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Evidence of expanded diversity in weeds as reservoir host of viruses in pepper fields across southwestern Nigeria

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ABSTRACT
This study aimed to investigate virus occurrence in weed species in the main pepper-growing areas in Southwest Nigeria. The weed samples collected from pepper farms were identified and subjected to Antigen-Coated Plate Enzyme-Linked Immunosorbent Assay using antibodies specific eight different viruses. Results showed that the Weed species collected contain 17 families, 33 genera and 36 plant species of which 83.33% of the plant species tested positive to one or more plant viruses. The results indicate that potato virus Y (PVY) and potato virus X (PVX) infected more weed species (24). Also, Ageratum conyzoides serve as host to 8 viruses while Alchornea cordata, Corchorus olitoris and Talinum triangulare serve as host to 7 viruses respectively. These results provide information on weeds as virus reservoirs and contribute to the knowledge of epidemiology of these diseases, enabling a proper weed management aiming at reducing the secondary spreading control of viruses.

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Control; diseases; epidemiology; genus; plant species; serological

Introduction

The nomenclature of viruses are often based on the plants they infect even though most of these viruses can infect more than one plant species (Goyal et al. 2015). Viruses cause many important plant viral diseases. Some or many of the viral diseases cause reduction in yield and quality of food crops (Kucharek et al. 2003; Arogundade et al. 2019a). Viral pathosystem in most horticultural crops are caused by interactions

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with susceptible host, virulent pathogen and a suitable environment (Islam et al. 2017).

In southwest Nigeria, reports have shown that the incidence of viral diseases of Capsicum species are generally high across the zone with a relatively high average of 78%. Viruses especially potato virus Y, tomato etch virus, cucumber mosaic virus, pepper veinal mottle virus and potato virus x are of high occurrence causing diseases in pepper fields in the zone (Arogundade et al. 2015). The high incidence is not unconnected with their ability to remain infective for many months in alternative weed hosts together with a good breeding environment for the vectors of the virus that aids effective transmission (Arogundade et al. 2015).

Weeds act as hosts of plant viruses and can be regarded as virus reservoir of a number of cultivated crops, hence, making viral infections difficult to control (Kucharek and Purcifull 2001; Arogundade et al. 2019b). Most viruses can overwinter in annual weeds through seeds, this play an important role in maintaining viral load from which vectors spread viruses in the next planting season (Norris and Kogan 2005; Arogundade et al. 2019b). The determination of the extent to which weeds actually contribute to reestablishment of diseases in crop plants still remain the major difficulty in integrated pathogen management (Wisler and Norris 2005).

Also, the science of plant virus ecology which takes into account plant virus populations and their hosts that causes economically important disease epidemics within a particular environment has received less attention (Islam et al. 2017). When studies on plant virus ecology is compared with studies on valuable agronomic and ornamental plants, the former is reported to be relatively scarce, except when the pathogen provides biological control of the weed (Wisler and Norris 2005). Studies on artificial inoculation in the greenhouse have been numerous for host range but such tests may not necessarily represent natural infections or epidemiology of these viruses. Few attempts have been made to list host ranges of the viral diseases found naturally on vegetables in Nigeria. Hence, this study provides information about weed species that serve as reservoir host of viruses that majorly infect vegetable crops especially pepper under natural field condition in south-western states of Nigeria.

Materials and methods

Study area

The study was conducted in the southwestern states of Nigeria, viz; Oyo, Ogun, Osun, Ondo, Ekiti and Lagos. The region falls under the rain forest (Oyo, ogun, Osun, Ondo and Ekiti) and Mangrove (Lagos) agroecological zones and it is located in the tropics of the country.

The site experiences more of wet seasons than dry seasons and rainfall which normally begins in the month of March and ends in November with its peaks in the month of June.

