Original Research Article

STUDIES ON THE DETECTION OF VIRUSES INFECTING CAPSICUM SPECIES IN THE NORTHERN ZONE OF CROSS RIVER STATE

ABSTRACT

The objective of this study was to determine the occurrence, detection, and geographical spread of viruses that infect pepper (Capsicum spp.) in six towns spanning two local government areas (Yala and Ogoja) in thenorthern zone of Cross River State. A total of 348 leaf samples from symptomatic and asymptomatic peppers were randomly collected from farmer's fields during a survey in 2023–2024. The samples were then preliminary tested using an antigen coated plate enzyme-linked immunosorbent assay (ACP-ELISA) with seven antibodies specific for ToLCV, Cowpea aphid borne mosaic virus (CABMV), Cucumber mosaic virus (CMV), Potato virus X (PVX), Tobacco mosaic virus (TMV), and Pepper veinal mottle virus (PVMV). The findings showed that the viruses that infect pepper in Yala had a high cumulative mean incidence, accounting for 56.9% (198 infected samples). Yache in Yala had the highest virus incidence at 90%, while Bansarain Ogoja had the lowest at 40%. At different rates, ToLCV, CMV, PVY, and PVMV were found in the samples; PVX, TMV, and CABMV were not found. The CMV virus was the most commonly discovered of them. Additionally, mixed infections between the viruses—between ToLCV and PVY in Yache and between CMV and PVMV in Bansara—were found. This is the first account of viruses found in pepper in the northern part of Cross River State. The findings show that the viruses that cause the infections are common and might potentially be a threat to the production of pepper in that region.

Keywords: ACP-ELISA; incidence; pepper; virus; northern Cross River State

Introduction

Viral infections have been a major threat to pepper cultivation in Cross River State for several years. Many farmers have abandoned pepper farming due to total crop losses as a result ofvirusesnegatively impacting the quality and yield of the pepper fruits [10, 20].Ironically, pepper is one of the most cultivated crops and an integral part of the diet in most homes in Cross River State.

Pepper belongs to the genus Capsicum and it is one of the members of the Solanaceae (Nightshade) family which comprises up to 200 species although there are five major cultivated species namely *C. annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. pubescens* L. and *C. baccatum* L. [22]. Capsicum is believed to originate from tropical Latin America and *C annuum* is the most widely grown vegetable crops globally [7, 11].

The capsicum berry ranges in size from large to little, highly pungent to bland, sweet to sour, and in form, colour, pungency, and flavour. Particularly, according to [8], the colour is associated with pigments such as red capsanthin and capsorubin, green chlorophyll, violet/purple anthocyanins, yellow/orange β- and β-carotene, zeaxanthin, lutein, and yellow/orange β-cryptoxanthin.

Peppers are generally consumed fresh or processed for use as vegetables and spice and are of great economic value.[15] 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. [9] reports that peppers are a source of vitamins, dietary fibre, iron, protein, phosphorus, potassium, manganese and copper. They have also been found to be rich in phytochemicals such as carotenoids, flavonoids and capsaicinoids.

The plant goes by various common names depending on locality. In the study communities such as Okpoma, Yache and Ugaga in Yala it is called “Ata” and in Ishibori, Bansara and Mbube in Ogoja local government areas, it is called “Usura”

[16] reports that there are 68 viruses that have been mentioned in connection with pepper out of which 20 species cause damages to the plants[17]. [24]has however reported that in Africa more than 45 viruses have been implicated to infecting pepper.

The majority of pepper crops in Nigeria, especially in Cross River State, show intricate indications of vein chlorosis, mosaic, mottle, leaf deformity, and stunting, which result in significant losses in plant vigour and productivity. According to [4], one of the biggest obstacles to pepper production is viral infections. Preliminary studies by [4][] have revealed that cucumber mosaic virus (CMV) and pepper veinal mottle virus (PVMV) were highly prevalent in Nigeria. Nonetheless, the majority of viral diseases that were purported to affect pepper were found based on how their symptoms manifested.

Determining the particular viral disease which commonly affect pepper plants will help with comprehension and knowledge of the best ways to manage them in order to avoid losses. The objective of the present study was to determine the incidence, severity of viruses infecting pepper plants commonly present in the locations studied in northern Cross River State.

