

Serological Detection and Distribution of Eggplant Mosaic and Eggplant Mottled Dwarf Viruses on Eggplant in Kaduna State

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Abstract:- Eggplant (*S. melongena*), an important and popular vegetable crop commonly grown in Nigeria and other parts of the world is usually affected by two pertinent viruses: Eggplant mosaic virus (EMV) and Eggplant mottled dwarf virus (EMDV). These two destructive viruses cause considerable yield loss in eggplant production. The need to guide the farmers through scientific information and technology suggested this targeted study on the two most prominent viruses affecting the crop. There are insufficient information pertaining to the crop and works on eggplant viruses and their alternative hosts in Kaduna State are not available. A serological survey and analysis was therefore carried out in order to evaluate the presence and distribution of the two viruses in Kaduna state of Nigeria. Most infected plants in the areas surveyed showed virus-like symptoms consisting predominantly of mottling, chlorosis, leaf distortion and stunted growth. A total of two hundred and seventy both of symptomatic and asymptomatic eggplant leaf samples were collected from three LGAs of the State. Twenty-one weed samples were also collected from the locations. The samples were screened using the Double Antibody Sandwich Enzyme linked Immunosorbent Assay (DAS-ELISA) for the presence of Eggplant mosaic virus (EMV) and Eggplant mottled dwarf virus (EMDV). Results showed that EMV and EMDV were detected in single infection (EMV 16.67%, EMDV 33.33%). Among the weed species tested, *Senna occidentalis*, *Isorbelina doka*, *Digitaria horizontalis* tested positive for EMV while *Ageratum conyzoides*, *Axonopus compressus*, *Euphorbia heterophylla* tested positive for EMDV. *Ageratum conyzoides* tested positive to both EMV and EMDV.

Keywords:- Eggplant, Kaduna, Virus, EMV, EMDV, Incidence, Farmers.

I. INTRODUCTION

Eggplant (*Solanum* spp.), also known as Brinjal in Southern Asia and Aubergine in Britain and France is one of the most important vegetable crops in Nigeria and other West African nations. It is one of the top ten vegetables in the world and the sixth most widely grown vegetable produced in the world after tomato, watermelon, cabbage, onion and cucumber (Oladosu *et al.*, 2021, USAID and PDDBA, 2006, Sabatino *et al.*, 2019). Eggplant is second to tomato as the most important *Solanaceae* fruit crop (David *et al.*, 2018). It is an economic flowering plant belonging to the family *Solanaceae* (Pessarakli and Dris, 2003), mostly cultivated in Asia with about 74% of the world's production (Shenia and Gangshuana 2018). It is an economically important crop in Asia, Africa and sub-tropical countries like India and central America (Aminifard *et al.*, 2010). It is found in many diets across several communities especially in Africa, the subtropics (India, Bangladesh, and Central America), Middle East and Southeast Asia (Oladosu *et al.*, 2021). Eggplant is perennial in nature but it is cultivated commercially as an annual crop (Eletta *et al.*, 2017).

There are about 2300 species of *Solanum* known worldwide and about 25 species represented in Nigeria (David *et al.*, 2018). The different species and varieties of eggplant vary particularly with respect to their morphology (plant growth habit, vigour, hairiness, fruit shape, fruit size, fruit colour and yield potential), physiology (flowering pattern and water use) and biochemical properties (fruit bitterness and glycoalkaloid content) (Oso and Borishade 2017). The species, *Solanum melongena* L (Brinjal, aubergine or the common eggplant) is the most popular specie and it is cultivated worldwide (Aliero, 2007, Knapp *et al.*, 2013). *Solanum aethiopicum* L (Scarlet eggplant) and *Solanum macrocarpon* L (Gboma eggplant) are the two horticulturally important eggplant species indigenous to Africa (Schippers, 2000). Eggplant is a vegetable with increasing popularity in the world (Pessarakli and Dris, 2003). It is widely cultivated across most of the African continent but more intensively in East and West Africa (Nwaiwu *et al.*, 2012). It is an indigenous tropical African crop grown for its nutritional, medicinal and economic values of the leaves and fruits, with various varieties of

economic importance commonly produced in Nigeria (Oso and Borisade, 2017).

