

## **Agro-ecological incidence and severity of *Pepper veinal mottle virus*, genus *Potyvirus*, family *Potyviridae*, on cultivated pepper (*Capsicum annuum* L.) in Nigeria.**

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### **Abstract**

A survey for the agro-ecological distribution of *Pepper veinal mottle virus* (PVMV) (Family Potyviridae, genus Potyvirus) its incidence and severity on cultivated pepper was conducted between 2003 to 2005 in six agro-ecological zones in Nigeria, comprising the major pepper producing areas of the humid forest, derived savanna, southern Guinea savanna, mid-altitude, northern Guinea savanna and Sudan savanna. The virus was isolated and its physical properties determined.

PVMV was confirmed to be present in cultivated pepper fields in the six agro-ecological zones surveyed but with significant difference in disease incidence and severity within the agro-ecological zones. Out of the three thousand suspected viral infected pepper leaves sample collected from the fields in the six agro-ecological zones, 88% were confirmed to be PVMV positive through the serological test while 12% were found to be negative.

The Electron micrograph photograph showed antiserum decorated PVMV particles, having flexuous filamentous particles of 750nm in length and 10 nm wide. The thermal inactivation point of the virus was 70°C and still infective up to the 12th day, while the dilution end point in which PVMV extract from *Capsicum annuum* L. was still infective was 10<sup>-4</sup>.

The incidences of PVMV diseases were observed to be high in the derived savanna and the humid forest compared with the other agro-ecological zones. The percentage PVMV disease incidence ranged between 39.14% with 34.48% severity in the Sudan savanna to 50.12% incidence and 43.85% severity in the derived savanna zone.

The high incidence and severity of PVMV in these two agro-ecological zones that are characterized by thick vegetation and warm humid climate, with the presence of many secondary host plants for the virus, suggest that ecological characteristics, climate and vegetation in the different ecological zones appeared to play a major role in determining the incidence and severity of PVMV infection on pepper in the fields.

Keywords: *Pepper veinal mottle virus*, Incidence, Severity, Pepper, Agro-ecological, Zones.

### **Introduction**

Pepper is cultivated principally in southwestern and northern Nigeria between latitude 10°N and 12°3'N in the northern Guinea savanna and Sudan ecological zones (Erinle, 1988). However, there is a sizeable production of pepper in the rain forest and derived savanna of southwestern Nigeria (Opoku-Asiama et al., 1987).

*Pepper veinal mottle virus* (PVMV) was first recognized as a distinct member of a group of viruses which was originally designated the *Potato virus Y* group but was later renamed the *Potyvirus* group (Harrison et al., 1971). PVMV occurs mainly in Africa although; it affects *Capsicum annuum* L. crops in Afghanistan (Lal and Singh, 1988) and India (Nagaraju and Reddy, 1980). The virus probably also occurs in *Capsicum* spp. in Sierra Leone and Zaire, (Huguenot et al., 1996). PVMV has been reported in several West African countries, and in some parts of Nigeria (Alegbejo and Uvah 1987; Fajinmi 2006).

There was a report that a strain of PVMV occurs naturally in *Telfairea occidentalis* (Cucurbitaceae) in Nigeria (Atri, 1986). Strains of the virus are also experimentally transmissible to at least 35 species of the Solanaceae and to nine species of five other families (Aizoaceae, Amaranthaceae, Apocynaceae, Chenopodiaceae and Rutaceae) (Ladipo and Roberts, 1977, Brunt et al., 1978; Prasada Rao et al., 1979; Igwegbe and Waterworth, 1982).

Symptoms expressed by the leaves of PVMV-infected plants include chlorosis of the veins, followed by systemic interveinal chlorosis, mottle, and small distortion of leaves and at times leaf abscission and fruit distortion occur (Brunt et al., 1978).

