

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

1. INTRODUCTION

Beans are among the most cultivated plants and are commonly eaten fresh or dried particularly *Phaseolus,* and it originated from the Americans. [1] Lima beans (*Phaseolus lunatus* L.), is among the five kinds of *Phaseolus* beans that was domesticated by pre-Columbian farmers [2]. Beans generally are an important source of protein and fibre around the world [3]. 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 [4]. They also play an important role in human nutrition, animal feed and the industrial production of citric acid [5]. Lima bean being edible is a cheaper source of protein compared with the expensive animal protein sources such as egg, meat and fish [6].

Reduced yields of this crop may arise as a result of errors in agro techniques, which are even greater when weather conditions are unfavourable for its growth and development [7]. Other possible causes of low yield are the numerous pathogenic microorganisms such as fungi and bacteria, viruses and nematodes [8]. 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 [8 and 9].

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) [1,8, 9, 10 ,11 Bhadramuthy and Bhat, 2009]). All these viruses can cause great damage to plant production, especially when vector population on the bean plants is high [12].

Despite the great potential of lima beans, it is highly underutilised in Nigeria. More so, from the basis of symptoms observed on bean plants which are mainly attributed to virus diseases, ranging from various forms of mosaic to distortions [13]. 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 have not also received the benefits of intensive research programme as devoted to cowpea and soybean where early detection of viral infection can guarantee the initiation of appropriate management measures become difficult. Thus, there is a need to unravel the prevalence of these viral diseases in the area that will help in their management and lead to an increase in the yield of the crop. Therefore, this study was carried out to detect the viruses associated with *Phaseolus lunatus* in Zango-kataf and Kaura Local Government Areas of Kaduna State, Nigeria with the view to providing baseline information on them for further research.

2. MATERIALS AND METHODS

2.1 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.

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 labelled polyethene bag and taken to the Virology Laboratory in the Department of Crop Protection, Ahmadu Bello University, Zaria for analyses.

2.2 Preparation of Samples

Thirty-eight leaf samples were tested for the presence of *Bean golden mosaic virus* (BGMV),

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

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.

2.3 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).

2.4 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 were 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 a paper towel, and 100 µl of the 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 the sample extraction buffer at 1:100 and used as trapping antibody. One hundred microlitres of the mixture were 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 Timko et al. [14].

2.5 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 plates 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 a 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 were introduced into the test wells and incubated at 37^{o}C for two hr; the content of the test wells was 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 the sample extraction buffer at a dilution ratio of 1:1000 and used as trapping antibody. One hundred microlitres of the mixture were 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 the 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 [14].

2.6 Data Analysis

The results from the tests were analysed using Analysis of variance (ANOVA) using Statistical Analysis System [15] at $P<=0.05$. Means were separated using Least Significance Difference (LSD).

3. RESULTS

3.1 Disease Incidence

Disease incidence of *Bean golden 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 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 the 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).

