THE EFFECT OF AGRO-ECOLOGICAL ZONES ON THE INCIDENCE AND DISTRIBUTION OF APHID VECTORS OF PEPPER VEINAL MOTTLE VIRUS, ON CULTIVATED PEPPER (*CAPSICUM ANNUUM* L.) IN NIGERIA

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ABSTRACT

The distribution of aphid vectors of *Pepper veinal mottle virus* (PVMV) was studied on cultivated pepper between 2003 and 2005 in the major pepper producing areas of the six agro-ecological zones in Nigeria. The aphids were isolated, identified and their transmission ability determined.

Population of aphid vectors increased progressively in all the agro-ecological zones from March at the onset of raining season reaching a peak in August and then declining from September at the on set of the dry season. The Humid forest and Derived Savanna agro-ecological zones recorded highest mean population of aphids / leaf and types of aphid's species compared with other agro-ecological zones. Six species of aphid, *Myzus persicae* (Sulzer), *Aphis gossipii* (Glover), *Aphis craccivora* (Koch), *Aphis spiraecola* (Patch), *Aphis fabae* (Blanchard), and *Rhopalosiphum maidis* (Fitch), were identified on the pepper fields surveyed in all the agro-ecological zones. There was no significant difference in the occurrence of *M. persicae*, *A. gossipii, A. craccivora*, and *R. maidis* while *A. spiraecola* and *A. fabae* that varied in occurrence in all the zones. Ability to transmit PVMV to a healthy pepper plants varied in all the identified aphid species but *R. maidis* was not able to transmit PVMV from infected to a healthy pepper plant. A more sustainable approach to controlling pepper viruses is by targeting the aphid vectors and preventing the vectors from reaching the crops because the aphid vectors which are virus specific are the major means of transmitting virus to healthy plant.

KEYWORDS: Agro-ecological Zones, Aphids, Distribution, Pepper, *Pepper veinal mottle virus*. Nigeria

INTRODUCTION

In Nigeria, pepper is cultivated principally in the rain forest and derived savannah of the southwest and in the northern guinea savannah and Sudan ecological zones of the north between latitude 10^0 N and 12^03'N [13] [33].

Pepper veinal mottle virus (PVMV), a *Potyvirus*, is a major constrain to the cultivation of pepper in some parts of Nigeria [3] [14] [16]. Pepper infected by PVMV shows varied symptom expressions and disease severity on the leaves, stem and flowers or fruits [17]. The virus causes severe and devastating destruction of pepper plants; and it has been reported that they are efficiently transmitted in nature by aphids, which are often difficult to control [20] [8] [16] [17].

It has been observed that in aphid-borne viruses, about 5% of the infections occur during the primary spread where the virus is introduced to the plant by aphids that pick up the virus from an external source such as a reservoir host. The other 95% of the infections are believed to occur during the secondary cycle where the virus is spread by aphids that acquired the virus within planting [24] [16].

Therefore, this study was targeted at studying the occurrence and distribution of aphid responsible for the transmission of *Pepper veinal mottle virus,* in cultivated pepper in the six agro-ecological zones of Nigeria.

MATERIALS AND METHODS

Aphid populations trapping was done from March, onset of rainy season in 2003-2005 after peppers were established in fields, and continued until November of the same year, when the long dry season begins. Ten pepper production fields were randomly selected in each agro-ecological zone. Average aphid population counts in 10×10 m areas were done. Population averages were determined.

Sixteen sampling sites were positioned in each pepper field: one sample site within 5 pepper plants were randomly selected from each farm. The trap type was green water pan trap. The water trap was prepared by spray painting square 25 by 25 by 8-cm plastic storage containers with emerald green. The water trap was placed on wooden platforms held 0.5m above the ground on steel fence posts, separated within 2m of each other at each sample site. Aphid samples were removed and preserved in 70% alcohol contained in screw capped sampling bottles with identification indicating location, time and date of collection.

Also irrespective of farm size and plant population, 20 infected pepper plants showing viral disease symptoms were randomly selected from each of the 10 selected farms. The aphids were dislodged to the surface of a white cardboard paper from 10 randomly selected leaves, with a camel hairbrush. Using a hand lens, the aphids, (the winged and wingless), on each leaf were counted and the average count per leaf was obtained per location. Aphid samples were removed and preserved in 70%

alcohol contained in a screwed capped sampling bottle with proper identification tag showing location, time and date of collection. Also, alive aphid samples were collected and reared on four week-old pepper plants in a screened aluminum wire mesh cages (90 X 60 X 60 cm wooden frames). They were later transferred to another set of 4 healthy pepper plants in netted cages in the screen-house for continuous rearing.