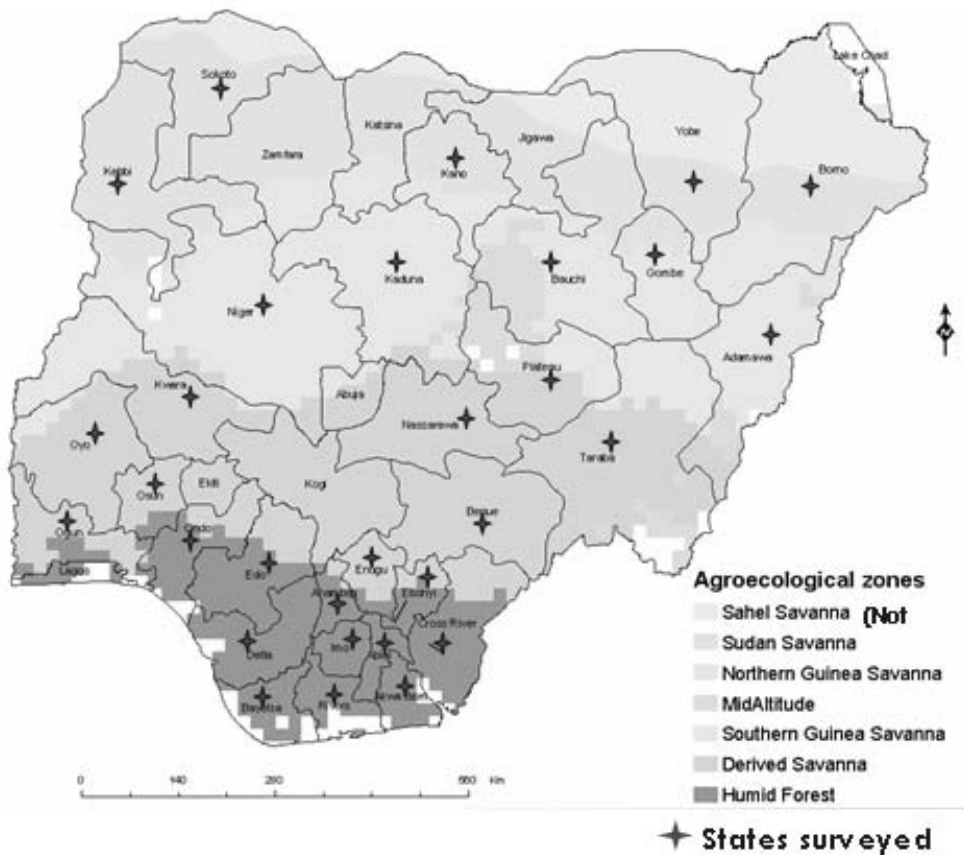
There have been reports of one hundred percent losses of marketable pepper fruit due to infection with pepper viruses causing whole field to be abandoned prior to harvest and in some areas making cultivation of pepper to be uneconomical in some parts of Nigeria (Alegbejo and Uvah 1987).

Therefore, this study was targeted at studying the incidence, severity and distribution of *Pepper veinal mottle virus*, genus Potyvirus family Potyviridae in cultivated pepper in the six agro-ecological zones of Nigeria.

### Materials and methods

Survey for *Pepper veinal mottle virus*, genus Potyvirus, virus disease incidence and severity was conducted during the 2003, 2004 and 2005 planting seasons in six agro-ecological zones of Nigeria (Humid forest, Derived Savanna, Southern Guinea Savanna, Mid-Altitude, Northern Guinea Savanna and Sudan Savanna). Thirty states within the six-agro ecological zones were surveyed to cover areas where pepper plants were cultivated.

The states were Ogun, Edo, Ondo, Delta, Imo, Abia, Anambra, Cross rivers, Rivers, Akwa Ibom, Bayelsa, Oyo, Osun, Enugu, Benue, Taraba, Kwara, Nassarawa, Plateau, Niger, Gombe, Sokoto, Kaduna, Kebbi, Kano, Borno, Adamawa, Yobe, Bauchi and Ebonyi (Fig 1.)



**Fig. 1** A map of Nigeria showing the Agro-ecological zones surveyed for the incidence and severity of *Pepper veinal mottle virus* (PVMV) diseases on cultivated pepper *Capsicum* sp. (Source: International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.)

**Sampling techniques:** Ten cultivated pepper farms of approximately 10 metres by 10 metres were randomly surveyed per state. On each site 10 plants were randomly sampled from the population of plants showing virus-induced symptoms. The symptoms were also scored for severity. Five hundred leaf samples from cultivated pepper plants

showing symptoms of mosaic, chlorosis, yellowing, stunting, mottle, necrosis, leaf deformation and leaf bunching were collected and stored in zip-lock plastic sampling bags per agro-ecological zones. Care was taken to avoid mix up by adequately labelling to reflect location and date of collection with an identifying tag number. All the samples were stored in an ice crest cooler for onward transportation to the virology Laboratory for serological analysis. Percentage virus disease incidence was calculated in each farm plot by counting the number of diseased plants divided by the total number of pepper plants within the farm multiplied by 100.

$$\text{Percentage Disease Incidence} = \frac{N - n}{N} \times 100$$

N = Total number of observations

n = Total number of plants with no disease symptoms.

