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SURVEY AND MOLECULAR DETECTION OF VIRUSES INFECTING PEPPER IN CROSS RIVER STATE

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ABSTRACT

Several viral diseases limit the production of pepper around the world. The aim of this study was to assess the prevalence and diversity of viruses infecting open field and backyard garden cultivation of pepper in the northern, central, and southern zones of the Cross River State, Nigeria. The survey was carried out in the cropping season of 2024 on cultivated pepper crops growing mostly on small farm holdings and backyard gardens scattered within the six local government areas of Yala, Odukpani, Yakurr, Etung, Akpabuyo, and Ogoja. ACP-ELISA was used to detect viruses across the state. The most prevalent virus in almost all locations was CMV, and the least prevalent virus was ToLCV. The majority of infected plants were pepper veinal mottle virus (PVMV) and Cucumber mosaic virus (CMV). A relatively high average of 72.4 % disease incidence and an average severity index of 3.0 was recorded across all locations of the state. The survey showed Yala had the highest mean incidence of 79.25 % and a severity index of 3.35 while Ogoja recorded the lowest mean incidence of 68 % and a severity index of 2.8. Mixed infections of ToLCV and PVY and PVMV and CMV were more common in 11 of the 18 locations. Analysis of variance for the mean disease incidence and mean severity index revealed that there was no significant difference across the different locations at $p > 0.05$. The findings demonstrate the predominance of these viruses on pepper in ross River State, underscoring the necessity for further research to identify more viruses that hinder pepper production.

Keywords: Cross River State, Pepper, Viruses, Mixed infections, CMV, PVMV, PVY, ToLCV

INTRODUCTION

Peppers (Capsicum spp.) are economically significant crops known for their culinary and nutritional value. They belong to the family Solanaceae and are produced mostly in tropical and subtropical regions of the world. The genus *Capsicum*, which has at least 13 species that originated in South America, and *C. annuum* are the most widely cultivated pepper species worldwide (Jo et al., 2022; Esmaeilzadeh and Koolivand 2022). Peppers are generally consumed fresh or processed for use as vegetables and spices and are of great economic value. Kumar et al. (2024) reports that they are used as spices, as an ornamental plant, as a source of extracts for use in various pharmaceutical or cosmetic products, as food colouring and in traditional medicine. Dhamodharan et al. (2022) reported that peppers are a source of vitamins, dietary fiber, iron, protein, phosphorus, potassium, manganese, and copper. They are also been found to be rich in phytochemicals such as carotenoids, flavonoids, and capsaicinoids.

In Cross River State, pepper is among the cultivated vegetable crops that are edible and used as spice in meals. It is an important and widely cultivated cash crop in the state that contributes significantly to the economy of rural dwellers, especially women. Despite their importance, pepper cultivation is threatened by diseases and pests (Arogundade et al., 2012).

Several viruses have been identified in pepper plants, primarily belonging to the Potyviridae, Tobamovirus, and Geminiviridae families. Waweru et al. (2019) has however reported that in Africa more than 45 viruses have been implicated to infecting pepper. Many farmers have abandoned pepper cultivation owing to severe crop losses caused by these viruses. The most prominent of these include pepper mottle virus (PepMoV), tobacco mosaic virus (TMV), and chili leaf curl virus (ChLCV). Pepper mottle virus (PepMoV) is a significant threat that causes mottling, stunting, and yield loss in the affected plants (López et al., 2016). Elvira et al. (2018) highlighted that PepMoV, which is transmitted by aphids, has become increasingly prevalent in many pepper-growing regions, necessitating integrated disease management practices.

Pepper veinal mottle virus (PVMV) and Tomato leaf curl virus (TLCV) are notable pathogens that affect crop yield and quality. PVMV, a member of the genus Potyvirus, infects pepper plants (Capsicum spp.). It is transmitted by vectors, primarily aphids, and can cause significant economic losses in pepper production (Maldonado et al., 2021). The symptoms of PVMV infection include mottling, leaf curling, and stunted growth, which can severely reduce fruit yield (Khan et al., 2020; Khan et al. 2021). Tomato leaf curl virus (ToLCV), a member of the family Geminiviridae, encompasses a group of viruses that predominantly affects solanaceous plants, especially pepper and tomato plants. The primary vectors for ToLCV are whiteflies, which play a crucial role in viral transmission (Datti et al., 2019). Infected plants exhibit characteristic symptoms, such as leaf curling, yellowing, and stunted growth, leading to reduced fruit quality and quantity (Tan et al., 2021).

