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# PROCEEDINGS

**Theme:**

# BIOTECHNOLOGY AS A CHANGE AGENT FOR NATIONAL DEVELOPMENT

# IDENTIFICATION OF WEED HOSTS OF MAJOR LEGUME VIRUSES IN NIGER STATE, SOUTHERN GUINEA SAVANNA OF NIGERIA

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## ABSTRACT

Field survey was conducted during the 2015 cropping season to identify and determine the distribution of legume viruses in Niger State Southern Guinea Savanna of Nigeria. A total of 27 locations were visited. Leaves were collected from weed plants showing virus and virus-like symptoms in fields of cowpeas, groundnuts and soybean. Samples were also collected from asymptomatic plants within the vicinity of infected plants. The antigen coated plate - enzyme linked immunosorbent assay (ACP –ELISA) method was employed for virus detection in the leaf samples. *Blackeye cowpea mosaic virus* (BICMV), *Cowpea mild mottle virus* (CPMMV) and *Cowpea mottle virus* (CPMoV). *Aeschynomene indica*, *Amaranthus caudatum* and *Centrosema pubescens* were positive to BICMV, *Aspilia africana*, *Cleome viscera*, *Euphorbia hirta* and *Heterotis rotundifolliai* were host of CPMoV while CPMMV was detected in *Chenopodium amaranticolor*, *Desmodium scorpiurus*, and *Vicia faba*. The detection of these viruses in weed species indicates their importance in the ecology, survival and the significant role they play in the epidemiology of the various virus diseases. The occurrence of BICMV; CPMoV and CPMMV in these weed species is believed to be the first report in the study area. Fields should be kept free of these weed species in order to control the spread of these viruses to avoid overwintering.

**Keywords:** Antigen coated plate - enzyme linked immunosorbent assay, legume, survey, symptoms, virus, weed hosts.

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## INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp), groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* [L.] Merril) are the major legumes in sub-Saharan Africa (Batiano, 2011), the crops contributes bulk of the protein in the diets of millions of people. The grains of these crops are a major source of plant proteins for man, feed for animals, and a source of income. Moreover, these plants play an important role in providing soil nitrogen to cereal crops such as maize, millet, and guinea when grown in rotation, especially in areas where poor soil fertility is a problem. Virus diseases are considered a major limiting factor for the productivity of legumes in the tropical and sub-tropical countries (Bashir, 2008).

One of the principal avenues by which these viruses are perpetuated is

overwintering in weeds. For instance, it has been documented that perennial grasses in the lowland ecology (fadama) might be the original host of *Rice yellow mottle virus* from which it spread to cultivated rice when conditions became favourable, especially after intensive cultivation (Salaudeen *et al.*, 2008), also, these weed species harbour plant viruses during the growing season and serve as sources of inocula for secondary spread. Earlier, Odedara *et al.* (2011) reported that broad leaves and grasses which occur abundantly around the legume fields could serve as alternative hosts and possibly be responsible for inoculum carry-over. Besides weeds competition with crop plants for space, nutrient and light and as host to insect pests. Various weed species have been implicated in epidemiology of viruses of some notable crops. Salaudeen *et al.* (2008) reported that the *Rice yellow mosaic virus* which causes yield losses between 25 and 100% was harboured by weed species in the family Cyperaceae.

Information on virus types and their weed hosts has a lot of implications on virus survival and epidemiology. The ultimate goal of such information is for designing sustainable management strategies against legume virus. The objective of this study therefore, was to identify the weed hosts of major legume viruses in Niger State, Southern Guinea Savanna of Nigeria.

## MATERIALS AND METHODS

### Description of the study area

Niger State is located in the Southern Guinea Savannah agro-ecological zone of Nigeria and lies between latitude 6° 8′ E and longitude 8° 44′ N of the equator. The site experiences distinct dry and wet seasons with an annual rainfall ranging from 1100 mm in the northern part to 1600 mm in the south with a mean of 1350 mm. The rainfall which peaks in September normally begins in April and ends in October. The temperature ranges between 35 and 37.5°C with relative humidity between 40 and 80% in January.