Collection and identification of samples

Weed Sampling was conducted during a survey of pepper fields to determine weeds that co-host viruses infecting pepper in southwest Nigeria from June to September in the 2010 and 2011 planting seasons. Symptomatic weed samples were collected with-in and around pepper farms in all locations surveyed for pepper viruses. A total of 280 samples exhibiting or not suggestive of symptoms of viral infection were randomly collected from 28 Local Government Area (LGA) in the 6 southwest states where pepper is produced. The samples were collected in a screwed cap sample bottle with silica gel lined with cotton wool. The plant samples collected were identified using the flora of West Africa (Hutchinson and Dalziel 1972) and a handbook of West African weeds (Akobundu and Agyakwa 1998). Plants that could not be identified using these floras were sent to the Obafemi Awolowo University, Ile-Ife, Department of Botany Herbarium (IFE herbarium) for identification.

Serological detection analysis of the samples viruses

The sampled leaves were subjected to antigen-coated plate enzyme-linked immunosorbent assay (ACP - ELISA) as described by Kumar (2009). The samples were tested for the presence of potato virus Y (PVY), potato virus X (PVX), pepper veinal mottle virus (PVMV), pepper mild mottle virus (PMMV), tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), tobacco etch virus (TEV) and tomato mosaic virus (ToMV) using homologous rabbit polyclonal antiserum. Absorbance values were quantified at 405 nm using a microplate reader (Micro Read 1000, ELISA Plate Analyser) at 4 hours after incubation. Values were accepted to be positive when the optical density reading was at least twice that of the negative control.

Results

The Weed species collected from the survey contain 17 families, 33 genera and 36 plant species (Table 1 and Figure 1). The different weed species expressed diverse virus like symptoms in the natural habitat (Plate 1). Enzyme-Linked immunosorbent Assay (ACP-ELISA) used to assay the plant species revealed that 83.33% of the plant samples

Table 1. The weeds identified in five states and intercepted viruses.

Family	Genera	Plant Species	Habit	State Represented	Virus	Virus Host
Acanthaceae	*Nelsonia*	*Nelsonia canescens*	Herb	Osun	PVY + PVX + TEV	3
Amaranthaceae	*Alternanthera*	*Alternanthera brasilianca*	Herb	Ekiti	CMV	1
		Alternathera sessilis	Herb	Oyo	PVX + PMMV + CMV + TEV	4
	Amaranthus	*Amaranthus spinosus*	Herb	Osun	–	0
Asteraceae	*Acanthospermun*	*Acanthospermun hispidium*	Herb	Oyo	PVY + PVX + CMV + TEV	4
	Ageratum	*Ageratum conyzoides*	Herb	Ogun, Ondo	PVY + PVX + PMMV + PVMV TMV + CMV + TEV + ToMV	8
	Aspilia	*Aspilia africana*	Herb	Ondo	PVY + PVX + PVMV + PMMV + CMV + TEV	6
	Bidens	*Bidens pilosa*	Herb	Oyo	PVY + PVX + CMV + TEV + ToMV	5
	Chromolaena	*Chromolaena odorata*	Shrub	Oyo, Osun	PVY, PVX, PVMV, CMV	4
	Synedrella	*Synedrella nodiflora*	Herb	Ondo, Osun	PVY + PVX + PMMV + CMV + TEV	5
	Tithonia	*Tithonia diversifolia*	Herb	Oyo, Osun, Ekiti	PVY + PVX + PMMV + CMV + TEV	6
	Vernonia	*Vernonia amygdalina*	Shrub	Ondo	PVY + PVX + CMV + TEV	4
Commelinaceae	*Commelina*	*Commelina bengalensis*	Herb	Ogun, Oyo	PVY + PVX + PMMV + PVMV + CMV + TEV	6
Convolvulcaeae	*Hewitta*	*Hewitta sublobata*	Herb	Osun	–	0
Cucurbitaceae	*Momordica*	*Momordica charantia*	Herb	Osun	–	0
Dioscoreacae	*Dioscorea*	*Dioscorea rotundata*	Herb	Ondo	PVY + PVX + PMMV + PVMV + TEV	5
Euphorbiaceae	*Acalypha*	*Acalypha ciliate*	Herb	Ekiti	PVY + CMV + TEV	3
	Alchornea	*Alchornea cordata*	Shrub	Ogun	PVY + PVX + PMMV + PVMV + TMV + CMV + TEV	7
	Jatropha	*Jatropha caucas*	Shrub	Ondo	PVY + PVX + CMV + TEV	4
	Manihot	*Manihot esculenta*	Shrub	Ekiti	–	0
Fabaceae	*Calopogonium*	*Calopogonium mucunoides*	Herb	Osun	PVY	1
	Centrosema	*Centrosema pubescens*	Herb	Oyo, Osun	PVY + PVX + PMMV + PVMV + CMV + TEV	6
Malvaceae	*Corchorus*	*Corchorus olitoris*	Herb	Ogun	PVY + PVX + PMMV + PVMV + TMV + CMV + TEV	7
	Sida	*Sida acuta*	Herb	Oyo	PVY + PVX + CMV + TEV + ToMV	5
		Sida cordifolia	Herb	Oyo	PVY + PVX + CMV + TEV	4
		Sida linifoia	Herb	Ekiti	–	0
Moraceae	*Ficus*	*Ficus exasperata*	Tree	Ogun	PVY + PVX + PMMV + PVMV + CMV + TEV	6
Onagraceae	*Ludwigia*	*Ludwigia abyyssinica*	Herb	Ondo	–	0
Poaceae	*Panicum*	*Panicum maximum*	Grass	Ondo	–	0
Rubiaceae	*Diodia*	*Diodia scandens*	Herb	Ekiti	–	0
	Spermacoce	*Spermacoce verticulata*	Herb	Ekiti	PVY + PVX + PVMV	3
Talinaceae	*Talinum*	*Talinum triangulare*	Herb	Ogun	PVY + PVX + PMMV + PVMV + TMV + CMV + TEV	7
Uticaceae	*Fluerya*	*Fluerya aestuans*	Herb	Oyo	PVY + PVX + PMMV + CMV + TEV + ToMV	6
	Pouzolzia	*Pouzolzia guineensis*	Herb	Ondo	CMV	1
Verbenaceae	*Lantana*	*Lantana camara*	Shrub	Ondo	PVY + PVX	2
	Stachytarpheta	*Stachytarpheta cayenensis*	Herb	Ekiti	PVX	1