Most pepper crops in Nigeria, especially in the Cross River State, show intricate indications of vein chlorosis, mosaic, mottle, leaf deformity, and stunting, which result in significant losses in plant vigor and productivity. Viral infections constitute a major limitation to pepper production, and preliminary studies have revealed that cucumber mosaic virus (CMV) and pepper veinal mottle virus (PVMV) are highly prevalent in Nigeria (Arogundade et al. 2012).

In this study, we assessed the prevalence and diversity of the primary viruses that infect pepper crops in the northern, central, and southern zones of the Cross River State to understand these viruses and develop effective management strategies.

MATERIALS AND METHODS

Sample collection

During the survey, symptoms observed were leaf deformation, leaf curling, foliage mosaic symptoms, and stunting (Fig. 3). Across the local government areas of study, the common names for pepper were 'Ata' (Yala), 'Ntokon' (Odukpani and Akpabuyo), 'Abene' or 'Akpana' (Ogoja), 'Ngare' (Etung), and 'Ekapon' (Yakurr). Cross River State lies between latitudes 4° 40', and 5° 05' North and longitudes 8° 15' and 8° 25 East of the Greenwich meridian in the south-south geo-political zone of Nigeria (Effiong and Ushie 2019). The state has an area of approximately 20,956 km2 and is generally characterized by a humid tropical climatic regime that supports the cultivation of various crops throughout the year (Figure 1). However, there is a peak cropping period in April and May, whereas the dry January and February periods are unsuitable for farming activities. Land preparation, planting, and weeding are the most labor-intensive farming operations for major crop cultivation.

The survey was carried out in the cropping season of 2024 on cultivated pepper crops (*C. annuum, C. frutescens and C. chinense*) growing mostly on small farm holdings and backyard gardens scattered within the six local government areas of Yala (Yache, Okpoma and Bansara), Odukpani (Akim Akim, Idere and Ikoneto), Yakurr (Nko, Idomi and Agoi Ibami) Etung (Bendeghe, Ajasso and Agbokim), Akpabuyo (Ikot Edem Odo, Ikot Eneyo and Ikot Nakanda) and Ogoja (Mbube, Ishibori and Ugaga) (Figure 1 and 2). Both symptomatic and asymptomatic leaf samples were collected from the field and were immediately placed in Ziplock bags then stored on ice. Subsequently, they were preserved at 4 °C and desiccated on calcium chloride.

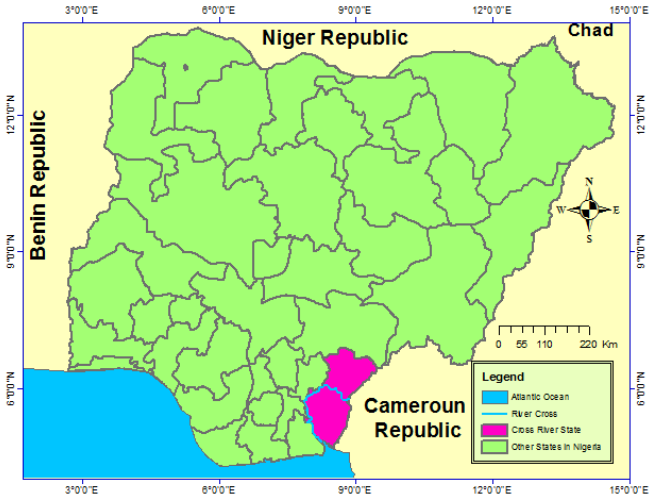

Fig. 1: Map of Nigeria showing location of Cross River State (Source: Effiong and Ushie 2019)

