The Incidence, Severity and Occurrence of Four Viruses Infecting Pepper (*Capsicum spp.*) in the Southern Guinea Savannah Agro-ecological Zone of Nigeria

Taiye Hussein ALIYU

Summary

The incidence, severity and occurrence of four viruses infecting pepper were determined in Kwara State, Nigeria. A disease survey and antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) were the tools deployed to achieve these objectives. The survey indicated the highest virus incidence (97%) in four locations, and the lowest incidence (16%) in three locations, with variations in severity scores. The ELISA result indicated the occurrence of all four viruses with the highest percentage occurrence of virus in the samples as follows: Pepper veinal mottle virus (36.3%), Blackeye cowpea mosaic virus (16.2%), Cowpea aphid borne mosaic virus (7.4%), and Cucumber mosaic virus in the locations (4.8%). The results indicate the prevalence of these viruses on pepper in the Southern Guinea savannah agro-ecological zone and therefore the need for constant studies to detect other viruses that limit pepper production.

Key words

pepper, virus, ACP-ELISA, agro-ecological zone

Department of crop protection, Faculty of agriculture, University of Ilorin, p.m.b. 1515, Ilorin-Nigeria
e-mail: aliyutaiyehussein@yahoo.com

Received: July 3, 2014 | Accepted: December 29, 2014

Introduction

Pepper belongs to the genus *Capsicum* that is a member of the family of *Solanaceae* and comprises of about 30 species. It is grown worldwide and constitutes the world's second most important vegetable crop after tomato (Yoon *et al.,* 1989). Pepper is widely used in many parts of the world for sauces, stew, soup and generally as a flavouring agent (Amusa *et al.,* 2004). Pepper is also suitable for diets of the obese and is useful in the control of cancer of the stomach and colon (Pamplona-Roger, 2007). Pepper also contributes to the standard of living of the farmers as the cultivation can generate income for the farmers (Drafor, 2014).

In Nigeria, pepper is cultivated principally in the rain forest and derived savanna of the southwest and in the southern guinea savanna and sudan ecological zones (Erinle, 1988). Nigeria cultivates an average yield of 1021kg/ha and is the third largest exporter of pepper (Bosland and Vativa, 2000).

Peppers are susceptible to a number of pests and pathogens causing considerable economic losses. The most economically important pests to peppers in West Africa are thrips, aphids, whitefly, root knot and fruit borers (Dagnoto *et al.,* 2013). The most economically important pepper bacterial diseases are bacterial wilt, bacterial leaf spot, and bacterial soft rot. Most threatening fungi for peppers in West Africa are *Phytophthora* root rot, Southern blight and anthracnose (James *et al*., 2010).

Virus diseases cause serious losses in the pepper industry and can become the most limiting factor affecting pepper production (Makkouk and Gumpf, 1974). They cause symptoms such as various forms of mosaic and distortions in plants with consequent reductions in crop growth and yield (Jin *et al*., 2009). About 68 viruses are known to infect peppers worldwide (Pernezny *et al*., 2003) and constitute a major limiting factor to vegetable production in most African countries (Nono-Womdim, 2003). Among these viruses, 11 were reported in Africa out of which Pepper Veinal Mottle Virus (PVMV) and Tomato Yellow Leaf Curl Virus (TYLCV) are the most widespread in the Western Africa sub-region (Dafalla, 2001).

In consideration of the importance of pepper as source of nourishment for a stable food security and a tool for income generation for farmers, it is important to assess the status of some viruses on the crop. This will help to proffer suitable control mechanisms for enhanced pepper production, reduce malnutrition and alleviate the poverty status of the farmers.

The objectives of the study was to survey for incidence, severity and occurrence of four viruses namely Pepper veinal mottle virus (PVMV), Blackeye cowpea mosaic virus (BICMV), Cowpea aphid borne mosaic virus (CABMV), and Cucumber mosaic virus (CMV) on pepper (*Capsicum spp.*) in Kwara State, Nigeria.

Materials and methods

Survey methodology. The survey was carried out in 2013/2014 cropping season on 20 pepper farm locations scattered within the four agricultural zones of the Southern guinea savanna agro-ecological zone of Kwara state, Nigeria. In each location, virus incidence was estimated based on fifty (50) plants observation per field.