The isolated aphids were mounted on slides and identified with the aid of light microscope at magnification of X40 objectives.

Insect transmission test

The isolated and identified aphids were tested for their ability to transmit PVMV in screened aluminum wire mesh cages (90 X 60 X 60 cm wooden frames) in the screen house.

Each aphid species collected from naturally infected pepper plants on the field were starved for 60 minutes in a Petri dish containing a layer of slightly moistened filter paper to prevent the insects from drying up. Each aphid species were then transferred with camel hair brush to the primary leaves of five healthy four week old bell fruit shaped pepper plants (*Capsicum annuum L.* "cv. *Tattasai*"), in five different screened aluminum wire mesh cage (90 X 60 X 60 cm wooden frames) for continuous rearing and to ensure that the aphids species were virus free before use [7] [22].

In another set of screened aluminum wire mesh cage (90 X 60 X 60 cm wooden frames) there were 8-week-old PVMV infected pepper plants (cv. *Tattasai*). 80 aphids that have been starved for 60 minutes as described earlier were transferred onto primary leaves of the virus infected pepper plants for about 60 minutes of acquisition feeding period, Five aphids were transferred on to each of five 4–week old bell fruit shaped pepper plant, "cv. Tattasai" seedlings replicated three times in a screened aluminum wire mesh cages of inoculation access period for 2 hours [7]. This procedure was repeated on all the isolated identified species of aphid. Infection and symptom development of the inoculated plants were observed for a period of two months and statistically analyzed [35].

Detection of PVMV on infected pepper plant transmitted by aphid vectors

The virus (PVMV) was detected by a Protein-A sandwich ELISA (PAS-ELISA) [26] [28] and through Polymerase chain reaction serological test [12] [21] [26]

Virus Detection

Protein-A sandwich ELISA (PAS-ELISA)

The same PAS-ELISA protocol was used for the detection of PVMV in the five hundred infected pepper leaf samples collected per agro-ecological zone and samples collected from pepper plants used for the insect transmission test. The PVMV antibody used was AAB 328 antiserum diluted 1:1000 with Phosphate Buffered Saline (PBS-T) (0.05% Tween 20: pH 7.4: 8.0g NaCl, 0.2g KH_2 PO_4, 1.1g Na_2 HPO_4 0.2g KCl, 0.2g NaN_3 in 1 Litre H_2O + 0.5ml Tween 20 (0.05%)) obtained 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^OC 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 37^0C 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 (Polyvynil pyrolidone) was added into each of the wells of the ELISA plate and incubated overnight at 4^0C. 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 37^0C 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 ($^1/_2$ PBS + 0.05% Tween 20 + 0.02% egg albumin + 0.2% PVP + 0.02g NaN_3) was added per well and the plate incubated at 37^0C 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_2O + 0.2g NaN_3 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 incubation with the substrate, the absorbance was measured at 405nm using multiscan ELISA reader. The samples were considered positive when the ELISA reading exceeded twice the reading of the healthy control.

Polymerase chain reaction (PCR)

Total nucleic acid extraction and amplification

The total nucleic acid extraction was done by using a modified DNA extraction protocol according to Dellaporta *et al.* (1983) and Gibbs and Mackenzie, (1997). Amplified PCR product. Neuclotide sequence at the 5'–rapid amplification of cDNA ends (RACE) using PVMV 5' RACE (5' GTGAGTGTTGTAGAAGCACGGG-3") to determine the partial sequence of PVMV and purified nucleic acid was amplified with a Primer sequence (5′-3′) (TC(G/A/T/C)A(T/C)CAT(G/A/T/C)ACCCACAT(G/A/T/C)CC and

ATGGTITGGTG(T/C)AT(A/T/C)GA(G/A)AA(T/C)GG) using a thermal cycler which has been adjusted to produce the following cycles:95°C for denaturation, 60°C for annealing and 72°C for extension.

The PCR reaction mixture per PCR micro tube used was made up of 1.5µl X 10 buffer, 0.9µl $MgCl_2$, 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_2O.

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 about 38°C,it was then poured into the gel tray that has been prefitted with a 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 $MgCl_2$, 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_2O) (PCR reaction mixture per PCR micro tube). A standard DNA molecular marker ladder of 1000 base pair was used and treated in a similar manner. The gel was run at 80-100 volt.

Staining the DNA

The gel was stained in a 1% ethidium bromide. 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 a Polaroid photograph of the bands in the gel was taken.

RESULTS

Aphid population count per leaf.