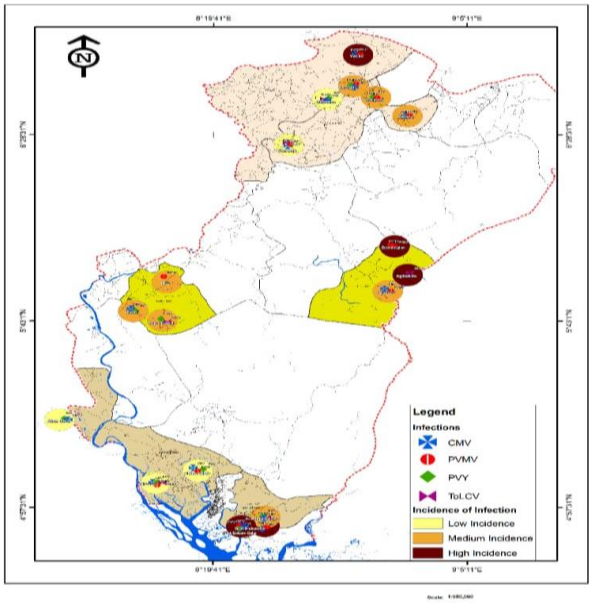

Fig. 2: Areas surveyed for the collection of virus-infected pepper leaf samples

Symptoms such as leaf deformation, leaf curling, foliage mosaic symptoms, and stunting symptoms (Fig. 3) were observed and photographed during the survey. Out of a total of 916 randomly collected symptomatic/asymptomatic leaf samples used for the survey, 360 symptomatic leaf samples was recorded across the three zones with 20 plants observed at each location and used for the purpose of percentage incidence of the virus which was calculated to estimate the incidence of the virus at each location and severity index. The severity score of the plants for disease incidence was calculated using Kumar's scale (2009). To calculate the average severity of the viral disease in the field, the mean of these scores was calculated.

1 = No obvious symptoms

2 = Few leaves of plant with mild mosaic, mottling, yellowing, or moderate necrosis (symptoms covering less than 25% of the plant); symptom recovery

3 = Moderate vein clearance, numerous leaves/plants with necrosis, mottling, yellowing and mosaic (symptoms covering 50% of the plant)

4 = Severe mosaic, mottling, yellowing and necrosis (symptoms affect whole plant)

5 = Severe stunting of whole plant and severe mosaic, mottling, yellowing and necrosis

6 = Severe stunting (entire plant), deformity, and mortality of the diseased plants, including severe mosaic, pouting, mottling, yellowing, and necrosis.

Antigen coated plate-enzyme-linked immunosorbent assay (ACP-ELISA)

For the detection of the viruses, antigen-coated plate enzyme-linked immunosorbent assay was employed, with polyclonal antibodies specific for the presence of Potato virus Y (PVY), Potato virus X (PVX), Pepper veinal mottle virus (PVMV), Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV), Cowpea aphid borne mosaic virus (CABMV), and Tomato leaf curl virus (ToLCV). To prepare the ELISA plate, 0.1 g of leaf sample was crushed in 1 ml of carbonate-coating buffer (0.015 M Na2CO3 and 0.0349 M NaHCO3). Next, 100 µl was dispensed into each well. The plate was incubated for 1 h at 37°C before being washed three times with phosphate buffered saline (PBS) containing 0.05% (v/v) Tween-20 (PBS-T), with a 3-minute interval between each wash. Polyclonal antiserum was cross-adsorbed in healthy pepper leaf sap extract (1:20 w/v) diluted in a conjugate buffer (PBS-T with 0.02% (w/v) egg albumin and 0.2% (w/v) PVP-40000). All antisera were diluted 1:1000 (v/v) in conjugate buffer, except for CMV, which was diluted 1:3000 (v/v). For virus detection, 100 µl polyclonal antisera was used. After one hour of incubation at 37°C, the ELISA plate was washed thrice with PBS-T. The secondary antibody used was 100 µl of alkaline phosphatase-conjugated anti-rabbit antibody diluted 1:15000 (v/v) in conjugation buffer. The plates were then incubated at 37°C for 1 h. After washing the plate thrice with PBS-T, 100 µl of 0.001 g/ml of p-nitrophenyl phosphate in 10% (v/v) diethanolamine buffer (pH 9.8) was added to each well and incubated at room temperature for one hour. Healthy pepper plants (*Capsicum* spp.) were used as the negative controls. After one hour, absorbance was measured at 405 nm using a Bio-Rad multiscan ELISA reader (ELx 800, Universal Microplate Reader). Samples were deemed viral positive if the ELISA reading was at least twice that of the healthy pepper leaf sap control.

Individual virus incidence was determined as a percentage of the total number of infected samples over the total number of leaf samples analyzed. The Statistical Package for Social Science (SPSS) v2008 was used to analyze incidence and severity. Analysis of variance (ANOVA) was performed at the 5% probability level.