Percentage incidence = (Number of infected plants (symptomatic plants) / 50) x 100

The severity score of the fifty plants counted for disease incidence was carried out using the scale as described by Kumar (2009). Mean of these scores (approximated to the nearest whole number) was expressed to determine the average severity of virus disease in the field.

1 = No visible symptoms

2 = Mild mosaic/mottling/yellowing/mild necrosis on few leaves /branches of a plant (symptoms on less than 25% of the plant); symptom recovery

3 = Moderate mosaic/puckering/mottling/yellowing/necrosis on many leaves/plants and vein clearing (symptoms cover 50% of the plant)

4 = Severe mosaic/puckering/mottling/yellowing/necrosis (symptoms on entire plant)

5 = Severe mosaic/puckering/mottling/yellowing/necrosis and severe stunting (entire plant)

6 = Severe mosaic/puckering/mottling/yellowing/necrosis and severe stunting (entire plant), deformation and death of the infected plants.

Elisa procedure. 50 leaf samples were collected from each location for ELISA testing. The moisture on the leaves was removed by blotting with absorbent paper and the leaves were cut into pieces and placed in a specimen bottle with Calcium chloride ($CaCl^2$) prior to ELISA testing. The samples were then tested for the presence of the four viruses.

Antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) was employed and the pepper leaves were tested using antibodies specific for Pepper veinal mottle virus (PVMV), Blackeye cowpea mosaic virus (BICMV), Cowpea aphid borne mosaic virus (CABMV), and Cucumber mosaic virus (CMV). The antibodies were obtained from the stock of the International Institute for Tropical Agriculture (IITA), Ibadan-Nigeria. The leaves were ground in coating buffer pH 9.6 (Na_2CO_3 1.59 g, $NaHCO_3$ 2.93 g dissolved in one litre of distilled water) at a ratio of 1:10 (w/v), 100 µl of the extract was dispensed per well. The plate was incubated in a humid box at 37^0C for 1hour. After incubation, the plate was washed by adding 200 ml of 10x phosphate buffered saline pH 7.4 (Na_2HPO_4 2.38 g, KH_2PO_4 0.4 g, KCl 0.4 g, NaCl 16.0 g and 10 ml of Tween – 20 made up of 2000 ml with distilled water) and washing the plate three times with the buffer by flooding for three minutes each time. The plate was held upside down and tapped firmly onto a folded paper towel to completely empty the wells. The same washing procedure was carried out after each incubation step.

Dried skimmed milk (200 µl of 3% in 100 ml PBS-T) was then added to each well. Antibodies were prepared by adding 1 g of healthy leaves to 20 ml of conjugate buffer (0.05 g of Albumin, 0.5 g of PVP, PBS-without Tween-20 at 10x filled up to 250 ml with distilled water). Hundred µl of antibody diluted in appropriate quantity of conjugate buffer (1:3000 e.g CMV) was dispensed into each well of the ELISA plate and then incubated at 37^0C for one hour. The plate was washed thrice with PBS-T, allowing three minutes for each wash. The enzyme conjugate, alkaline phosphate, was diluted by adding 1 µl anti-rabbit alkaline in 1500 ml conjugate buffer and mixed thoroughly. Then 100 µl of this was dispensed into each well of the ELISA plate and then incubated at 37^0C for one hour. The p-nitrophenylphosphate

(PNPP) substrate solution was prepared at a concentration of 1 mg/ml with substrate buffer. One hundred µl of PNPP solution was added to each well and the plate incubated in the dark for one hour at room temperature to allow for colour development.

The optical density (OD) values were measured at absorbance of 405 nm (A_{405}), using a BIO-RAD Micro plate Reader (ELx800, Universal Micro plate Reader). An optical density value greater than three times the mean of the negative controls i.e. virus - free plants, was considered as positive (Garcia *et al*., 2014).