Disease severity index: Virus disease severity was scored in each pepper farm surveyed by using a modified formula-grading scheme from Merritt et al., (1999) and Steel and Torrie, (1980), for disease severity;

1. No disease symptoms
2. Leaf mottling
3. Chlorosis/leaf mottling
4. Stunting/severe mottling / leaf bunching
5. Leaf defoliation.

$$\text{Disease Severity} = \frac{1 \times P_1 + 2 \times P_2 + 3 \times P_3 + 4 \times P_4 + 5 \times P_5 \times 100}{N (G - 1)}$$

Where: P<sub>1</sub> P<sub>2</sub> to P<sub>5</sub> = Total number of observed plants in each disease symptoms grading per farm site in each state within the agro ecological zone surveyed.

G = Number of grading = 5

N = Total number of observations

Host range test: Five gram of PVMV infected leaf samples collected from the field was homogenized with 10mls of coating buffer (coating buffer + 2% polyvinyl pyrrolidone (PVP): 1.59g of Na<sub>2</sub>CO<sub>3</sub>, 2.93g of NaHCO<sub>3</sub>, 0.2g of NaN<sub>3</sub>, in 1 litre of distilled water: pH 9.6) with a sterilized pestle and mortar and then filtered through mesh cheesecloth (Green. 1991; Thottappilly, 1992)

The following indicator test plants were inoculated: *Celosia argentea* (L), *Chenopodium amaranticolor*, *Chenopodium murale*, *Chenopodium quinoa*, *Chromolaena odorata*, *Commelina nudiflora* (L), *Cochorus olitorius*, *Lycopersicon esculentum* (MiLL), *Nicotiana clevelandii*. (Gray), *Nicotiana glutinosa* (L), *Nicotiana rustica* (L), *Nicotiana tabacum* (L), *Physalis floridana* (Rydb), *Physalis micrantha* (L), *Talinum triangulare*, *Vinca rosea* (L). Mechanical inoculation was made in a screened greenhouse and insects were controlled by periodic spraying with insecticide (Karate, I.C.I).

Four apical leaves of each of the test plants were mechanically inoculated with the sap extracted from PVMV infected leaf samples. The leaves were dusted with 600-mesh carborundum (Silicon carbide) abrasive and each leaf was inoculated with virus saturated cotton swap renewing the inoculum in the pad frequently whilst supporting the leaf with the other hand. Heavy pressure was avoided and same area was not gone over twice. After inoculation, test plants were rinsed with sterilized water from a squeeze bottle. Hands were washed with detergent between inoculations to prevent contamination. The inoculated plants were kept in a netted cage in the screen house and observed for four weeks for symptom development. Serological test was also carried out on the inoculated plants using Protein-A sandwich Enzyme Linked Immunosorbent Assay (PAS-ELISA) (KPL Technical Guide for ELISA Protocols (on line 1999/2000 edition) and IITA Virology laboratory modified protocols) to confirm the presence of PVMV. The sap from inoculated indicator test plants showing viral symptoms were then re-inoculated mechanically following the same procedure on new set of indicator test plants and healthy 4-week-old pepper plants to confirm infection and symptom expression.

## Virus Detection

Protein-a sandwich ELISA (PAS-ELISA): KPL Technical Guide for ELISA Protocols (on line 1999/2000 edition) and IITA Virology laboratory modified protocols for PAS ELISA was also used for the detection of the presence of PVMV, both from the five hundred infected pepper leaf samples collected per agro-ecological zone and samples collected from pepper plants used for the insect transmission test and also from leaf of indicator plants samples used for host range test.

The PVMV antibody used was AAB 328 antiserum diluted in ratio 1:1000 with Phosphate Buffered Saline (PBS-T) (0.05% Tween 20: pH 7.4: 8.0g NaCl, 0.2g KH<sub>2</sub> PO<sub>4</sub>, 1.1g Na<sub>2</sub> HPO<sub>4</sub> 0.2g KCl, 0.2g NaN<sub>3</sub> in 1 Litre H<sub>2</sub>O + 0.5ml Tween 20 (0.05%)) collected from the Virology Laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan.