### Collection of samples

A farm survey was conducted in major legume producing areas of Niger State with crops aged 6 – 9 weeks after planting (September and October, 2015). The state has 25 Local Government Areas (LGAs), grouped into three Agricultural zones. A multi-stage sampling procedure was employed to select three LGAs from each of the three zones, giving a total of 9 LGAs. Three villages/locations from the nine LGAs were randomly selected for the survey to give a total of 27 locations. Twenty leaf samples with symptoms such as leaf mottling, mosaic, leaf curling, distortion, chlorotic spot and stunting were collected from weed species growing from the edges and within the legume fields. Weed species were botanically identified and classified by their morphological characteristics as

described by Akobundu and Agyakwa (1987). The leaf samples were preserved over silica gels in air-tight vial tubes. In each location, samples were collected at approximately 5 to 10 km from the previous sampled farm. Geographical location of each field (longitude and latitude) was recorded using Global Positioning System (GPS- 4300) equipment (Ethrex Garmin GPS, Taiwan).

**Serological detection of legume viruses**

The sampled leaves were subjected to antigen - coated plate enzyme-linked immunosorbent assay (ACP - ELISA) as described by Kumar (2009) and absorbance values were quantified at 405 nm using a microplate reader (MRX, Dynex Technologies, Inc., USA) after overnight. Values were accepted to be positive when the optical density reading was at least twice that of the mean for the negative control.

## RESULTS

**Prevalence of characteristic virus symptoms**

The results showed that mosaic was the most prevalent symptom in all the locations surveyed. Leaf mottling was the next most rampant virus symptom observed during the survey. Leaf curl was another frequently encountered symptom followed by chlorosis, but not as rampant as the mosaic and leaf mottling symptoms. Stunting of plants, leaf puckering and deformation, dead of plants and others were also recorded but at specific locations only (Fig 1).

**Weed host of legume viruses**

Results obtained from the identification of weeds that are hosts to legume crops in Niger State, Southern Guinea Savanna of Nigeria using ACP - ELISA are shown in Table 2. The results show the presence of viruses in some specific locations. *Blackeye cowpea mosaic virus* (BICMV), *cowpea mild mottle virus* (CPMMV) and *cowpea mottle virus* (CPMoV) were the only viruses detected in weeds. These viruses occurred in both single samples and mixtures of two or more collected leaves at the different locations surveyed. Specifically, BICMV was found in Dabiri, Farin Shinge, Gidan Kwano and Tatiko, CPMMV was detected in Farinn Shinge and Manigi while CPMoV was found in Awuru, Gidan Kwano, Lemu, Manigi, Mokwa and Tatiko. Similarly, *Aeschynomene indica* (Linn.), *Amaranthus caudatum* L., *Centrosema pubescen* Benth., *Corchorus* spp, (Jacq.) Willd. and *Aspilia Africana* (Pers.) C.D Adams species, BICMV and *Cleome viscera* L were detected in the area hosting BICMV, CPMMV and CPMoV respectively

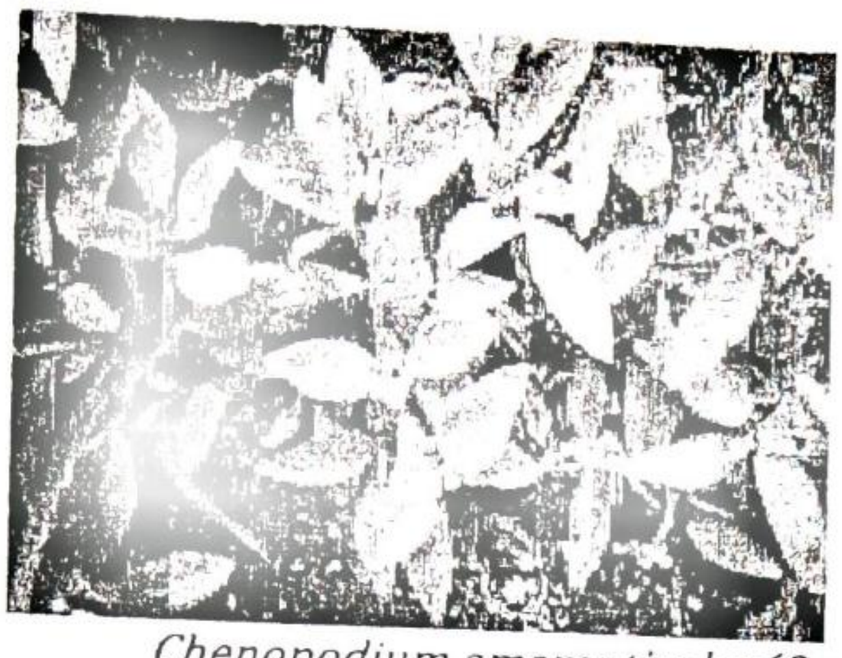
Table 1. GPS coordinates for locations where farm were surveyed