Results and discussion

Symptoms, incidence and severity

The pepper varieties exhibited virus symptoms such as shoe stringing, mosaic, mottling, vein clearing, stunted plants, fruit deformation, leave curl and leaf bunching. Observations similar to these were made by Achiangia *et al.* (2013) on pepper varieties sampled in the Western highlands of Cameroon. Viral symptoms are thought to be spread by aphids as a result of their feeding activities. Traditionally, it has been accepted that viral disease symptoms could be caused by a toxic effect of some virus components. Unfortunately, however, the molecular basis of this effect is known in very few cases (Mur *et al.,* 2008).

The results in Table 1 showed that virus incidence was the highest at Owu-isin, Ijara-isin, Araromi-opin and Ojoku with 97%, followed by Ajasse (92%), Odo-ore (87%), Ogbondoroko (80%), Ekudaji and Agbeku (78%), Ilemona (77%), Ijabe (70%), Otte (48%), Shao (19%), Laduba, Kiama and Iponrin (18%), Oke-oyi (17%) and Ilesha-Baruba, Okuta and Patigi (16%).

The result showed that virus incidence was higher in the wetter areas (southern parts) that have abundance of grasses and wild plants compared to the drier (northern parts) of the State. The virus severity scores for the locations (Table 1) also indicated that Owu-isin, Ajasse, Araromi-opin, Iyara-isin and Agbeku had the highest severity score of 5, followed by Ekudaji, Ijabe, Odo-ore and Ojoku (4), Ogbondoroko, Ilemona and Kiama (3). Laduba, Otte, Patigi, Iponrin, Oke-oyi, Ilesha-baruten, Okuta and Shao had lowest severity scores of 2.

Mathews and Dodds (2008) reported that most plant viruses have weeds or other alternative natural hosts that provide a reservoir of viruses from which economically important crop plants may become infected. Sivalingam and Varma (2007) noted that ornamental plants and wild plants near crop field seem to be infected with the viruses of cultivable plant species and play a key role in the development of virus disease epidemics.

The abundance of flora in the locations within the southern part of the State, could therefore account for the higher virus incidence observed. According to Hily *et al.* (2012) plant viruses require alternate hosts to continue virus-host-vector association, which is crucial for the sustainability of the viral pathogens in the absence of the original crop host. It is probable to assume therefore that the alternate host contributed to the infection on the pepper plants and could therefore be the reason to for the high virus severity scores in some locations.

The serological result of the occurrence of the viruses in pepper leaf samples are presented in Table 2. The results indicated that PVMV was the most prevalent virus and occurred in samples from all 20 locations. The percentage occurrence of PVMV ranged from 6.3% in Ilesha-Baruba to 36.3% in Owu-Isin. BICMV was detected in seven locations of Owu-Isin (13.3%), Ijabe (11.4%), Ajasse (9.1%), Ijara–Isin (16.2%), Kiama (6.4%), Ojoku (8.9%) and Okuta (1.8%). CABMV was detected in Otteh (4.6%) and Shao (7.4%), while only samples from Ajasse (11.3%) were positive to CMV.

Table 1. Incidence and Severity of pepper viruses

Ser. No	Location	Longitude Latitude	Incidence (%)	Severity Score
1	Owu-Isin	8.2855N 5.026E	97	5
2	Ekudaji	8.4167N 4.96670E	78	4
3	Laduba	8.3833N 4.5667E	18	2
4	Ogbondoroko	8.4000N 4.600E	80	3
5	Ilemona	8.1167N 4.667E	77	3
6	Ijabe	8.033N 4.700E	70	4
7	Odo-Ore	8.3333N 5.033E	87	4
8	Ajasse	8.1745N 4.7355E	92	5
9	Araromi-Opin	8.14065N 5.3292E	97	5
10	Otte	8.3100N 4.387E	48	2
11	Ijara-Isin	8.250N 5.033E	97	5
12	Patigi	9.2333N 6.2666E	16	2
13	Agbeku	8.4000N 4.9833E	78	5
14	Kiama	9.6060N 3.955E	18	3
15	Iponrin	8.4925N 4.570E	18	2
16	Oke-Oyi	8.5833N 4.7166E	17	2
17	Ojoku	8.500N 4.6500E	97	4
18	Ilesha-Baruba	8.914N 3.424E	16	2
19	Okuta	9.222N 3.206E	16	2
20	Shao	8 .353N 4 ·3335E	19	2