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

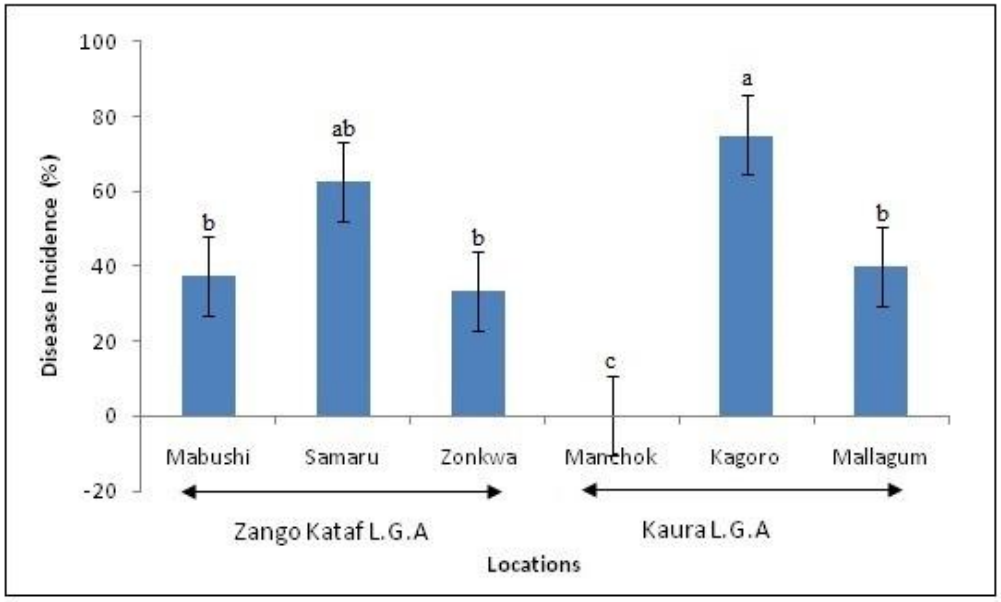

Fig. 2. Incidence of *Bean golden mosaic virus* in Zango-Kataf and Kaura Local Government Areas in Kaduna State, 2012

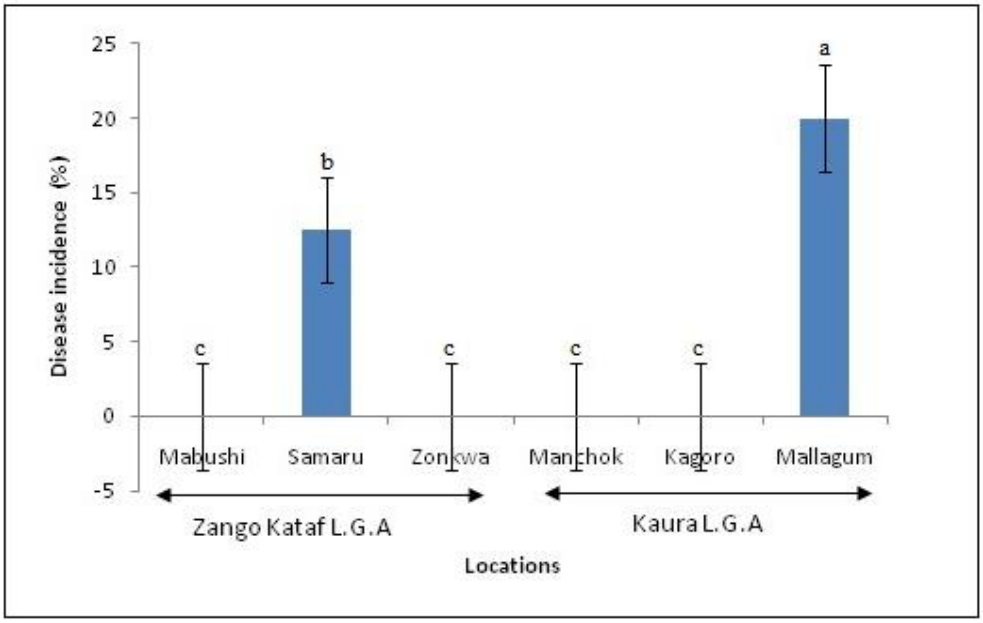

Fig. 3. Incidence of *Bean common mosaic virus* in Zango-Kataf and Kaura Local Government Areas of Kaduna State, 2012

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).

4. DISCUSSION

Disease survey, sampling and serological detection test remain vital keys to understanding the present status of disease as well as its distribution. 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 [16]. Based on the symptoms observed during the present study and which are similar to the symptoms described by Hagedorn et al. [17], the following four viruses: BGMV, BCMV, BYMV and CMV were suspected to be associated with Lima beans.