There were no significant differences in results between years. Occurrence of aphid vectors varied within months (Fig 1). Populations of aphid vectors increased from March, onset of rainy season, reaching a peak in August and then declining from September towards the dry season.

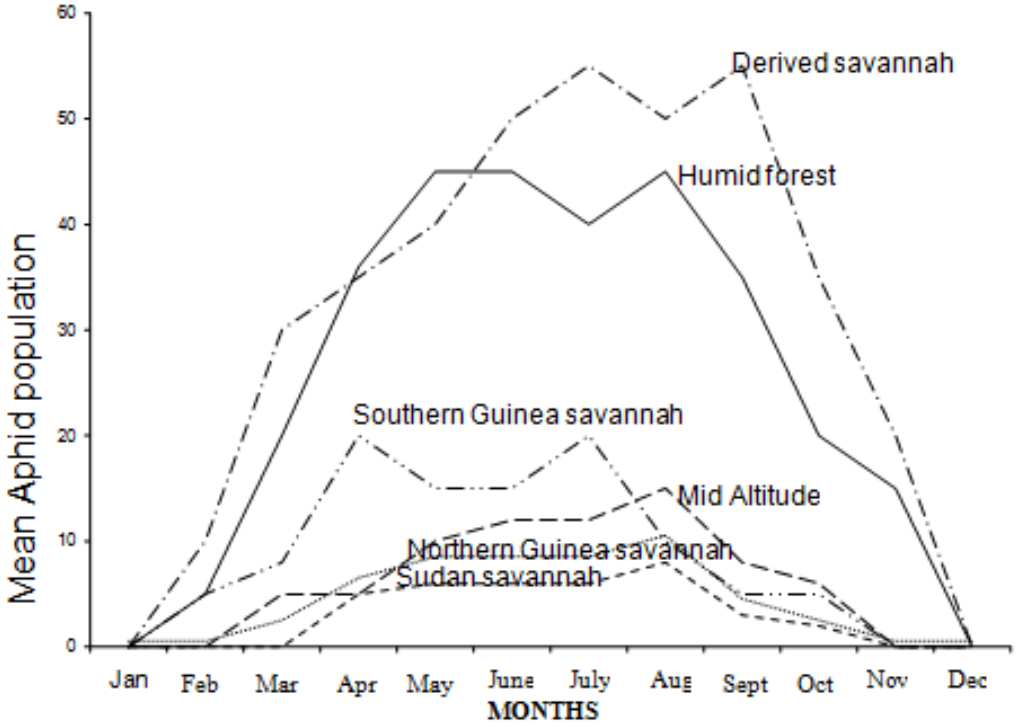

Fig. 1: Mean monthly aphid population on virus infected pepper plants in six agro-ecological zones of Nigeria.

The highest average aphid's population per leaf was found in the Derived Savanna zone followed by the Humid Forest. The population count of the aphids was significantly lower in the Mid Altitude and Sudan savanna agro-ecological zones (Table 1).

Six species of aphids were identified in all the pepper fields surveyed in the agro-ecological zones; these were; *M. persicae* (Sulzer), *A. gossipii* (Glover), *A. craccivora*

(Koch), *A. spiraecola* (Patch), *A. fabae* (Blanchard), *R. maidis* (Fitch) (Table 1). Whereas *M. persicae* (Sulzer) and *A. gossipii* (Glover) were found in all the zones, other species were absent in some zones (Table 1).

TABLE 1: Occurrence of aphid species and their populations/leaf in each agro-ecological zone

Agro-ecological zones	*Myzus persicae*	*Aphis gossipii*	*Aphis craccivora*	*Aphis spiraecola*	*Aphis fabae*	*Rhopalosphum madis*
Humid forest	4a,b[a]	3a	1a	2a	2a	2a
Derived savanna	6a	4a	1a	2a	1a,b	2a
Southern Guinea savanna	3b	2a	0a	2a	0b	1a,b
Mid Altitude	3b	3a	0a	2a	0b	0b
Northern Guinea savanna	3b	2a	1a	0a	2a	1a,b
Sudan savanna	2b	2a	0a	1a,b	1a,b	0b
Standard error+	0.4	0.3	0.16	0.2	0.2	0.28

[a] Means in a same column followed by the same letter are not significantly different, $P<0.05$, Duncan's multiple range test.

There were no significant differences in occurrence of *A. gossipii*, *A. craccivora*, and *A. spiraecola* in different agro-ecological zones. The occurrence of *A. fabae* was similar in the humid forest, derived savanna, northern Guinea savanna and Sudan savanna; there were none in the southern Guinea savanna and mid-altitude and this number was similar to the derived savanna and Sudan savanna zones.