Source: Field survey 2013/2014

The ELISA result shows the prevalence of the four viruses in the Southern guinea savanna agro-ecological zone of Kwara State and the occurrence of PVMV as the most widespread. The four viruses detected in the study are among those reported by Arogundade *et al.* (2012) to be occurring on pepper in Nigeria. The widespread nature of PVMV in the study area could be due to pepper being the main host of PVMV. Also, since PVMV is mostly transmitted by seeds, its high prevalence could be as a result of seed recycling. These findings are in agreement with studies by Lana *et al.* (1975) and Fajinmi (2006), who also found PVMV to be the most widespread naturally occurring pepper virus in

Table 2. Viruses detected in the locations

S/No	Location	Viruses			
		CMV	PVMV	BICMV	CABMV
1	Owu-Isin	--	++ (36.3%)*	++ (13.3%)	--
2	Ekudaji	--	++ (21.1%)	--	--
3	Laduba	--	++ (10.6%)	--	--
4	Ogbondoroko	--	++ (19.8%)	--	--
5	Ilemona	--	++ (22%)	--	--
6	Ijabe	--	++ (14.3%)	++ (11.4%)	--
7	Odo-Ore	--	++ (11.1%)	--	--
8	Ajasse	++ (4.8%)	++ (16.9%)	++ (9.1%)	--
9	Araromi-Opin	--	++ (21.5%)	--	--
10	Otte	--	++ (19.2%)	--	++ (4.6%)
11	Ijara-Isin	--	++ (31.7%)	++ (16.2%)	--
12	Patigi	--	++ (22.1%)	--	--
13	Agbeku	--	++ (12.6%)	--	--
14	Kiama	--	++ (7.4%)	++ (6.4%)	--
15	Iponrin	--	++ (19.8%)	--	--
16	Oke-Oyi	--	++ (13.3%)	--	--
17	Ojoku	--	++ (19.6%)	++ (8.9%)	--
18	Ilesha-Baruba	--	++ (6.3%)	--	--
19	Okuta	--	++ (9.3%)	++ (1.8%)	--
20	Shao	--	++ (9.7%)	--	++ (7.4%)

Note: CMV=Cucumber mosaic virus, PVMY=Pepper veinal mottle virus, CABMV= Cowpea aphid borne mosaic virus, BICMV=Blackeye cowpea mosaic virus. ++ denotes presence of virus, -- denotes absence of virus. * Figures in parentheses are percentages of virus occurrence in sample.

Nigeria and Appiah *et al.* (2014) who attributed the high rate of infection with PVMV observed in a pepper to the recycling of seeds, which may have resulted in the accumulation of viruses.

Mixed infection of two or more of the viruses occurred in nine locations: Owu-Isin (PVMY + BICMV), Ijabe (PVMY + BICMV), Ajasse (CMV + PVMY + BICMV), Otte (PVMY + CABMV), Ijara-Isin (PVMY + BICMV), Kiama (PVMY + BICMV), Ojoku (PVMY + BICMV), Okuta (PVMY + BICMV) and Shao (PVMY + CABMV). Shoyinka *et al.* (1997) are of the opinion that the possibility of 3-5 viruses infecting a single plant is not uncommon in nature. Mixed viral infections usually result in more severe disease symptoms due to synergistic interactions. Murphy and Bowen (2006) have also reported mixed viral infection on pepper and tomatoes. Findings similar to this have also been made by Abdalla *et al.* (1991), Méndez-Lozano *et al.* (2003) and Vanitharani *et al.* (2004).

Cucumber mosaic virus (CMV), Cowpea aphid borne mosaic virus (CABMV) and Blackeye cowpea mosaic virus (BlCMV) are considered important virus diseases of cowpea and other leguminous crops (Huguenot *et al.,* 1996; Thottappilly and Rossel, 1992). The detection of these viruses on pepper is a clear indication of its spread from hitherto known hosts to now pepper. The high incidence of aphid vectors on pepper has been well documented by Horvath and Nienhaus (1982). In Nigeria, some farmers intercrop cowpea with pepper, therefore, there is the chance of aphid transmitted cowpea viruses unto pepper.