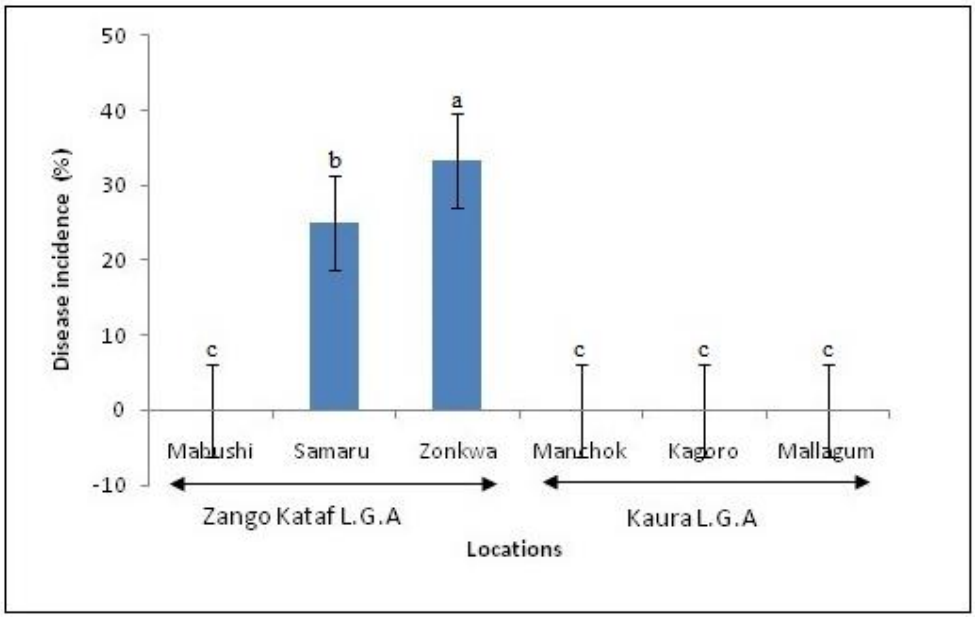

Fig. 4. Incidence of *Bean yellow mosaic virus* in Zango-Kataf and Kaura Local Government Areas of Kaduna State, 2012

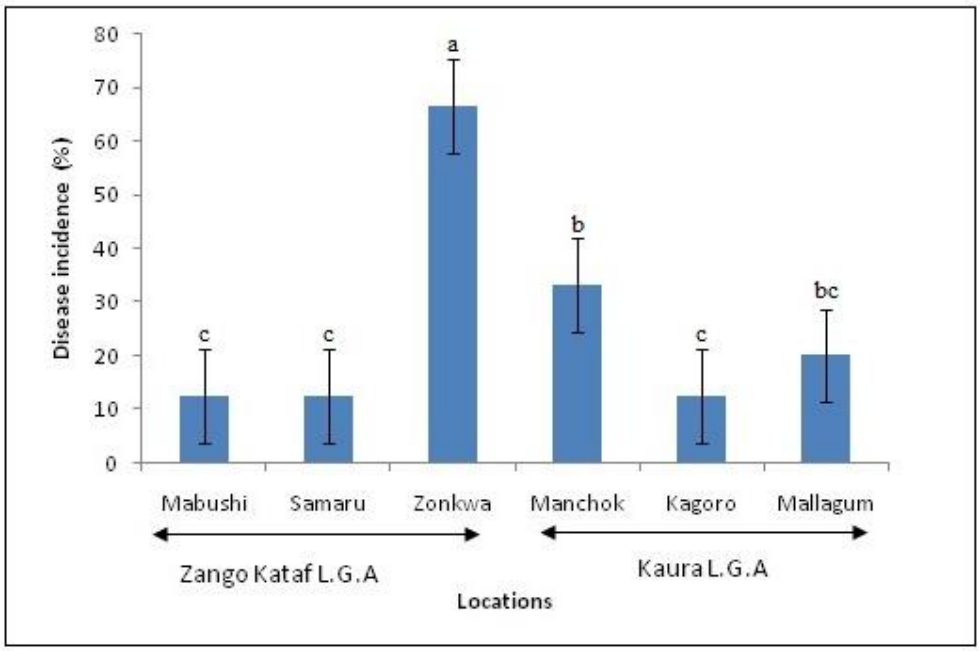

Fig. 5. Incidence of *Cucumber mosaic virus* in Zango Kataf and Kaura Local Government Areas of Kaduna State, 2012