PVY- potato virus Y, PVX- potato virus X, PVMV- pepper veinal mottle virus, PMMV- pepper mild mottle virus, TMV- tobacco mosaic virus, CMV- cucumber mosaic virus, TEV- tobacco etch virus, ToMV- tomato mosaic virus

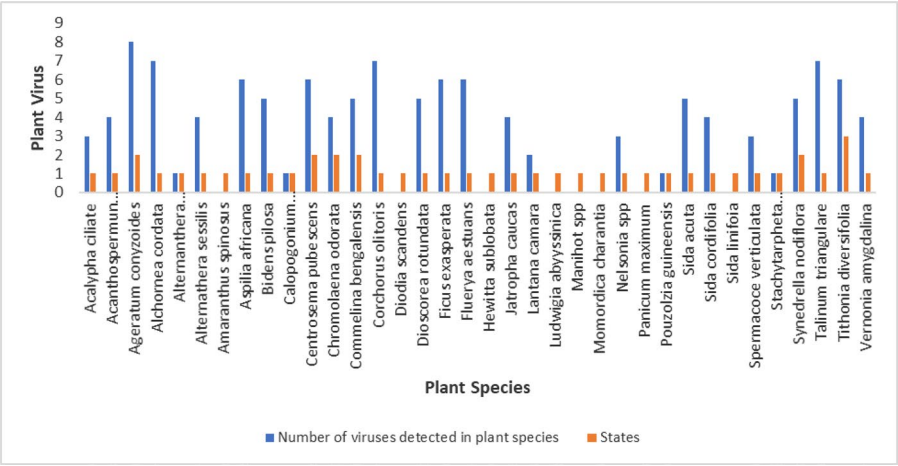

Figure 1. The plant species composition in each state and frequency to serve as virus host.