| Town | Longitude/Latitude | Elevation |
|---|---|---|
| Paiko Jazu | 9 ◦ 25.672 N/ 006 ◦ 39.447 E | 353.0 m |
| Tungan Makum | 09 ◦ 29.291 N/ 006 ◦ 37.384 E | 277.4 m |
| Kuta | 09 ◦ 51.479 N/ 006 ◦ 42.740 E | 320.2 m |
| Shiroro Dam | 09 ◦ 55.582 N/ 006 ◦ 48.797 E | 379.0 m |
| Zungeru | 09 ◦ 46.300 N/ 006 ◦ 08.846 E | 121.0 m |
| Dabiri | 09 ◦ 35.895 N/ 006 ◦ 01.129 E | 97.1 m |
| Lemu | 09 ◦ 22.290 N/ 006 ◦ 01.703 E | 136.9 m |
| Gidan Gwari | 09 ◦ 14.440 N/ 006 ◦ 09.054 E | 118.5 m |
| Zachinta | 09 ◦ 07.678 N/ 005 ◦ 54.765 E | 153.3 m |
| Kosteni | 09◦ 11.925 N/ 005◦ 25.464 E | 144.4 m |
| Awuru | 09◦ 49.977 N/ 004◦ 35.019 E | 162.3 m |
| Ba'aburasa | 09◦ 53.462 N/ 004◦ 23.749 E | 254.2 m |
| Manigi | 09◦ 44.196 N/ 005◦ 28.901 E | 215.4 m |
| Makera | 09◦ 37.281 N/ 005o 21.621 E | 204. 7 m |
| Zugurma | 09◦ 28.909 N/ 004◦ 57.315 E | 148.4 m |
| Bokani | 09◦ 26.958 N/ 005◦ 93.490 E | 163.1 m |
| Mokwa | 09◦ 18.884 N/ 005◦ 07.122 E | 206.9 m |
| Rafin Gora | 10 ◦ 06.304 N/ 005 ◦ 24.155 E | 288.2 m |
| Farin shenge | 10 ◦ 24.453 N/ 005 ◦ 30.859 E | 346.3 m |

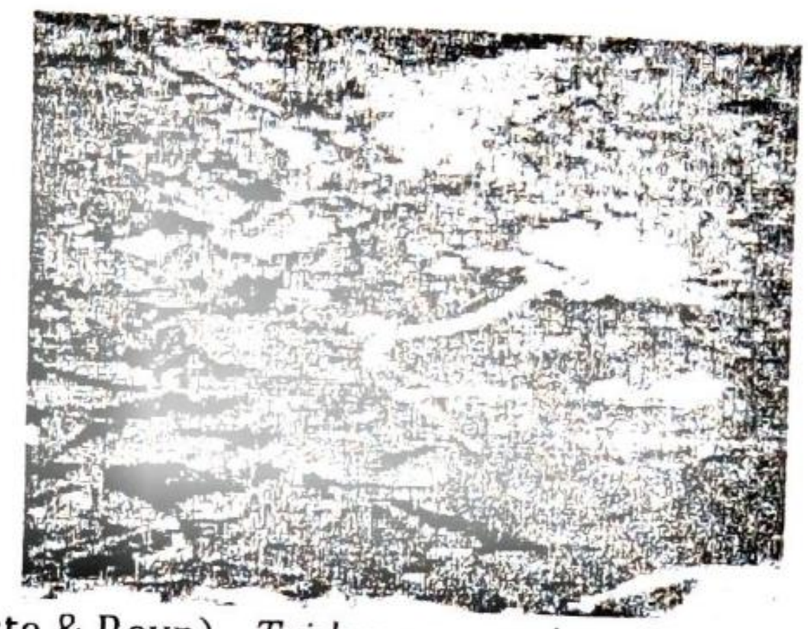
Table 2. Reaction of weed species in Enzyme Linked Immunosorbent Assay (ELISA) in Niger State Southern Guinea Savan Nigeria in the year 2015.