Virus indexing protocols: One hundred micro liter of protein A at 1µg/ml in coating buffer was dispensed into each well of ELISA plate. The plate was then incubated at 37°C for 2 hours. The plate was washed three times with PBS-T after the incubation period. 100µl of PVMV polyclonal (AAB 328) antiserum diluted 1:1000 in PBS-T was added to each of the ELISA plate and then incubated at 37°C for 2hours. After incubation the ELISA plate was washed three times with PBS-T.

One hundred micro liter of antigen (e.g. sap) ground in PBS-T +2% PVP (Polyvinyl pyrrolidone) was added into each of the wells of the ELISA plate and incubated overnight at 4°C. The plate was washed three times with PBS-T and 100µl of PVMV polyclonal (AAB 328) antiserum diluted 1:1000 in PBS-T was added into each of the wells. The plate was further incubated at 37°C for 2hours after which it was washed three times with PBS-T. 100µl of protein, A- alkaline phosphatase conjugate diluted 1:1000 in conjugate buffer (½ PBS + 0.05% Tween 20 + 0.02% egg albumin + 0.2% PVP + 0.02g NaN<sub>3</sub>) was added per well and the plate incubated at 37°C for 2hrs. The plate was washed three times with PBS-T. 200µl of 0.5 -1mg/ml of p-nitrophenyl phosphate substrate in substrate buffer (97ml diethanolamine + 800ml H<sub>2</sub>O + 0.2g NaN<sub>3</sub> add HCl to give pH 9.8) was added per well and incubated at room temperature for 30 minutes to one hour.

For all incubations plates were covered with ELISA cover plates to avoid edge effects and to maintain uniform temperature. Healthy pepper plants (*Capsicum* sp.) were used as negative control while PVMV infected *Capsicum* sp were used as positive control.

After one hour the absorbance was measured at 405nm using multiscan ELISA reader. The samples were considered positive when the ELISA reading exceeded that of the healthy control by or was at least twice the reading for the healthy control.

### **Polymerase chain reaction (PCR)**

The total nucleic acid extraction: The total nucleic acid extraction was done by using a modified DNA extraction protocol according to Dellaporta et al. (1983).

Two leaf discs (40mg) of PVMV infected pepper leaf sample were ground with 500µl extraction buffer (100ml of 1M Tris- HCl pH 8.0, 100ml of 0.5M EDTA pH 8.0, 100ml of 5M NaCl made up to 950ml with deionized water, autoclaved for 15minutes, and allowed to cool to approximately 50°C. Then 1% (w/v) Polyvinyl pyrrolidone (PVP) (40,000MW), dissolved by mixing was added and 700µl β-Mecarpto ethanol was added with Kontes pestle in a microfuge tube).

The extracted sap was poured into 1.5ml Eppendorf tube and 33µl of 20% Sodium dodecyl sulphate (SDS) plus 10mg/ml RNAase (1-2µl) pH 7.2 (100mg RNAase A (pancreatic RNAase) in 10ml 10mM Tris-HCl 15mM NaCl. Heat to 100°C (in a beaker of boiling water) for 15 minutes, cool slowly to room temperature. Dispense aliquot and store at -20 °C) was added. The sample in the Eppendorf tube was mixed thoroughly for another one minute and then incubated at 65°C for 10 minutes in hot water bath and allowed to cool to room temperature (approximately 2minutes). Ice-cold 160µl of 5M potassium acetate was added and mixed by gently inverting 5-6 times. The sample was then centrifuged at 13,000rpm for 10 minutes. 400µl of the supernatant was carefully transferred to a new 1.5ml Eppendorf tube. Ice-cold 200µl of Isopropanol (-20°C) was added and mixed by gently inverting 8-10 times. It was then kept at 4°C for 15-20minutes after which it was centrifuged at 13,000rpm for 10 minutes to precipitate the nucleic acid. The supernatant was decanted and the last drop of isopropanol was removed by placing the tube face down on paper towel. The nucleic acid was washed by adding 500µl of 70% ethanol and centrifuged at 13,000rpm for 5-10minutes. The ethanol was decanted and the nucleic acid air-dried. The nucleic acid was re-suspended in 500µl high salt TE buffer (50mM Tris-HCl pH 8 / 10mM EDTA, 25ml of Tris-HCl 1M pH 8.0 (121.1g of Tris base, 42ml of HCl), 10ml of EDTA 0.5M pH 8.0 (186.1g of EDTA, 18g of NaOH, 1 litre of de-ionized water, adjust pH to 8.2 and autoclave), make up to 500ml sterilized de-ionized water.). The sample was diluted 200times with the TE buffer and stored at 4°C as stock nucleic acid before amplifying.