Eggplant is mainly grown in mixed cropping system. It is usually grown in compound or backyard farms using household organic refuse or farm yard manure (Oso and Borisade, 2017). The production and consumption of *S. melongena* is on the increase in East Africa although it is popular in South Africa (Adeniji and Aloyce, 2012). The name “Garden egg” was derived from the shape of the fruits of some varieties which are white and shaped like chicken eggs (Chen *et al.*, 2001; Anyaegbu *et al.*, 2013). It is called Brinjal in India while Europeans call it Aubergine (Doijode, 2001). In Nigeria, it is commonly called ‘Garden egg’; the Igbos call it anara or Afufa (David *et al.*, 2018); the Yorubas call it ikan while the Hausas call it yalo (Schippers, 2000; Ndagana *et al.*, 2020). The four cultivar groups of *S. aethiopicum* are the shum group, the kumba group, the gilo group and the aculeatum group (Schippers, 2000). The Gilo, Kumba and Shum groups are the most important groups in Africa. The Gilo and Kumba groups are cultivated for their fruits particularly in the humid zone of West Africa while the Shum group is produced for its leaves in the savannah area (Nwaiwu *et al.*, 2012). Although excessive rainfall affects both vegetative growth and flower formation, the plant is well adapted to both wet and dry season cultivation (Degri, 2014). It is one of the most traded indigenous vegetables in the local markets (Majubwa *et al.*, 2015). It also offers gainful employment among the rural households and its cultivation is not limited to any age or sex (Anuebunwa, 2007).

A number of pests and diseases attack eggplants in the field (Horna and Gruere, 2006). The damage drastically reduces yields and affects the quality and quantity of the produce (Onu *et al.*, 2016). In order to obtain high yield of this crop in Nigeria, there is a great need to work together for the development of Eggplant varieties that can meet the challenges of the present and the future. An understanding of the viruses that infect Eggplants is a pre-requisite for formulating effective control measures. Disease management relies strongly on a fast and accurate identification of the causal agent. This study carried out serological detection and distribution of two very destructive viruses attacking eggplants in some local government areas in Kaduna State with a view to appraising the methodology of infections and proffering solutions for protecting the crop.

Eggplant is cultivated all year round in different parts of Nigeria and serves as the main source of income for many rural farmers and households (Omotesho *et al.*, 2017). The crop has been classified as a low status vegetable in spite of its great importance and uses (David *et al.*, 2018). Production is also constrained by a wide range of pests and diseases reducing the production as well as production quality (Omotesho *et al.*, 2017). Plant virus epidemics pose a worldwide challenge to achieving satisfactory yields and produce quality (Jones, 2006). Reports show that eggplants are infected by several viruses causing significant damage due to plant stunting, crinkle, mottling accompanied by leaf and fruit abnormalities (Rakib *et al.*, 2011).

In Nigeria, several viruses have been reported to infect eggplant. Such viruses as *Eggplant severe mottle virus* (ESMoV), *Pepper veinal mottle virus* (PVMV), *Eggplant green mosaic virus* (EGMV), *Eggplant Mosaic Virus* (EMV) and *Eggplant Mottled Dwarf Virus* (EMDV) (Alegbejo, 2015; Babaie and Izadpanah, 2003; Danesh and Lockhart, 1989; Ladipo *et al.*, 2008; Skotnicki, 1993). *Eggplant mottled dwarf virus* (EMDV) and *Eggplant mosaic virus* are two among other eggplant viruses reported as very severe and highly damaging to eggplants (CABI, 2018).

Eggplant production has a huge impact on the cultural, economic, nutritional and environmental lives of people. There has been inadequate research and insufficient information pertaining to this crop (Nwaiwu *et al.*, 2012). In Kaduna and Kano states, two-third of the population is involved in agriculture, the majority being small-scale farmers with approximately 2-3 hectares (Plaisier *et al.*, 2019). Moreover, in the northern part of Nigeria, eggplant is dominant in their farming systems (Ndagana *et al.*, 2020). The crop contributes a lot to food and income generation for farmers in the states under study. Weeds are potential initial sources of viral inoculums in fields (Prajapat *et al.*, 2014). They harbour viral vectors consequently contributing to the persistent occurrence of viral diseases in agro ecosystems (Asala *et al.*, 2014). There is a need to identify the weed species in order to select the most efficient and economical method of managing them in eggplant production (Marques *et al.*, 2017).