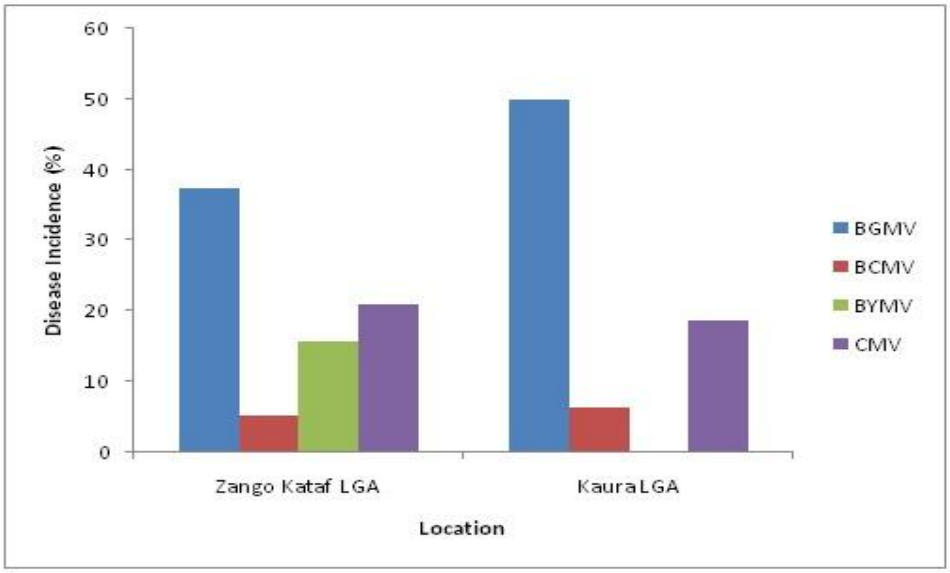

Fig. 6. Incidence of the four virus diseases in Zango-Kataf and Kaura Local Government Areas of Kaduna State, 2012

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. Report by Petrovic et al. [18] in Serbia, involving three viruses Bean common mosaic necrosis virus (BCMNV) Alfafa mottle virus (AMV), and BCMV and found BCMV to be the most infecting pathogen with 30.5%. In the present report, BCMV was encountered only in two locations with incidences of 12.5 and 20% respectively which is at variance with the report of Petrovic et al.[18].

The four viruses encountered in the present work had been reported on beans generally [19, 20 and 21]. Thus, BGMV was reported as a viral disease of *P. lunatus* by Schwartz et al. [22]. Similarly, CMV is a naturally occurring virus of *P. lunatus* in Nigeria as stated by Taiwo et al. [23]. 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 Northwestern Nigeria. The implication of this finding as reported by [9], 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. This particularly so as the incidence of BGMV and CMV were very high as against those reported elsewhere on lima bean [18].

Similarly, BYMV, CMV and BCMV are reported to be among the naturally occurring and most common viruses of the *Phaseolus* spp. in Africa (3,24This 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. As reported by Ghorbani et al. [25], BCMV particularly can be transmitted mechanically and also by whiteflies and can overseason on many plant species such as Chenopodiaceae, Solanaceae, Malvaceae and Amaranthaceae families and the surveyed areas abound with these agents. The workers [25] also stated that CABMV and CMV were etiological agents of lima bean mosaic virus in Brazil. In the present report, however, BGMV and CMV were the predominant mosaic agents in 33% each of the samples from the two LGAs.

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Again, the singular incidence of these four viruses, reveals varying patterns of their distribution across the locations of each LGA where leaf samples were collected. This suggests that distinct environmental conditions of

each location, as well as the varying vector populations of these diseases, could be responsible for the observed varying patterns. 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 [9]. Another compounding problem about the production of lima bean in these areas is the dual cropping of cowpea by the farmers in the surveyed areas.. This is because Beserra et al. [26] reported that the viruses that infect cowpea and common bean should be considered potential pathogens of lima bean, mainly because the crops belong to the same botanical family and are cultivated simultaneously in the same areas.

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. Thus, 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 [11]

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 variyous 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, which also needs to be undertaken.

5. CONCLUSION AND RECOMMEN-DATIONS

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 background information for in-depth research on the distribution of these viruses in other lima bean-producing areas of Nigeria. The study has also provided information on the need for surveillance and management strategies to checkmate aphids and thrips populations in the surveyed areas, in order to prevent the buildup of BCMV which is the most known production problem of lima bean worldwide.

Interestingly, the present study shows that emphasis should be laid mostly on further diagnostic studies and management strategies for BGMV, BYMV and CMV as they recorded a higher incidence than BCMV in the surveyed areas. Information from these studies will bring about increased and profitable cultivation of lima beans not only in Kaduna State but the whole of Nigeria.

Further research on virus studies on lima bean in the surveyed areas of Kaduna state is suggested. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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