Nucleic acid amplification: The nucleic acid was amplified with a primer pair (Gibbs and Mackenzie, 1997) using the PCR machine (Thermal cycler) which has been automated to profile 78 (95°C (Denaturation) 60°C, (Annealing) 72°C (Extension)) (International Institute of Tropical Agriculture (IITA) Ibadan Oyo State Nigeria). The PCR reaction mixture per PCR micro tube was made up of 1.5µl X 10 buffer, 0.9µl MgCl<sub>2</sub>, 1.2µl 2.5mM Deoxynucleotide

triphosphate (dNTP), 1.0µl Primer PTY Forward, 1.0µl Primer PTY Reverse, 0.16µl *Thermus aquaticus* (Taq) polymerase, 2.0µl Extracted DNA sample template, 7.24 dH<sub>2</sub>O.

### Agarose gel electrophoresis

**Gel preparation:** One percent agarose was dissolved (by heating in mixrowine oven for five minutes at medium setting) in an appropriate volume of Tris-acetate-EDTA buffer (TAE) (0.04 M Tris acetate, 0.001 M EDTA, pH 8.0). When the agarose cooled to touch (about 38°C). It was then poured into the gel tray that has been prefitted with comb. The gel was immersed into the electrophoresis tank containing TAE buffer. The comb was then removed to expose the wells formed.

**Loading of sample and running the gel:** Loading buffer (7.5µl) (0.25% bromophenol blue, 0.25% Xylene cyanol FF and 30% glycerol in water) was added into each comb well in the gel with the 15µl amplified nucleic acid (1.5µl X10 buffer, 0.9µl MgCl<sub>2</sub>, 1.2µl 2.5mM Deoxynucleotide triphosphate (dNTP), 1.0µl Primer PTY Forward, 1.0µl Primer PTY Reverse, 0.16µl *Thermus aquaticus* (Taq) polymerase, 2.0µl Extracted DNA sample template, 7.24 dH<sub>2</sub>O) (PCR reaction mixture per PCR micro tube). A standard DNA molecular marker of 1000 base pair (1kilobyte DNA ladder) was used and treated in similar manner. The gel was run at 80-100 volt.

**Staining the DNA:** The gel was lowered carefully into a solution of 1µl (1%) ethidium bromide to stain the DNA. Thereafter the gel was placed in water to remove excess ethidium bromide. The DNA band in the gel was observed under ultra violet light and its position related to the fractions of the DNA molecular marker used. A Polaroid photograph of the bands in the gel was taken. The expected band size was between 0.6- 1.0 kilobyte.

**Electron microscopy:** Symptomatic leaf samples of PVMV infected pepper leaf (*Capsicum* sp.) dried with calcium chloride in MacCartney bottles were sent to (Dr. Stephan Winter, Head,) DSMZ Plant Virus Division <sup>c/o</sup> Messeweg 11/12 38104 Braunschweig Germany for the virus isolation, identification and electron microscopy study.

Determination of the stability of the PVMV sap extract in causing infection. The stability of the PVMV sap extracts at different temperatures, and at different dilutions and their longevity invitro were determined using the method as described by Green (1991). Thermal inactivation point. This is the temperature required to completely inactivate the virus in crude sap during a 10- minute exposure (Green, 1991).

Fifteen grams of virus infected leaf sample was homogenized with 30mls of extraction buffer (Phosphate buffer solution: 8.0g of NaCl, 0.2g of KH<sub>2</sub>PO<sub>4</sub>, 1.1g of Na<sub>2</sub>HPO<sub>4</sub>, 0.2g of NaN<sub>3</sub>, in 1 litre of distilled water and 0.5ml Tween 20 (0.05%) pH 7.8) with a sterilized pestle and mortar and then filtered through mesh cheesecloth. Streptomycin (0.01%) was added to the sap extracted to prevent bacterial contamination. Using a sterilized pipette, 12 screw-capped test tubes were each filled with 2mls of the virus infected sap extract.