Results

Incidence and severity of symptoms

During the survey, the plants were photographed and exhibited symptoms, such as leaf deformation, leaf curling, foliage mosaic symptoms, and stunting symptoms (Fig. 3). In total, 360 symptomatic leaf samples were collected from the three zones. The survey showed a high mean disease incidence and severity across the sampled locations namely- Etung, Yala, Akpabuyo, Yakurr, Ogoja and Odukpani local governments, with Yala having the highest mean incidence of 79.25 % and a severity index of 3.35. The Etung and Akpabuyo local government areas also recorded a high incidence and severity index of 72.75 % and 3, respectively, for Etung and 73.25 % and 3.05 Akpabuyo. The Ogoja local government recorded the lowest mean incidence of 68 % and a severity index of 2.8 (Table 1).

Fig. 3: Leaves of pepper showing typical symptoms of mosaic, mottling observed across locations

Table 1. Incidence and severity of viral diseases of pepper in six LGA of Cross River State, Nigeria.

Yala		Ogoja		Yakurr		Odukpani		Etung		Akpabuyo	
Mean incidence (%)	Mean severity	Mean incidence (%)	Mean severity	Mean incidence (%)	Mean severity	Mean incidence (%)	Mean severity	Mean incidence (%)	Mean severity	Mean incidence (%)	Mean severity
94	2.8	78	3.4	70	2.0	50	3.4	46	3.0	70	2.4
76	4.0	70	2.8	94	4.0	68	2.8	96	2.0	58	3.8
84	3.4	68	2.4	72	3.6	88	3.0	20	2.8	86	3.0
60	2.8	74	3.0	60	2.4	58	3.2	92	3.2	62	2.8
74	4.6	46	2.8	46	2.8	96	2.4	74	2.4	76	2.0
68	2.4	62	3.2	100	3.0	64	2.8	94	3.8	90	3.4
86	3.0	54	2.6	34	2.2	100	2.0	90	4.2	88	4.4
92	3.8	92	2.2	84	3.2	44	4.0	70	2.6	56	2.6
79.25	3.35	68.0	2.8	70.0	2.9	71.0	2.95	72.75	3.0	73.25	3.05

Prevalence and distribution of viruses

For virus prevalence studies, 916 leaves with virus-like symptoms were collected and tested across the state, with 45.4 % of the samples testing positive. Results obtained implicated four viruses across the sampled areas of the state on pepper plants to include: tomato leaf curl virus (ToLCV), cucumber mosaic virus (CMV), pepper veinal mottle virus (PVMV), and potato virus Y (PVY). The most prevalent virus in almost all locations was CMV, and the least prevalent virus was ToLCV (Table 2). All four viruses were found to be present in only two locations, Ikoneto and Ikot Edem Odo, in Odukpani and Akpabuyo local government areas, respectively.

Table 2: Distribution and prevalence of pepper viruses in Cross River State

LGA	LOCATION	ToLCV	PVY	CMV	PVMV	Number of samples	Negative samples
Yala	Okpoma	X	X	8 (9.52)	6 (7.14)	84	70 (83.3)
	Ugaga	X	4 (8.7)	11 (23.9)	7 (15.2)	46	24 (52.2)
	Yache	8 (11.8)	12 (17.6)	16 (23.5)	X	60	32 (53.3)
Odukpani	Akim-Akim	X	9 (12.5)	15 (20.8)	7 (9.7)	72	41 (56.9)
	Ntan-Obu	X	10 (26.3)	16 (42.1)	X	38	12 (31.6)
	Ikoneto	5 (17.9)	2(7.1)	4(14.3)	3(10.7)	28	14 (50.0)
Yakurr	Nko	X	X	X	16 (42.1)	38	22 (57.9)
	Idomi	X	12(25)	16 (33.3)	X	48	20 (41.7)
	Agoi-Ibami	10 (18.5)	22 (40.7)	X	X	54	22 (40.7)
Etung	Agbokim	15 (30)	X	X	X	50	35 (70.0)
	Ajasso	X	X	14 (18.9)	36 (48.6)	74	24 (32.4)
	Bendeghe	X	5 (12.5)	X	3 (7.5)	40	32 (80.0)
Ogoja	Ishibori	9 (15)	X	7 (11.7)	12 (20)	60	32 (47.1)
	Bansara	X	5 (16.7)	2 (6.66)	5 (16.7)	30	18 (60.0)
	Mbube	X	X	12 (20)	8 (13.3)	60	40 (66.7)
Akpabuyo	Ikot Edem Odo	5 (8.93)	8 (14.3)	13 (23.2)	6 (10.7)	56	24 (42.9)
	Ikot Eneyo	1 (2.94)	3 (8.82)	6 (17.7)	X	34	24 (70.6)
	Ikot Nakanda	X	X	19 (43.2)	13 (29.5)	44	12 (27.3)

Virus detected by antigen coated-plate enzyme-linked immunosorbent assay (ACP-ELISA); X, Virus-negative; PVY, Potato virus Y; PVMV, Pepper veinal mottle virus; CMV, cucumber mosaic virus; ToLCV, Tomato leaf curl virus.