| Weed species | Growth habit | Polyclonal antibody | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | CABMV | BICMV | CMV | SBMV | CPMoV | CYMV | CPMMV |
| *Aeschynomene indica* (Linn.) | A | 0.113 | 0.613* | 0.121 | 0.204 | 0.139 | 0.250 | 0.211 |
| *Ageratum conyzoides* (Linn.) | A | 0.230 | 0.182 | 0.214 | 0.119 | 0.291 | 0.244 | 0.117 |
| *Amaranthus caudatum* L. | A | 0.210 | 0.839* | 0.118 | 0.299 | 0.237 | 0.201 | 0.481* |
| *Aspilia Africana* (Pers.) C.D Adams | A | 0.221 | 0.231 | 0.091 | 0.201 | 0.382* | 0.110 | 0.291 |
| *Cenchrus biflorus* Roxb. | A | 0.229 | 0.197 | 0.239 | 0.103 | 0.154 | 0.249 | 0.102 |
| *Centrosema pubescen* Benth | A | 0.211 | 0.892* | 0.101 | 0.199 | 0.127 | 0.102 | 0.110 |
| *Chenopodium amaranticolor* (Coste & Reyn) | A | 0.221 | 0.710* | 0.229 | 0.120 | 0,101 | 0.212 | 0.429* |
| *Cleome viscera* L. | A | 0.091 | 0.118 | 0.197 | 0.217 | 0.451* | 0.228 | 0.102 |
| *Desmodium scorpiurus* (Sw.) Desv. | A | 0.110 | 0.401* | 0.120 | 0.193 | 0.207 | 0.229 | 0.494* |
| *Euphorbia hirta* (Linn.) | A | 0.211 | 0.291* | 0.111 | 0.129 | 0.429* | 0.221 | 0.098 |
| *Heterotis rotundifollia* (SM) | P | 0.162 | 0.221 | 0.102 | 0.226 | 0.562* | 0.081 | 0.170 |
| *Hyptis suaveolus* (Poit) | A | 0.110 | 0.129 | 0.298* | 0.190 | 0.281 | 0.153 | 0.461* |
| *Laportea aestuans* (L.) Chew. | A | 0.211 | 0.105 | 0.203 | 0.214 | 0.221 | 0.092 | 0.117 |
| *Physalis angulata* (Linn.) | A | 0.119 | 0.049 | 0.106 | 0.117 | 0.162 | 0.114 | 0.215 |
| *Talinum triangulare* (Jacq.) Willd. | P | 0.225 | 0.104 | 0.217 | 0.182 | 0.135 | 0.139 | 0.231 |
| *Tridax procumbens* Linn. | A | 0.219 | 0.193 | 0.221 | 0.210 | 0.107 | 0.183 | 0.216 |
| *Vicia faba* L. | A | 0.238 | 0.249 | 0.148 | 0.292 | 0.119 | 0.232 | 0.459* |
| Diseased | | 2.138 | 2.377 | 2.586 | 2.140 | 2.584 | 0.262 | 0.452 |
| Healthy | | 0.138 | 0.124 | 0.132 | 0.154 | 0.142 | 0.127 | 0.191 |
| Buffer | | 0.128 | 0.141 | 0.138 | 0.167 | 0.141 | 0.165 | 0.214 |

CABMV = *Cowpea aphid borne mosaic virus;* BICMV = *Blackeye cowpea mosaic virus;* CMV = *Cucumber mosai*
SBMV = *Southern bean mosaic virus;* CPMMV = *Cowpea mild mottle virus;* CMoV = *Cowpea mottle virus;* CP
*Cowpea yellow mosaic virus.* A = annual: P = perennial: * positive.