Using a sterilized pipette, 2mls of sap extract virus isolate was carefully dispensed into a screw-capped test tube. Each tube was then immersed in a heated shaker water bath for 10 minutes at different temperatures of 35<sup>0</sup>C to 100<sup>0</sup>C at 5<sup>0</sup>C interval. The heated shaker water bath was covered with a transparent glass cover at each temperature level to maintain a uniform temperature within the water bath during the 10 minutes period. After each time limit at each temperature level, the tubes containing the virus sap extract were allowed to cool down to room temperature and then used to inoculate three replicates of five test plant (a 4-week old "Tattasai" pepper) seedlings replicated three times. The test plants were then observed for symptom development between 3 days to 30 days. Serological test using PAS-ELISA was also used to determine the presence of PVMV in inoculated test plants. The temperature range at which virus activity ceased was recorded for the virus isolate. Longevity *in vitro*. This is the length of time the virus is infective in crude sap kept at room temperature [25°C – 27°C].

Three replicates of five test plants (a 4-week old "Tattasai" pepper) replicated three times were inoculated with sap from individual test tubes at different days for 12 days, the test plants were then observed for symptom development between 3 to 30 days from the day of inoculation. Also serological test using PAS-ELISA was used to determine the presence of PVMV in inoculated test plants. The day at which virus activity ceased was recorded for the virus isolate.

**Dilution end point:** This is the highest dilution of plant sap in which the virus is still infectious. Ten grams of PVMV virus infected leaf sample was homogenized with 20mls of extraction buffer (Phosphate buffer solution: 8.0g of NaCl, 0.2g of KH<sub>2</sub>PO<sub>4</sub>, 1.1g of Na<sub>2</sub>HPO<sub>4</sub>, 0.2g of NaN<sub>3</sub>, in 1 litre of distilled water and 0.5ml Tween 20 (0.05%) pH 7.8) with a sterilized pestle and mortar and then filtered through mesh cheesecloth.

Serial ten-fold dilutions were then made from this original sap. 2mls of extract was pipetted from the original sap extract into the first of the eight tubes. Using a sterilized pipette, 1.8ml of sterilized distilled water was pipetted into each of the remaining 7 tubes. From the first tube 200µl of the sap was pipetted and transferred to the second tube to

give a dilution of 1/10. From the second tube (1/10 dilution) 200µl of the extract was transferred into the third tube (1/100 dilution) and this was repeated for the remaining tubes up to dilution of 1/10,000,000.

Three replicates of five test plants [4 – week old bell shaped pepper seedlings] replicated three times were inoculated with sap samples for each serial dilution, the test plants were then observed for symptom development between 3 to 30 days from the day of inoculation. Also serological test using PAS-ELISA was used to determine the presence of PVMV in inoculated test plants. The dilution rate at which virus activity ceased was recorded for the virus isolate.

## Results

**The Incidence and severity of *Pepper veinal mottle virus* diseases:** Similar trends were obtained in the results in the years under study with no significant difference. Sixty eight percent of the farms visited were observed to be under sole pepper cropping system while 32% had mixed-cropping system. The pepper plants were either intercropped with maize, cassava, yam or plantain. Mixed pepper cropping system was observed to be mostly practiced in the humid forest and the derived savanna, where 86% of the farmers' grew pepper with other crops on the same parcel of land and 14% of the farmers practiced sole pepper cropping system. But in the southern Guinea savanna, mid-altitude, northern Guinea savanna and Sudan savanna only 10% of the farmers practiced mixed pepper intercrop.

All the farms visited recorded the presence of PVMV. Out of the three thousand suspected viral infected pepper leaves sample collected from the fields in the six agro-ecological zones, 88% were confirmed to be PVMV positive through the serological test while 12% were found to be negative.

All the pepper farms surveyed showed significant difference in PVMV disease incidence and severity within the agro-ecological zones (Table 1). The percentage PVMV disease incidence ranged between 39.14% in the Sudan savanna to 50.12% disease incidence in the derived savanna zone (Table 1). The incidences of virus diseases were observed to be high in the derived savanna and the humid forest compared with the other agro-ecological zones (Table 1). The derived savanna zone recorded a mean percentage PVMV disease incidence (50.12%) and severity (43.85%) while the Sudan savanna zone recorded the lowest disease incidence (39.14%) and severity (34.48%) (Table 1).

**Tab. 1** Mean percentage of *Pepper veinal mottle virus* incidence and severity in six agro-ecological zones of Nigeria surveyed

Zones	Mean % Disease Incidence	Mean % Disease Severity
Humid Forest	48.54 <sub>b</sub>	42.69 <sub>a,b</sub>
Derived savanna	50.12 <sub>a</sub>	43.85 <sub>a</sub>
Southern Guinea savanna	40.41 <sub>e</sub>	36.88 <sub>c</sub>
Mid Altitude	46.0 <sub>c</sub>	42.9 <sub>a,b</sub>
Northern guinea savanna	44.0 <sub>d</sub>	41.30 <sub>b</sub>
Sudan savanna	39.14 <sub>e</sub>	34.48 <sub>d</sub>
Standard error ±	0.56	0.49

Means followed by the same alphabet in each column are not significantly different from each other at 0.05 level.