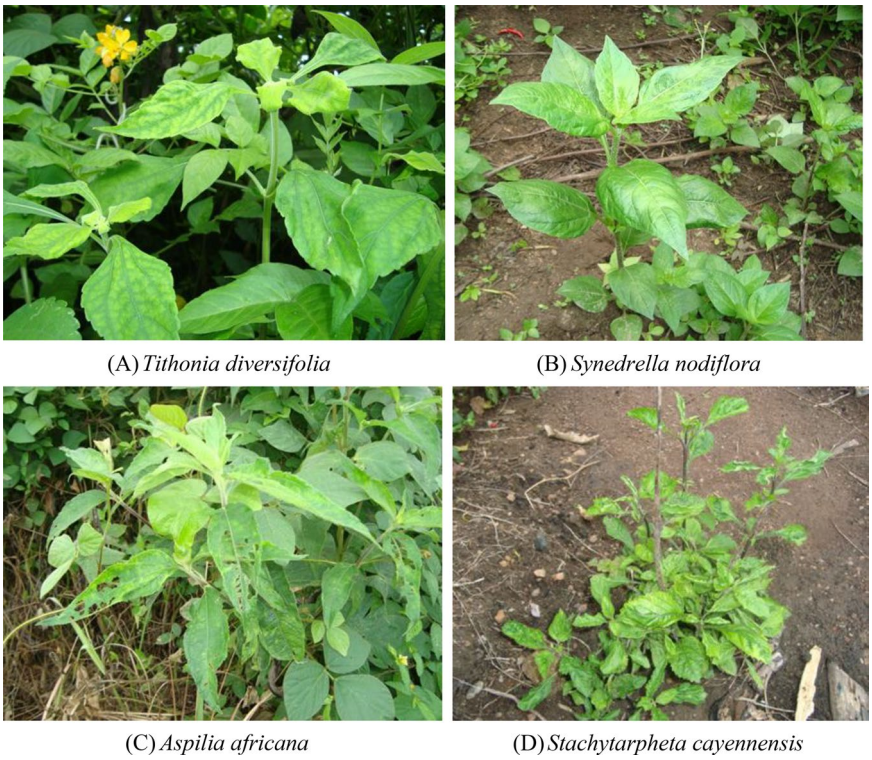

Plate 1. Some weed species on the field showing symptoms of viral infection.

collected tested positive to one or more of PVY, PVX, PVMV, PMMV, TMV, CMV, TEV and ToMV (Table 1 and Figure 2).

The samples of weed flora within and around pepper fields in Ogun state include *T. triangulare, C. olitorus, Commelina bengalensis, A. conyzoides, A. cordata and Ficus exasperata*. *Talinum triangulare, A. cordata* and

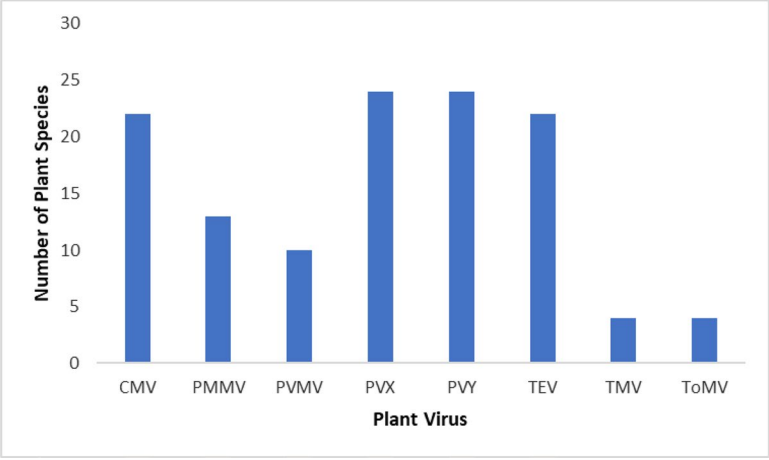

Figure 2. Distribution of intercepted viruses on identified weed species. PVY- potato virus Y, PVX- potato virus X, PVMV- pepper veinal mottle virus, PMMV- pepper mild mottle virus, TMV- tobacco mosaic virus, CMV- cucumber mosaic virus, TEV- tobacco etch virus, ToMV- Tomato mosaic virus

C. olitorus served as alternate host to seven (7) viruses each - PVY, PVX, PVMV, PMMV, TMV, CMV and TEV-, *F. exasperata* serve as host to six (6) viruses - PVY, PVX, PVMV, PMMV, CMV, and TEV-, *A. conyzoides* served as host to five (5) viruses - PVY, PVX, PVMV, CMV and TEV -, while *C. bengalensis* served as host to only PVY (Table 1).