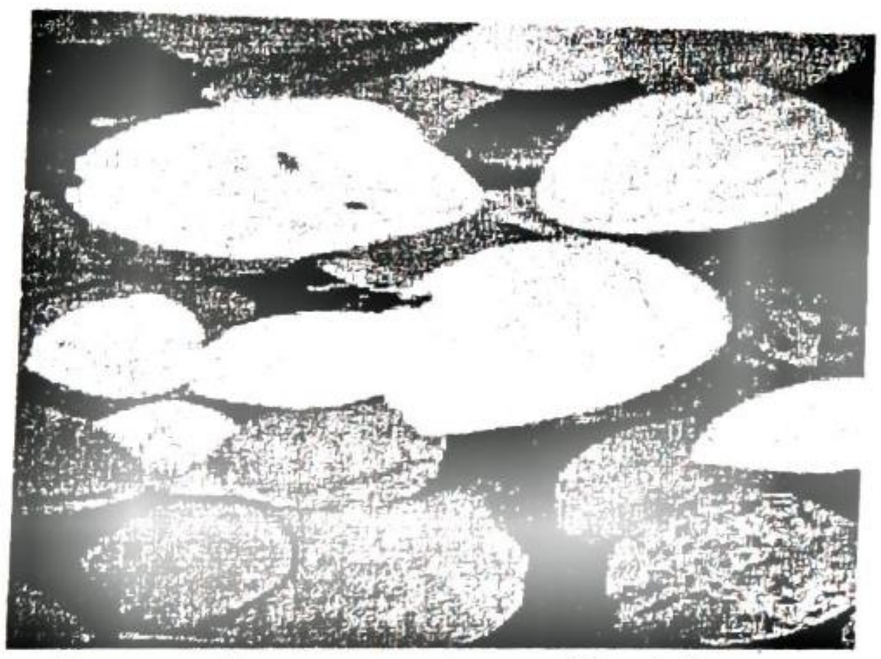


*Chenopodium amaranticolor* (Coste & Reyn)

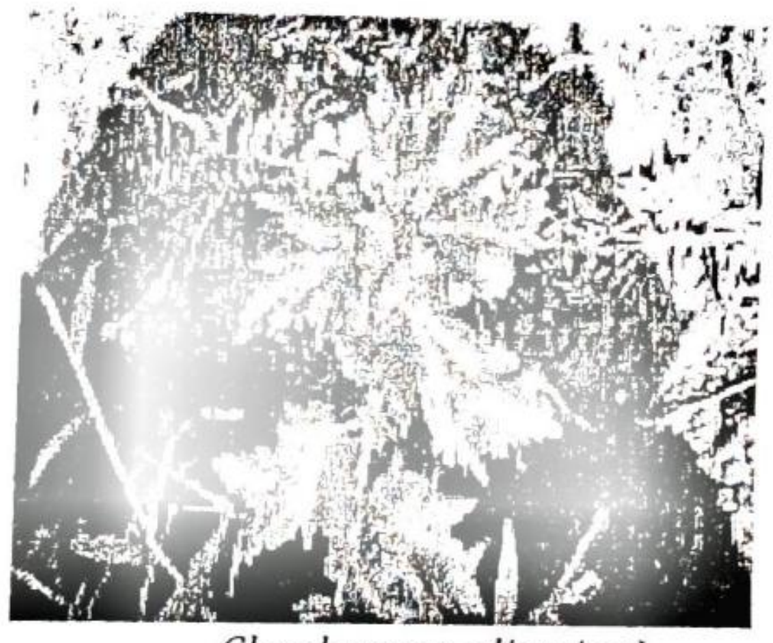


*Tridax procumbens* Linn.