Viral isolates from the field samples of infected pepper plants collected were able to induce distinct symptoms on inoculated healthy pepper plants and a range of indicator plants used. PVMV induced a systemic leaf mottling on *Nicotiana clevelandii* (Gray), leaf mottling and necrotic spot on *N. benthamiana* and systemic symptoms on *N. rustica* L. and *N. glutinosa* L.; *Physalis floridana* (Rydb) and *Lycopersicon* spp., On *Physalis micrantha*, PVMV induced premature abscission of leaves, partial defoliation on chronic infected plants (systemic) and local necrotic spot. Local necrotic spot were induced on *Chenopodium amaranticolor* (Costs and Reyn), *C. quinoa*. (Willd), *C. murale* L. and it caused slight leaf mottling of *Chromolina odorata* L. (Table 2).

**Tab. 2** Symptoms expression of a host plant range mechanically inoculated with PVMV.

	Family	Symptoms
<i>Celosia argentea</i> L.	Amaranthaceae	-
<i>Chenopodium amaranticolor</i> Cost and Reyn.	Chenopodiaceae	Nec.
<i>Chenopodium murales</i> L.	Chenopodiaceae	Nec.
<i>Chenopodium quinoa</i> Willd.	Chenopodiaceae	Nec.
<i>Chromolaena odorata</i> (L.) R.M King and H.Rob	Asteraceae	M,
<i>Commelina nudiflora</i> L.	Commelinaceae	-
<i>Corchorus olitorus</i> L.	Tiliaceae	-
<i>Lycopersicon esculentum</i> Mill., nom.cons.	Solanaceae	S, M
<i>Nicotiana benthamiana</i> L.	Solanaceae	C, Nec., S
<i>Nicotiana clevelandii</i> Gray	Solanaceae	S, V, M,C
<i>Nicotiana glutinosa</i> L.	Solanaceae	M

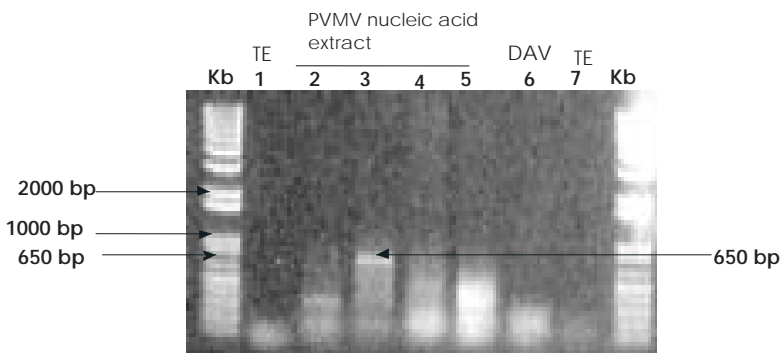
	Family	Symptoms
<i>Nicotiana megalosiphon</i> L.	Solanaceae	S, V, M,C
<i>Nicotiana rustica</i> L.	Solanaceae	S, M
<i>Nicotiana tabacu</i> . C.V. xanthi	Solanaceae	-
<i>Petunia hybrida</i> L.	Solanaceae	C, S, M
<i>Physalis floridana</i> Rydb.	Solanaceae	Nec.
<i>Physalis micrantha</i> . L.	Solanaceae	S, M,Nec
<i>Solanum nigrum</i> L.	Solanaceae	Nec.
<i>Talinum triangulare</i>		Nec.
<i>Vinca rosea</i> . L.	Apocynaceae	-

Keys: C = Chlorosis; S = Systemic; V = Vein banding/Vein Clearing; Nec. = Necrotic lesion; M = Leaf molting; - = No symptoms;

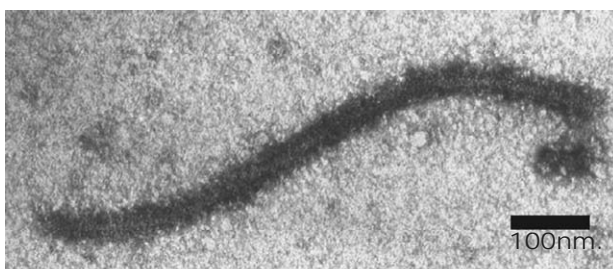
PVMV - Pepper veinal mottle virus (genus Potyvirus. Family Potyviridae)

**Virus detection:** All the farms visited recorded the presence of PVMV. Out of the three thousand suspected viral infected pepper leaves sample collected from the fields in the six agro-ecological zones, 88% were confirmed to be PVMV positive through the serological test while 12% were found to be negative. The PVMV antiserum (AAB 328), diluted at ratio of 1:1000 PBS-T was very effective in detecting the PVMV in viral infected leaf samples through PAS-ELISA serological test.