M. persicae (Sulzer) was found in all the agro-ecological zones but at varied degree of occurrence. There was no significant difference in the occurrence of *M. persicae* in southern Guinea savanna, mid-altitude Northern Guinea savanna and Sudan savanna. The Derived savanna recorded the highest incidence of *M. persicae* (Sulzer) which varied significantly from other agro-ecological zones except Humid forest (Table 1)

Fewer *R. maidis* were isolated on pepper plants and maize plants in a maize pepper intercrop, generally beneath leaf bracts of the maize cob and towards the point of attachment to the stem of the plant. Other species of aphids identified on maize were; *Aphidius colemani* (Viereck), and *A. gossipii*.

Insect transmission test

All aphid species except *R. maidis* transmitted PVMV from infected to healthy plants...'

(Table 2). 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)

TABLE 2: Ability of aphid specie isolated from cultivated pepper plants to transmit *Pepper veinal mottle virus* from pepper

	Myzus persicae			*Aphis gossipii*			*Aphis craccivora*			*Aphis spaericola*			*Aphis fabae*			*Rhopalosiphum maidis*		
	Susceptibility[a]			Susceptibility			Susceptibility			Susceptibility			Susceptibility			Susceptibility		
	REPLICATIONS			REPLICATIONS			REPLICATIONS			REPLICATIONS			REPLICATIONS			REPLICATIONS		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
	4/5	3/5	4/5	4/5	4/5	5/5	2/5	2/5	2/5	3/5	3/5	3/5	2/5	2/5	2/5	0/5	0/5	0/5
PVMV	3/5	3/5	4/5	3/5	4/5	4/5	3/5	2/5	3/5	4/5	3/5	2/5	2/5	3/5	2/5	0/5	0/5	0/5
	4/5	5/5	3/5	4/5	5/5	3/5	1/5	2/5	2/5	3/5	3/5	2/5	3/5	2/5	3/5	0/5	0/5	0/5
Percent	73.33			80			42.22			57.77			46.66			0		
Mean	SE+4.71			SE+4.71			SE+4.0			SE+4.0			SE+3.33			SE+0		

SE + Standard error

[a] Numerator is the number of plants that became infected; denominator is the number of plants inoculated

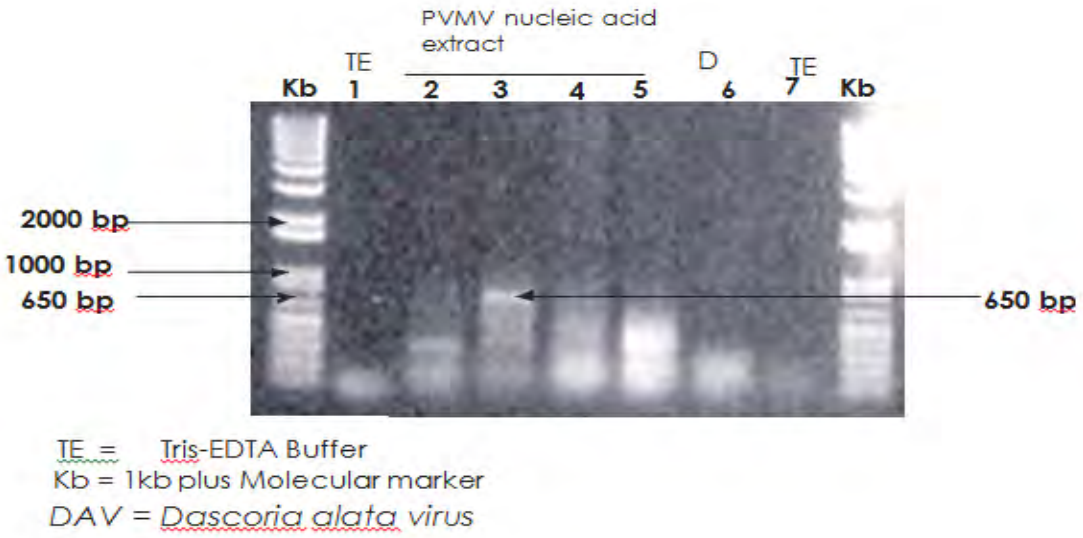

Fig 2: Polymerase Chain Reaction of total nucleic acids extracted by the modified Dellaporta (1983) extraction method Amplification is 650bp (0.65 kb) nucleic acid of *Pepper veinal mottle virus* genus *Potyvirus* Family *Potyviridae*

DISCUSSION

The high incidence of aphid vectors on pepper plant in the Derived savanna and the Humid forest agro-ecological zones compared with other agro-ecological zones might have been influenced by the climate and vegetation of these zones. These zones are characterized by thick vegetation and warm humid climate, coupled with the presence of many secondary host plants for the aphid vectors. This could 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.