*Hyptis suaveolus* (Poit)

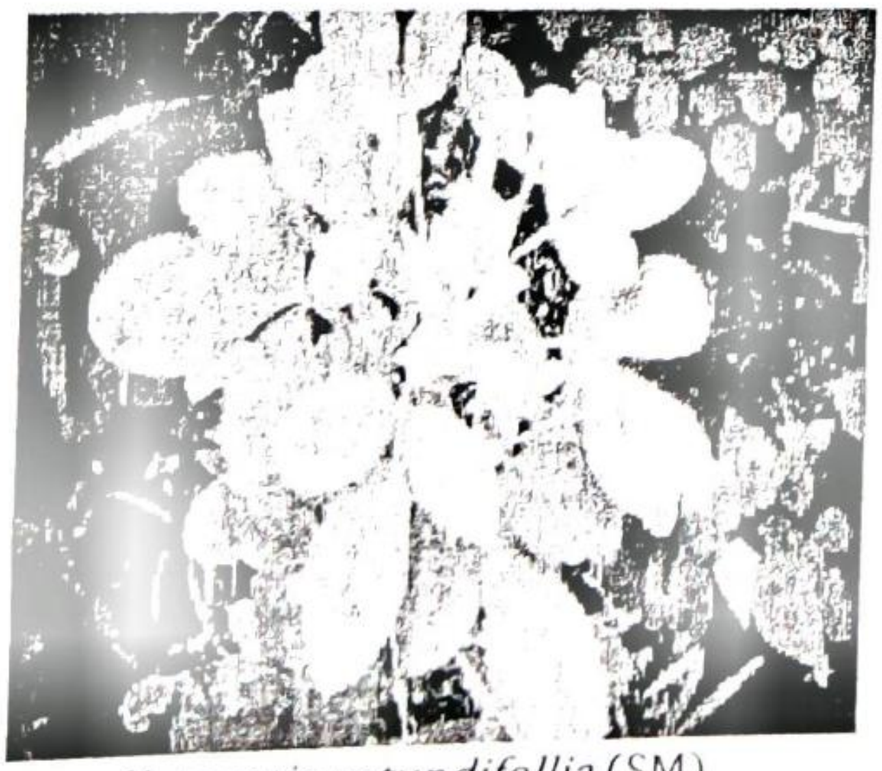


*Desmodium scorpiurus* (Sw.) Desv.



*Chochorous oliterius* L.

*Cenchrus biflorus* Ro



*Heterotis rotundifollia* (SM)

*Cleome viscera* L.

*Amaranthus caudatum* L.

Plate 1: Some weed samples with symptoms of virus infections during the 2015 survey

## DISCUSSION

The virus symptoms (leaf mottling, mosaic, leaf curling, distortion, chlorotic spot and stunting) observed on the sampled weeds were in agreement with the earlier findings on viral infected legumes in the tropic (Shoyinka *et al.*, 1997). Occurrence, distribution and spread of this pathogen might probably be influenced by environmental factors, presence of suitable and susceptible hosts and presence and activities of vectors. Incidence and distribution of the viruses were natural and may have stemmed primarily from seed infection and weed hosts as observed from the cropping pattern (Alabi *et al.*, 2010).

BICMV and CPMoV incidence recorded at Lemu and Dabiri that are separated by less than 5 km collaborates the findings of Aliyu *et al.* (2012) who discovered two different viruses from the same genus co-existing in the nearby field in Kwara State, Nigeria. This implies that subsequent mutation and replication of the viruses could simply result in several serotypes with varying degree of pathogenicity on the one hand and multiple infections of legume crops on the other hand. The occurrence of CPMoV and CPMMV in naturally infected cowpea is believed to be the first report from Niger State. BICMV was detected at Awuru and Gidan-Kwano in mixture with CPMoV.