Information on plant-virus interaction is important for the future of agricultural production and food safety by predicting future emergence events or epidemics and by supporting the application of appropriate control measures (Hancinsky *et al.*, 2020).

II. OBJECTIVES

The objectives of this study are

- To determine the occurrence and distribution of *Eggplant Mosaic Virus* and *Eggplant Mottle Dwarf Virus* associated with eggplant in Kaduna and Kano States.
- To determine the presence of the two viruses in weeds within and in close proximity to eggplant fields.

III. MATERIALS AND METHOD

A. Survey

Surveys were conducted in the 2017/2018 and 2020/2021 dry seasons to determine the incidence of *Eggplant mosaic virus* (EMV) and *Eggplant mottled dwarf virus* (EMDV) on eggplants and weeds in Kaduna state. Three Local Government Areas (LGAs) known for cultivation of Eggplant were selected. They are: Kaduna south, Igabi and Giwa LGAs. Three farms from each of the three LGAs were surveyed. In 2021 due to security problems in Giwa LGA, Kudan LGA was surveyed. Figure 1 depicts the local government areas surveyed.



Fig. 1: Local Government Areas Surveyed in Kaduna State

During the survey, data on age of plants, crop protection measures taken, cultivar type, cropping pattern, history of cultivation, surrounding crops and source of seed were obtained by the use of questionnaires administered to the farmers. The co-ordinates and elevation were recorded with the use of a Global Positioning system (GPS). The disease incidence was recorded as a percentage of the number of diseased plants to the total number of plants examined as shown in the formula below

Disease incidence (%)

$$= \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

In each field, 3×3 m² sized-quadrant was set up at four corners and at the centre of the field. Six leaf samples were collected from each quadrant; four symptomatic and two asymptomatic per quadrant making a total of thirty samples from each farm (twenty symptomatic and ten asymptomatic). Ninety eggplant leaf samples were collected per LGA; two hundred and seventy eggplant leaf samples were collected. The samples were carefully labeled, wrapped in polyethylene bags, placed in an ice chest and transported to the Virology Laboratory of the Department of Crop Protection, Ahmadu Bello University, Zaria. Twenty-one weed leaf samples were collected within each farm and about 2 meters around the farms. The weed samples were also labeled, wrapped and placed in an ice chest alongside the eggplant leaf samples. The weed species were identified at the Herbarium in the Department of Botany, Ahmadu Bello University, Zaria.

B. Laboratory Analysis of Samples

The eggplant and weed samples were analysed using the Double Antibody Sandwich Enzyme linked Immunosorbent Assay (DAS-ELISA) protocol. The Double Antibody Sandwich format of Enzyme-Linked Immunosorbent Assay (DAS-ELISA) was used for the two viruses as recommended by the manufacturer, German Collection of Microorganisms and Cell cultures, Braunschweig, Germany. *Andean Potato Latent virus* (APLV) - specific antiserum (DSMZ RT-0978) was used to detect the presence of *Eggplant mosaic virus* in the leaf samples. For *Eggplant mottled dwarf virus*, EMDV-specific antiserum (DSMZ RT-0836) was used for the detection of the virus in the leaf

samples. Wells of the microtitre plates were coated with 200µl of polyclonal antibody diluted at 1:1000 in carbonate coating buffer.