The aphids exhibited fluctuations in abundance that was related to the vegetation of the ecological zones, with the derived savanna recording the highest mean aphid types and population per leaf compared with other agro-ecological zones. The vegetation type coupled with minimum adequate rainfall and enough light intensity might have contributed to the reproductive tendencies of the aphid species. It has been reported that light intensity and day length played an important role in the reproductive capacity of aphids [1]. With increasing day length, the reproductive rate increases thereby increasing aphid population but the effect of light intensity is yet to be understood [1]. Season and temperature variation have been described to have a major effect in the abundance of aphid population because it plays a major role in reproduction ability and mortality of aphids [30] [25].

The environment has been reported to be a major abiotic factor affecting aphid's population dynamics [31] [29]. The presence of many alternative secondary hosts for the breeding of the aphids and the viruses made the population per leaf of the aphids and aphids type to be more in the Derived savanna agro-ecological zone. It was

reported that during low rainfall, aphid migrants colonize a wide range of secondary host, on which apterous females are produced which reproduce parthenogenetically [11]. Rapid rates of population occur, resulting in dense colonies and in response to overcrowding, they continuously colonize fresh herbaceous secondary host plant [11].

In considering the possible role of aphid-borne virus, the survival of the virus is dependent on having an efficient aphid vector. Aphid density per leaf has been described to have a major effect in disease incidence and severity on a plant [18] [19]. They stated that plants with high aphid vector numbers increases the probability of virus transmission and subsequent increase in disease incidence and severity. This kind of observation was observed in the Derived savanna and the Humid forest agro-ecological zones that both recorded high aphid population per leaf and high disease incidence, and severity.

Rainfall may not likely be the reason for the sudden decline in aphid's population per leaf in other agro-ecological zones but sparse vegetation cover and other climatic conditions such as high temperature, low humidity and the long dry season from September and March before the onset of the rains might have caused the aphids to die as a result of non-availability of food source for survival. Sparse vegetation cover coupled with limited number of secondary hosts for the aphids and the virus, nutritional stress or nutritional factor and overcrowding have been reported to have an effect on aphid population [9] [10] [32]. To support this argument that the cause might be nutritional, it has been shown that aphid infestation on *Solanum integrifolium* (L) changes peroxidase, esterase, and protein content of the plant in proportion to the level of infestation [34].

Out of the six species of aphids identified to be associated with the field cultivated peppers, *Myzus persicae* (Sulzer) and *Aphis gossypii* (Glover) showed the highest transmission ability. Their occurrence in all the farms surveyed in all the six agro-ecological zones indicated their efficiency in spreading PVMV diseases of pepper. They have both been implicated to have the ability to transmit non-persistently viruses that infect peppers [8] [2] [6] [15] [16]. Their isolation in all the agro-ecological zones confirmed their ability to adapt and breed efficiently in different agro-ecological zones in Nigeria.

Myzus persicae (Sulzer) has been reported to be highly polyphagous on hosts, which are in over 40 different families, including *Brassicaceae, Solanaceae, Poaceae, Fabaceae, Cyperaceae, Convolvulaceae, Chenopodiaceae, Compositae, Cucurbitaceae* and *Umbelliferae* [4]. *Myzus persicae* (Sulzer) has been identified to be an important aphid virus vector; it has been shown to transmit well over 100 plant virus diseases, in about 30 different families, including many major crops [5] [23] [19]

The relationship of *Rhopalosiphum maidis* (Fitch) with pepper could not be established by this study because of its inability to transmit PVMV from infected pepper plant to healthy plant, though it has been reported to have the ability to transmit *Potyviruses* [8]. *Aphis craccivora* (Koch) and *Aphis fabae* (Blanchard) were

another set of isolated aphid species that exhibited the ability in transmitting PVMV but not as efficiently as *Myzus persicae* (Sulzer)and *Aphis gossypii*(Glover).

CONCLUSION

The management and control of PVMV disease is the prevention of crop losses [27]. Though it is very difficult to eliminate viruses from infected plants directly, management of PVMV disease should be targeted at the aphid vectors. It is highly suggested that the ability of the aphid vectors from spreading the virus within and across cultivated crops should be prevented or eliminated. Therefore, to have an effective control of PVMV diseases on cultivated peppers, control should be directed and targeted towards these aphid vectors.

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