Ten weed species from Ondo state identified as *A. conyzoides, Discorea rotundata, Lantan, camara, Synedrella nodiflora, Aspilia africana, Vernonia amygdalina, Jatropha caucas, Panicum maximum, Ludwigia abyssinica* and *Pouzolzia guineesis* were infected with various viruses when tested with ACP-ELISA. *Agaratum conyzoides* served as host to eight (8) viruses - PVY, PVX, PVMV, PMMV, TMV, CMV, TEV and ToMV-, *D. rotundata* Served as host to PVY, PVX, PVMV, PMMV and TEV, *L. camara* was infected with PVY and PVX, *S. nodiflora* was infected with PVY and PMMV, *A. africana* was infected with PVY, PVX, PVMV, PMMV, CMV and TEV, *V. amygdalina* was infected with PVY, PVX, CMV and TEV, *J. caucas* was infected with PVY, PVX, CMV and TEV. *Panicum maximum* and *L. abyssinica* were not infected with any of the viruses assayed while *P. guineesis* was only infected with CMV (Table 1).

The weed flora identified within and around pepper fields in Oyo state were *T. diversifolia*, *Alternathera sessilis, C. odorata, Sida acuta, Fluerya aestauans, Centrosema pubescens, C. bengalensis, Bidens pilosa, Sida cordifolia* and *Acanthospermun hispidium*. *Tithonia diversifolia* served as host to PVY, CMV and TEV, *A. sessilis* served as host to PVX, PMMV, CMV and TEV, *C. odorata* served as host to PVY, PVX, CMV and TEV, *S. acuta* served as host to PVY, PVX, CMV, TEV and ToMV, *F. aestauans*

served as host to PVY, PVX, PMMV, CMV, TEV and ToMV, *C. pubescens* served as host to PVY, PVX, PVMV, PMMV, CMV and TEV, *C. bengalensis* was infected with PVY, PVX, PMMV, CMV and TEV, *B. pilosa* served as host to PVY, PVX, CMV, TEV and ToMV, *S. cordifolia* served as host to PVY, PVX, CMV and TEV, *A. hispidium* served as host to PVY, PVX, CMV and TEV (Table 1).

The weed flora in Osun state include *C. pubescens, Amaranthus spinosus, C. odorata, Hewitta sublobata, Synedrella nodiflora, Nelsonia* spp., *T. diversifolia, Momordica charantia* and *Calapogonium mucunoides*. *Amaranthus spinosus*, *Hewitta sublobata* and *M. charantia* did not test positive to any of the viruses assayed using ACP-ELISA. However, *C. pubescens* and *C. odorata* were infected with PVY and CMV while *S. nodiflora* was infected with PVY, PVX, CMV and TEV, *Nelsonia* spp. was infected with PVY, PVX and TEV, *T. diversifolia* was infected with PVY, PVX and CMV while *C. mucunoides* served as host to only PVY (Table 1).

Weeds identified within and around pepper fields in Ekiti state and assayed using ACP-ELISA were *Spermacoce verticulata, T. diversifolia, Stachytarpheta cayennensis, Alternanthera brasilianca, Diodia scandens, Sida linifolia, Acalypha ciliate* and *Manihot* spp. *Spermacoce verticulata* was host to PVY, PVX and PVMV, *T. diversifolia* was infected with PVY, PVX, PVMV, PMMV, CMV and TEV, *Stachytarpheta cayennensis* and *Alternanthera brasilianca* was infected with only PVX and CMV respectively and *Acalypha ciliate* was infected with PVY, CMV and TEV while *S. cayennensis*, *Diodia scandens* and *Sida linifolia* were not host to any of the viruses assayed (Table 1).

Number of infected plant species was high for PVY and PVX (24) followed by CMV and TEV (22), while TMV and ToMV showed the least number of infected weed species (4) (Figure 2). The diversity of weed species that serve as alternative hosts for Pepper-infecting viruses contributing as inoculum sources to secondary dissemination, for infection of Pepper fields, in South west Nigeria varies. *Tithonia diversifolia* occurred in and around pepper fields in three states *A. conyzoides*, *C. pubescens, C. odorata*, *C. bengalensis* and *Synedrella nodiflora* occurred in two states respectively. The results presented in this study indicate that the weeds *A. conyzoides*, *A. cordata*, *C. olitoris*and *T. triangulare* can serve as alternative host to 8-7 viruses (Figure 1).