In the Polymerase chain reaction (PCR), there was successful amplification of the PVMV fragment (0.65 kilobytes) using the nucleic acid molecule from PVMV infected leaf samples. Thus the extraction protocol could be considered to be appropriate for the PCR. (Fig. 2). The Electron micrograph photograph showed antiserum decorated PVMV particles, having flexuous filamentous particles of 750nm in length and 10 nm wide (Plate 1).



**Fig. 2** Polymerase Chain Reaction of total nucleic acids extracted by the modified Dellaporta (1983) extraction method Amplification is 650bp (0.65 kb) nucleic acid of Pepper veinal mottle virus genus Potyvirus Family Potyviridae; Kb = 1kb plus Molecular marker; DAV = *Dascoria alata* virus.



**Plate 1** Electron micrograph of a decorated particle of *Pepper veinal mottle virus* isolated from pepper plant infected with PVMV (Courtesy of Dr. Stephan Winter, Head, DSMZ Plant Virus Division Messeweg 11/12 38104 Braunschweig)

The stability of the *Pepper veinal mottle virus* in sap extract: The thermal inactivation point of PVMV was observed to be 70°C and the virus was still infective up to the 12th day. The dilution end point in which PVMV extract from *Capsicum annum* L. was still infective was found to be at dilution of 10<sup>-4</sup>.

Discussion and conclusion: In all the agro-ecological zones surveyed, characteristic symptoms of PVMV disease, mild mottle, mosaic, vein banding, ring spots, various types of necrosis, leaf discoloration, deformation, blistering and severe stunting of the whole plant were observed on pepper plant infected with PVMV but at varied degree of severity which was similar to earlier described symptoms by Atiri (1992).

Climate and vegetation in the different ecological zones appeared to have played a major role in determining the incidence and severity of PVMV infection on pepper in the fields. The high incidence of PVMV especially in the Derived savanna and the Humid forest agro-ecological zones might not be unconnected with the ability of the virus to remain infective for many months in alternative weed host coupled with a good breeding environment for the vectors of the virus that aids effective transmission. These zones are characterized by thick vegetation and warm humid climate (Fajinmi 2006), with the presence of many secondary host plants for the virus. This would have encouraged the rapid multiplication of the aphid and the virus itself and subsequent increase in the efficiency and the ability of the aphid species to successfully transmit the virus non-persistently.

Proximity of pepper plants to certain important weed hosts has contributed greatly to the spread of viral diseases of pepper (Alegbejo 1987). The weeds include *Solanum nigrum*, *S. gracil*, *Physalis angulata*, *Vigna rosea*, *Vigna sinensis*, *Commelina nudiflora*, *Petunia hybrida*, *Physalis floridana*, *P. micrantha* and *Solanum incanum* (Alegbejo 1987). The ability of the isolated PVMV strain to cause infection on alternative host plants tested suggest for the adaptive ability of the virus on weed host plants in the two agro-ecological zone characterized by thick vegetation and warm humid climate, with the presence of many secondary host plants for the virus and vectors (Aldyhim, and Khalil. 1993) which aided its infective capability and spread of the virus on pepper plants where its incidence and severity had been observed to be high.

There is every possibility of significant pathogenic variability within the PVMV strain isolated as suggested by Gilbert, et. al., (2005), this might have contributed significantly to the adaptive nature of the virus in these agro-ecological zones. Also the physical properties of the virus that showed that the virus was still capable of causing infection below 65°C confirmed the ability of the virus to be able to survive the climatic condition of both the derived savannah and the humid agro-ecological zones but could not survive very well the climatic conditions of the other agro-ecological zones that are characterized with harsh climatic conditions characterized with high temperatures and sparse vegetation.

Therefore the ability of any virus to adapt, cause severe infection and spread in any given environment depend greatly on the vegetation, climatic and ecological characteristics of such area.

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