Conclusion

The study showed that the incidence and severity levels of the viruses varied across locations within the Southern Guinea savannah agro-ecological zone of Nigeria. PVMV was the most occurring, with other viruses like CMV, BICMV and CABMV positively identified occurring on pepper samples. The viruses occurred singly and in mixed infections of two or three viruses. The study therefore suggests the need for regular virus disease survey since viruses are not static epidemiologically. If this is done, it would aid decision planning for effective and sustainable virus disease control on pepper.

References

Abdalla, O.A., Desjardins, P.R., Dodds, J.A. (1991). Identification, Disease Incidence, Distribution of Viruses Infecting Peppers in California. *Plant Disease* 75:1019-1023.

Achiangia, N. P., Ngimoh, N.W., Annih, M.G. (2013). Relative Prevalence, Incidence and Severity of two viruses infecting cultivated pepper in the Western Highlands of Cameroon. *Agricultural Science Research Journals* 3(8):261-266.

Amusa, N.A., Kehinde, I.A., Adegbite, A.A. (2004). Pepper (*Capsicum frutescens*) fruit anthracnose in the humid forest region of south-western Nigeria. *Nutrition and Food Science* 34 (3):130–134.

Appiah, A.S., Quartey, E.K., Amoatey, H.M., Nunekpeku, W., Owusu-Ansah, M., Ofori, S. (2014). Response of Nine Cultivars of Pepper (*Capsicum* spp.) to Infection by Four Viruses under Natural Field Conditions in the Coastal Savanna Zone of Ghana. *Research Journal of Applied Sciences, Engineering and Technology* 7(5):903-907.

Ara, M.R.M., Masud, M.H., Akanda, A. M. (2012). Detection of Plant Viruses in some Ornamental Plants that act as alternate hosts. *The Agriculturists* 10(2):46-54.

Arogundade, O., Balogun, O.S., Kareem, K.T. (2012). Occurrence and distribution of pepper veinal mottle virus and cucumber mosaic virus in pepper in Ibadan, Nigeria. *Virology Journal* 9:79 doi:10.1186/1743-422X-9-79. http://www.virologyj.com/content/9/1/79.

Bosland, P.W., Votava, E.J. (2000). Peppers: Vegetable and Spice Capsicum. CABI Publishing, New York.

Dafalla, G.A. (2001). Situation of tomato and pepper viruses in Africa. In: Proceedings of a Conference on "Plant Virology in Sub Saharan Africa". d'A. Hughes J.BO Odu (eds.), International Institute of Tropical Agriculture, June 4-8, Ibadan, Nigeria, pp 18-24.

Dangnoto, S., Yaro-Diarisso, N., Sanogo, P.N., Adetula, O., Dolo-Nantoumé, A., Gamby-Touré., K., Traoré-Théra, A., Katilé, S., Diallo-Ba, D. (2013). Overview of pepper (*Capsicum* spp.) breeding in West Africa. *African Journal of Agricultural Research* 8(13):1108-1114.

Drafor, I. (2014). Gender and small-farmer commercialisation: The case of two farming communities in Ghana. *Journal of Development and Agricultural Economics* 6(4):184-192.

Erinle, I. D. (1988). Present status and prospects for increased production of tomato and pepper in Northern Nigeria. Paper presented at the International Symposium on Integrated Management practices. Taiwan, Taiwan March 21st – 26th.pp.10

Fajinmi, A.A. (2006). The incidence, spread and possible control strategies of Pepper veinal mottle potyvirus (PVMV) disease on pepper (*Capsicum annuum*) in Nigeria. PhD thesis, University of Ibadan Oyo State Nigeria.160p.

Hily, J.M., Garcı´a, A., Moreno, A., Plaza, M., Wilkinson, M.D. (2014). The Relationship between Host Lifespan and Pathogen Reservoir Potential: An Analysis in the System Arabidopsis thaliana-Cucumber mosaic virus. *PLoS Pathog* 10(11): e1004492. doi:10.1371/journal.ppat.1004492.