Discussions

Pepper is reported to be susceptible to over 40 viruses, multiple infection scenarios are the most common phenomena on the farmers' field (Kim et al. 2010; Arogundade et al. 2015). The results from this study suggest further the important need to employ efficient management control

strategies for weed elimination within, as well as, in the surrounding areas of pepper fields aiming at reducing virus source and then, the chances of infection of the crop. Despite the relatively high weed species composition in Osun the plant samples which serve as host to viruses in pepper fields were lowest compared to other states. This might be as a result of environmental factor, availability of susceptible vectors and alternate host.

The variance in plant species and virus composition within the studied states showed plant diversity that serve as host of viruses. The family: Asteraceae has the largest species composition and the species host at least three viruses. The family is known to compose one of the largest invasive species, also, serving as an alternate virus host on crops field (Sekar 2012; Aguiar et al. 2018; Noba et al. 2017). *Ageratum conyzoides* belong to the family *Asteraceae* and this study confirmed that the plant species serve as alternative host to eight (8) viruses. According to Roossinck (2013) invasive species are often robust in the environment and host many viruses even at times increasing the vectors population. Seven weed species were present in pepper farms in more than 2 states of the 6 states in the study area and most of the weed species in the study areas had multiple virus infections. Studies have shown that invasive species or weeds on agricultural field are most times naturalized weeds or sometimes native plants (Cooper and Jones 2006). Jones 2009 also reported that plant viruses generally often have a wide range of hosts belonging to different plant families by infecting species-rich native plant communities.

The prominence of PVY, PVX, CMV and TEV viruses on the weed species in the study areas on pepper fields was in agreement with the report of Power and Flecker (2003) which stated that PVY, PVX, PMMV, CMV TEV and PVMV had a broad number of host species. CMV and PVY have been reported as major viruses in vegetable grown fields (Cicek and Yorganc 1991; Ozaslan 1998; Hiskias et al. 1999; Arogundade et al. 2015, 2019a). Additionally, PVY had been reported as an important plant virus that can cause significant damage on horticultural plants (Sharma et al. 1989; Hiskias et al. 1999; Özdağ and Sertkaya 2017). The high occurrence of PVX in this study might be as a result of high host range of PVY and CMV recorded as previous study by Özdağ and Sertkaya (2017) have shown that PVX had missed infectivity with some viruses which include CMV and PVY. Draper et al. (2002) also reported that PVX + PVY is more important than respective single infection.

Moreover, the lower incidence of CMV in weed species than PVY and PVX may be attributed to none availability of proper inoculum, weed host and aphid vector, this agrees with the statement of Ali et al. (2012) who reported low incidence of CMV in their study despite the wide host range of the virus., which was attributed to absence of CMV

inoculum sources, infected seed and aphid vectors. However, majority of the plants that tested positive to TMV and ToMV in this study were glabrous while all pubescents plants in this study were negative for these two viruses, this might account for their low host range. Studies have also shown that the prevalence of viruses is largely dependent on environmental factors which include abiotic factors and biotic factors like susceptible hosts and availability of vectors (Alabi et al. 2010; Ahmed et al. 2019). The plant families, Convolvulaceae, Cucurbitaceae, Onagraceae and Poaceace collected from this study did not serve as host to any of the viruses indexed and the low species distribution in the family might be responsible for this.

In addition to pepper, there are reports on other crops which have also stated the importance of weeds as potential host to viruses which infect crops that are economically important and contributing to disease occurrence during the growing season, and also to virus dissemination (Papayiannis et al. 2011; Solórzano-Morales et al. 2011; Ali et al. 2012; Papayiannis et al. 2012; Asala et al. 2014).

Importantly, weeds have ability to thrive in the event of drought in the field, and sustain themselves in the absence of preferred hosts, becoming an important initial source of virus inoculum, which can be spread not only to commercial crops but also to infect other weed plants after harvesting periods (Asala et al. 2014).

Disclosure statement

No potential conflict of interest was reported by the authors.

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