C. Serological detection of Eggplant viruses in Eggplant leaf samples

Eggplant leaf and weed samples were tested for the presence of *Eggplant mosaic virus* and *Eggplant mottled dwarf virus*. The samples were prepared using the mortar and pestle method described by Hughes *et al.*, (2004). One gram each of the leaf samples was ground in a sterile mortar with a pestle using 1 in 10 ml of sample extraction buffer to obtain a homogenous solution. Positive and negative control were pipetted onto each plate. The plates were covered and placed in the incubator (Gallenkamp, model 1H-105, made in England) at 37°C for 2 hours. Afterwards the plates were washed three times with phosphate-buffered saline (PBS-Tween 20) by flooding the plates and allowing them to stand for three minutes, draining and tap drying over tissue paper. Two hundred microlitre aliquots of the test samples were dispensed into each well of the microtitre plates and incubated in a refrigerator at 4°C overnight. The plates were washed by flooding with PBS-Tween three times as described earlier. Two hundred microlitre enzyme conjugate diluted at 1:1000 in conjugate buffer were dispensed into each well. The plates were covered and incubated at 37°C for 2 hours and washed as before. Two hundred microlitre aliquots of poly-nitrophenyl phosphate (pNPP) dissolved in substrate buffer (one milligram of pNPP per milliliter of substrate buffer) was added to each well and incubated at room temperature in the dark for 1 hour. The results were determined by visual observation and with the use of BIORADI mark Micro-plate reader (Made in India) at 405nm. Readings twice the values of the negative control were considered positive.

D. Statistical Analysis

The serological results were analyzed using descriptive statistics to describe the occurrence and distribution of the viruses in the two states.

IV. RESULTS AND DISCUSSION

Many of the symptoms observed on the eggplants were similar to that of *Eggplant mosaic virus* and *Eggplant mottled dwarf virus* infection. Mottling, chlorosis, leaf deformation and stunting were observed on the leaves of the eggplants. Some of the weeds also exhibited symptoms of mottling and chlorosis. Reports states that symptoms of *Eggplant mottled dwarf virus* on eggplants include mottling, vein clearing and leaf deformation accompanied by mild to severe stunting (Aramburu *et al.*, 2006, CABI, 2018). *Eggplant mosaic virus* causes mosaic symptom on the leaves of Eggplants (Gibbs and Harrison, 1969). Stunting and deformation can also be observed on the plants (Shalaby, 1997). Some of the samples tested negative to these viruses.

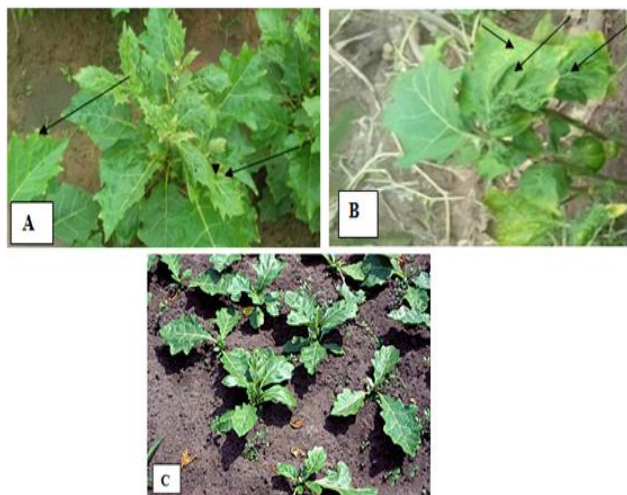


Plate 1: Eggplant infected with Eggplant mosaic virus with arrows showing chlorotic symptoms (A); Eggplant mottled dwarf virus with arrows showing leaf deformation and stunting (B); compared with healthy plant (C)

In 2018 there was no record of infection in Kaduna south LGA (Figure 2), although disease symptoms were observed on the plants. A report by Jeong et al (2014) noted that plants can show symptoms similar to those of virus infection when they respond to unfavourable weather conditions, nutrition deficiencies, infection by other types of pathogens, pest damage or damage by abiotic agents. Eggplant mottled dwarf virus was detected in the three LGAs visited in 2021 (Figure 3). However, Eggplant mosaic virus was detected in only one LGA (Figure 3). Crops of Solanaceae family like pepper, tobacco and tomato can harbour these viruses (TNAU, 2016), this could also be a factor in the high incidence rate of disease in some farms visited in Igabi and Giwa LGAs (Figure 2) as it was observed that eggplants were grown with pepper and tomato as surrounding crops in some farms in Igabi LGA while in many of the farms in Giwa LGA, eggplant was grown as a sole crop as well as a surrounding crop. According to Taiwo and Owolabi, (2004), monoculture can lead to inoculum build-up. Majority of the farms in Kaduna south LGA were observed to be highly symptomatic. Irregular weeding was observed as well. This shows in the high incidence rate of Ereservist MDV in the locality (Figure 3). Regular weeding prevents disease build-up by eliminating plants that harbour viruses. Weed species can serve as a reservoir of plant viruses (Hancisky et al., 2020). Seed-borne infection could arise from uncertified seeds (Ayo-John and Odedara, 2017). Some of the farmers sourced their seeds from previous harvest while others obtained them from the market. Continuous cropping which was also practiced by some of the farmers may have led to a high disease incidence. According to a report by Ayo-John and Odedara, (2017), continuous cropping could cause disease build-up. However in some farms where crops of different families were successively cultivated, the incidence of disease was found to be lower. A lower percentage incidence of EMDV was recorded in Igabi LGA (Figure 3), it was observed that eggplant was planted in succession to maize and rice in some of the farms within the locality. The surrounding crops were maize. Maize act as tall barrier crop