Horvath, J., Nienhaus, F. (1982). Separation of viruses from inhibitor containing pepper leaf extracts (*Capsicum annuum L.* L.) with controlled poreglass exclusion Chromatography. *Acta Phytopatholog Academic Science Hung* 17:41-45.

Huguenot, C., Furneaux, M.T., Clare, J., Hamilton, R.I. (1996). Serodiagnosis of pepper veinal mottle in West Africa using specific monoclonal antibodies in DAS-ELISA. *Journal of Phytopathology* 144:29–32.

James, B., Atcha-Ahowé, C., Godonou, I., Baimey, H., Goergen, G., Sikirou, R., Toko, M. (2010). Gestion intégrée des nuisibles en production maraîchère : Guide pour les agents de vulgarisation en Afrique de l'Ouest. Institut International d'Agriculture tropicale (IITA), Ibadan, Nigeria. p. 120

Jin, R., Pan, J., Xie, H., Zhou, B., Xia, X. (2009). Separation and quantitative analysis of capsaicinoids in chili peppers by reversed-phase argentation LC. *Chromatographia* 70:5-6.

Kumar, L. (2009). Methods for the diagnosis of Plant Virus diseases, laboratory Manual. IITA. p. 94

Lana, A.O., Gilmer, R.M., Wilson, G.F., Shoyinka, S.A. (1975). An unusual new virus, possibly of the potato virus group from pepper in Nigeria. *Phytopathology* 65:1329–1332.

Makkouk, K.M., Gumpf, D.J. (1974). Further identification of naturally occurring virus diseases of pepper in California. *Plant Disease Reperteur* 58:1002-1006.

Mathews, D. M. and Dodds, J. A. (2008). First report of Angelonia flower break virus in *Nemesia* spp. and other ornamental plants in California. *Plant Disease* 92(4):651.

Méndez-Lozano, J., Torres-Pacheco, I., Fauquet, C.M., Rivera-Bustamante, R.F. (2003). Interactions between geminiviruses in a naturally occurring mixture: Pepper huasteco virus and Pepper golden mosaic virus. *Phytopathology* 93:270-277.

Mur, L. A. J., Kenton, P., Lloyd, A. J., Ougham, H., Prats, E. (2008). The hypersensitive response; the centenary is upon us but how much do we know? *Journal of Experimental Botany* 59:501–520.

Murphy, J.F., Bowen, K.L. (2006). Synergistic disease in pepper caused by the mixed infection of Cucumber mosaic virus and Pepper mottle virus. *Phytopathology* 96:240-247.

Nono-Womdim, R. (2003). "An overview of major virus diseases of vegetable crops in Africa and some aspects of their control". pp. 213-232. In*: Plant virology in Sub-Saharan Africa*, Proc. Conf. Organized by IITA. J.d'A. Hughes and J.Odu, (eds.). International Institute of Tropical Agriculture, Ibadan, Nigeria.

Pamplona-Roger, G.D. (2007). Healthy Foods, Editorial Safeliz, Spain p 375.

Pernezny, K., Robert, P.D., Murphy, J.F., Goldberg, N.P. (2003). Compendium of pepper diseases. *The American phytopatholgical Society* 1:24-25.

Shoyinka, S.A., Thottappilly, G., Adebayo, G.G., Anno-Nyako, F.O. (1997). Survey on cowpea virus incidence and distribution in Nigeria. *International Journal of Pest Management* 43(2):127-132.

Sivalingam P. N., Varma A. (2007). Nontomato natural hosts of tomato infecting begomoviruses in north-western India. *Indian Journal of Virology*, 18(1):20-27.

Thottappilly, G, and Rossel, H.W. (1992). Virus diseases of cowpea in tropical Africa. *Tropical Pest Management* 38:337-348.

Vanitharani, R., Chellappan, P., Pita, J.S., Fauquet, C.M. (2004). Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of post transcriptional gene silencing. *Journal of Virology* 78:9487-9498.

Yoon, J.Y., Green, S.K., Tshanz, A.J., Tsou, S.C.S., Chenge, L.C. (1989). Pepper Improvement for the tropics, Problems and Approach In: Tomato and Pepper Production in the Tropics. Asian Vegetable Research and Development Centre, AVRDC Shantine Taiwan. pp. 86–90.

acs79_35