All the locations spanning the six local governments from which samples were collected had at least one virus detected with Nko (PVMV) and Agbokim (ToLCV). Locations with detection of multiple infections include Ugaga (PVY, CMV and PVMV), Yache (ToCLV, PVY and CMV), Akim Akim (PVY, CMV and PVMV), Ishibori (ToCLV, CMV and PVMV), Bansara (PVY, CMV and PVMV) and Ikot Eneyo (ToCLV, PVY and CMV). This study also found mixed viral infections in pepper plants. Mixed infections were common between ToLCV and PVY in Yache, Agoi Ibami, Ikoneto, and Ikot Edem Odo they constituted 9.72 % of the total infected pepper plants. Mixed infections between PVMV and CMV were more common in 11 of the 18 locations and constituted 23.5 % of infected plants. Analysis of variance for the mean disease incidence and mean severity index revealed that there was no significant difference across the different locations at $p > 0.05$.

DISCUSSION

ELISA test has been the preferred method of diagnosis in field survey of plant viruses due to its quality of being quick, reliable, sensitive and economical

(Vinayarani et al., 2011; Almeida et al., 2018; Kapoor et al., 2018).

A relatively high average of 72.4 % incidence and an average severity index of 3.0 was recorded across all locations of the state. The high average incidence and severity index of the viruses across the locations corroborates with the findings of Arogundade et al. (2015) in which an average incidence of 78 % and severity index of 2.8 was found in the South West region of Nigeria. The environmental conditions of both the South West region and Cross River State are greatly influenced by the South West Trade Wind Gutiérrez-Sánchez et al. (2023) has drawn a parallel on how environmental conditions modulate virus transmission however this this is at variance with the results from the analysis of variance that revealed that there was no significant difference across all locations for disease incidence and severity index.

Four viruses (PVMV, ToLCV, PVY and CMV) were detected across the state from the 416 samples that tested positive out of 916 with PVMV and CMV being the most prevalent. This is in agreement with the survey of Bolou Bia et al. (2018) in Cote d'Ivoire which revealed the prevalence of PVMV and CMV of which they attributed to the effect of environmental conditions, climate and soil directly or indirectly affecting virus prevalence.

The occurrence of mixed infections between ToLCV and PVY (9.72 %) and CMV and PVMV (23.5 %) was reported across the locations. Mixed infections have been reported in pepper plants in south west Nigeria (Fajinmi 2010, Arogundade et al. 2015), in the southern guinea savannah of Nigeria (Aliyu 2014) and in Benin (Zohoungbogbo et al. 2022). Mixed viral infections have been known to produce severe disease symptoms because of their synergistic interactions. These interactions have been known to be mostly between viruses of different species especially between a begomoviruses with either a potyviruses or criniviruses (Sánchez-Sánchez et al. 2024) or among viruses that share vectors (Ekpiken et al. 2021; Eyong et al. 2021). The results obtained on mixed infection in this study where there was a lower incidence of ToLCV and PVY compared to CMV and PVMV is at variance with the findings of Tamborindeguy *et al*. (2023) who reported that the mixed infections of a begomoviruses and potyviruses are the most reported cases of synergistic interactions. Webster et al. (2015) have suggested that the incidence of mixed infection provides an opportunity for genetic recombination among the viruses present.

Ali et al. (2018) has suggested that an integrated approach for management of the viral disease of pepper crops will include the adoption of cultural practices, growing of resistant cultivars and biological control agents. Bautista et al. (2020) has reported the use of breeding and biotechnological methods in reducing the impact of viral infections on pepper.

CONCLUSION

This study was carried out with the aim to determine the virus incidence and severity index of the pepper plants in Cross River State, Nigeria. The preferred method of diagnostic for the detection of the viruses in determining virus incidence was the ELISA technique. Our result showed that CMV was common, followed by PVMV, PVY, and ToLCV. The viruses were found singly and in multiples.

Viruses are a major risk to pepper production across the globe. Gaining a thorough understanding and handling of these viral infections is essential for maintaining pepper farming. Ongoing research is required to create resistant strains, develop virus free and high yielding pepper seeds, and apply effective integrated pest management approaches for the control of the viruses.

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