as well as a non-host. This could serve in lowering the disease incidence in the location. Differences in the percentage incidence of both viruses in the different locations could be attributed to factors such as source of initial inoculum, host species and insect vectors found in the areas (Aguar et al., 2012).

Some of the farms had a high weed population. Weeds have a significant effect on disease incidence. They serve as pest, vector or are reservoir of a pathogen (Wisler and Norris, 2017). There is a possibility of an epidemic if adequate management methods are not applied (Alfaro- Fernandez et al., 2016). *Physalis angulata*, *Euphorbia heterophylla*, *Axonopus compressus* and *Ageratum conyzoides* tested positive for EMDV. *Euphorbia heterophylla* has been reported as a host of EMDV (<https://secure.fera.defra.gov.uk>). This is in accordance with the report of Hancisky et al., (2020), stating that weed species can serve as reservoir of plant viruses. *Senna occidentalis*, *Isorbelina doka*, and *Digitaria horizontalis* tested positive for EMV. *Ageratum conyzoides* also tested positive for EMV. When present in or around farms, weeds can be harmful especially when they are carriers of viruses with a wide host range (Khan and Dijkstra, 2006). This is the first report of these weed species as weeds that harbour EMV and EMDV in Nigeria.

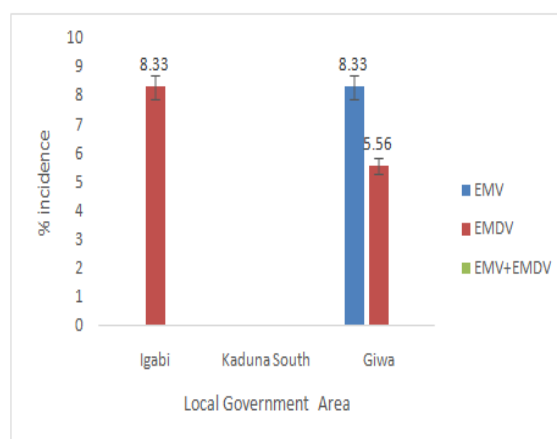


Fig. 2: Incidence of Viruses in Kaduna State during the 2018 Dry Season

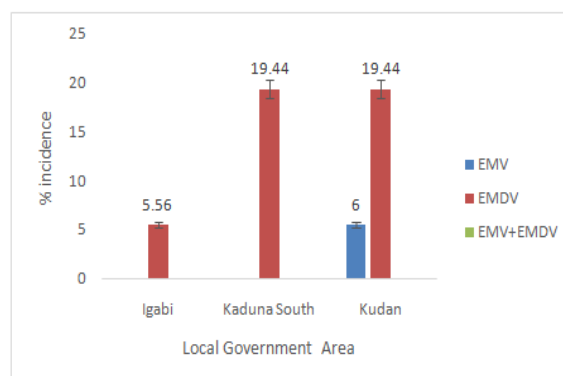


Fig. 3: Incidence of Kaduna state during the 2021 dry season

V. CONCLUSION

This study, for the first time determined the occurrence and distribution of *Eggplant mosaic virus* and *Eggplant mottle dwarf virus* associated with eggplant in Kaduna State. The results indicate the presence of the two viruses in some of the weed species notably; *Ageratum conyzoides*, *Euphorbia heterophylla*, *Physalis angulata* tested positive for EMDV while *Digitaria horizontalis*, *Senna occidentalis* and *Isorbelina doka* tested positive for EMV. *Ageratum conyzoides* tested positive for the two viruses.

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