CPMoV incidence was prevalent in Tatiko, Dabiri, Manigi, Awuru, Lemu and Mokwa. Although, Awuru is located at the riverine area which partially agrees with that of Alegbejo (2015), who reported a high incidence of the pathogen in riverine areas of the middle belt of Nigeria which has a Southern savanna climate and where a lot bambara groundnut is grown. Further, this finding shows that the pathogen could also be spread to other areas within the Southern savanna which are not riverine. The seed borne nature of CPMoV and recent detections suggest that the virus could be spreading through seeds to other parts of the Southern guinea Agro-ecological zone of Nigeria. CPMMV has been reported to occur naturally in the middle belt of Nigeria which Niger State is inclusive (Alegbejo, 2015). Odedara (2011) reported CPMoV as seed-borne virus that is considered as major constraint to yield in legume fields, because emerging plants are quickly exposed to virus inocula with greater damage at the early stages of crop plant development. and this shows how important the virus could be in the ecological zone.

Possibly, the negative reaction of some of the weed leaf samples to ELISA implies that they belong to entirely different virus types (non-legume viruses). The detection of virus in some of the weed species indicates their importance in the ecology and survival of the pathogen. Odedara (2011) reported that non-crop hosts of viruses can be particularly troublesome if they act as overwintering hosts and remain unknown since bioassay must be carried out to confirm their presence. The weed species which were implicated in the fields reveal that they can harbour these pathogens during the growing season and serve as sources of

inocula for secondary spread. It also indicates that these viruses can survive on these weed species during the off season and then serve as sources of primary inoccula at the beginning of the new season. Earlier, Alegbejo (2015) reported that the more important natural reservoirs of these pathogens are the annual weeds from the Leguminaseae which can host the organisms all the year round and for extended numbers of years. Although, Odedara (2011) maintained that the viruses can also survive in infected dry leaves of the susceptible annual leguminous weed species, resulting in accumulation of virus inocula.

*Aeschynomene indica* (Linn.), *Amaranthus caudatum* L., *Centrosema pubescen* Benth. were among the common weed species in which viruses were detected in the study area during the survey. Earlier, Hampton and Thottappilly (2003) reported some of these forage legume species to be naturally infected by these viruses. CPMMV and CPMoV were detected in *Corchorus* spp, (Jacq.) Willd. and *Aspilia Africana* (Pers.) C.D Adams, which is in contrary to the earlier work of Alegbejo (2015), who reported them as a non-host. This might be due to the difference in the pathogenicity of the virus strains involved. The detection of BICMV in *Cleome viscera* L., contradicts the findings of Odedara *et al.* (2011) that earlier reported the weed as non-host of the virus could also be attributed to the pathogenicity of the viruses strains assayed. Also, CPMMV infecting *Euphorbia hirta* (Linn.) and *Heterotis rotundifollia* (SM) were earlier reported as hosts of BICMV only (Alegbejo, 2015). This is the first report of natural virus infection of these weeds, *Hyptis suaveolens* [Poit], (CPMMV), *Aeschynomene indica* L. and *Centrosema pubescens* Benth (BICMV) and *Cleome viscera* L. (CPMoV

## CONCLUSION AND RECOMMENDATION

Legume production in Niger State suffers from high virus infection with resultant substantial loss in grains. Virus diagnosis showed that three important legume viruses (BICMV, CPMoV and CPMMV) were prevalent in some specific locations surveyed. *Aeschynomene indica* (Linn.), *Amaranthus caudatum* L., *Centrosema pubescen* Benth., *Corchorus* spp, (Jacq.) Willd. and *Aspilia Africana* (Pers.) C.D Adams species, BICMV and *Cleome viscera* L were detected in the area hosting BICMV, CPMMV and CPMoV respectively There is the need, therefore, for constant monitoring of legume fields through regular field sanitation and disease surveys to identify new and emerging weeds as a good starting point for legume viruses management in the study area. However, additional research is needed, particularly on those weed samples which exhibited weak positive and negative reactions. This could be of agricultural importance for food security.

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