Materials and Methods

Sample collection

Thesurvey was carried out in cropping season of 2023/2024 on cultivated pepper crops (*C. annuum*, *C. frutescens* and *C. chinense*)mostly small farm holdings and backyard gardens scattered withinthe two local government areas of Yala (Yache, Okpoma and Bansara) and

Ogoja (Mbube, Ishibori and Ugaga).Leafsamples were taken from plants exhibiting indications of potential viral origin.Samples were collected, immediately stored in ziplockbags on ice, and transported to the lab. After that, they were preserved at 4 °C while desiccated on calcium chloride

Based on twenty (20) plants observed at each location, the percentage incidence of the virus was calculated to estimate the incidence of the virus at each location. The severity score of the twenty plants that were counted for disease incidence was calculated using [14] scale. Tocalculate the average severity of virus disease in the field, the mean of these scores wasexpressed.

1 = No obvious symptoms

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

3 = Moderate vein clearance, numerous leaves/plants with necrosis, mottling, yellowing and mosaic (symptoms covering50% 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; also includes severe mosaic, pouting, mottling, yellowing, and necrosis.

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

Leaf samples were packed separately and stored on dry ice in the field before being transported to the laboratory for further examination. Leaf samples were collected, refrigerated at 4 degrees Celsius, and tested. For the test, antigen-coated plate enzyme-linked immunosorbent assay was employed, with 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). 100 µl was then dispensed into each well. The plate was incubated for 1 hour 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 conjugate buffer (PBS-T with 0.02% (w/v) egg albumin and 0.2% (w/v) PVP-40000). All antisera were diluted at 1:1000 (v/v) in conjugate buffer, except for CMV, which was diluted at 1:3000 (v/v). For virus detection, 100 µl polyclonal antisera were used. After one hour of incubation at 37°C, the ELISA plate was washed three times with PBS-T.The secondary antibody used was 100 µl of alkaline phosphatase conjugated anti-rabbit antibody diluted at 1:15000 (v/v) in conjugation buffer. The plate was incubated at 37°C for 1 hour. After washing the plate three times 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 per well and incubated at room temperature for one hour. Healthy pepper plants (Capsicum spp.) were employed as the negative control. After one hour, the absorbance was measured at 405 nm using a BIO-RAD multiscan ELISA reader (ELx 800, Universal Microplate Reader). The 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 expressed as a percentage of the total number of infected samples over the total leaf samples analyzed.

Results

Incidence and severity of symptoms

The elicited symptoms of the virus were varied and ranged from vein clearing to leaf malformation. Others include shoe stringing, fruit malformation, leaf mosaic, leaf mottling, leaf curl and in severe cases stunted growth (Figure 1). The sampling sites are as illustrated in Figure 2.

Figure 1: Symptoms of diseased peppers observed in this study**.** (A) severe symptom and mosaic on stunted plant; (B) Leaf curling on leaves; (C) Normal fruits of *Capsicum annuum*.

Table 1 shows the results on virus incidence and severity index of some communities in Yala and Ogoja local government areas. The mean percentage incidence of the virus in Okpoma, Bansara and Yache all in Yala was 44%, 40% and 90% while severity was 2.8, 2.6 and 4.6 respectively. The average disease incidence of the virus at Yala was 58% while severity was 3.3. Furthermore, the mean percentage incidence of the virus in Mbube, Ishibori and Ugaga all in Ogoja was 68%, 60% and 54% while severity was 3.6, 3.2 and 3.0 respectively. The average disease incidence of the virus at Ogoja was 60.66% while severity was 3.26 (Table 1). The highest disease incidence was recorded in Yache (90 %) while the lowest was in Bansara(40 %) both in Yala local government area. The average disease incidence revealed a slightly higher incidence in Ogoja (60.66 %) as against 58 % as in Yala. Furthermore, the highest severity was obtained in Yache (4.6) while the lowest of 2.6 occurred in Bansara (Table 1).

The resultsfurther revealed that out of the 328 samples collected for the detection of viruses, four viruses were detected namelyToLCV, CMV, PVMV, and PVY. The samples showed no reactivity with TMV, CABMV, or PVX antibodies, indicating that these viruses were not present in the peppers examined (Table 2). Data obtained from the survey revealed that in Yala local government area, out of the 84 samples collected in Okpoma, viruses detected included CMV (8) and PVMV (6). The remaining 70 samples were categorized as negative samples as they did not react with the antibodies.

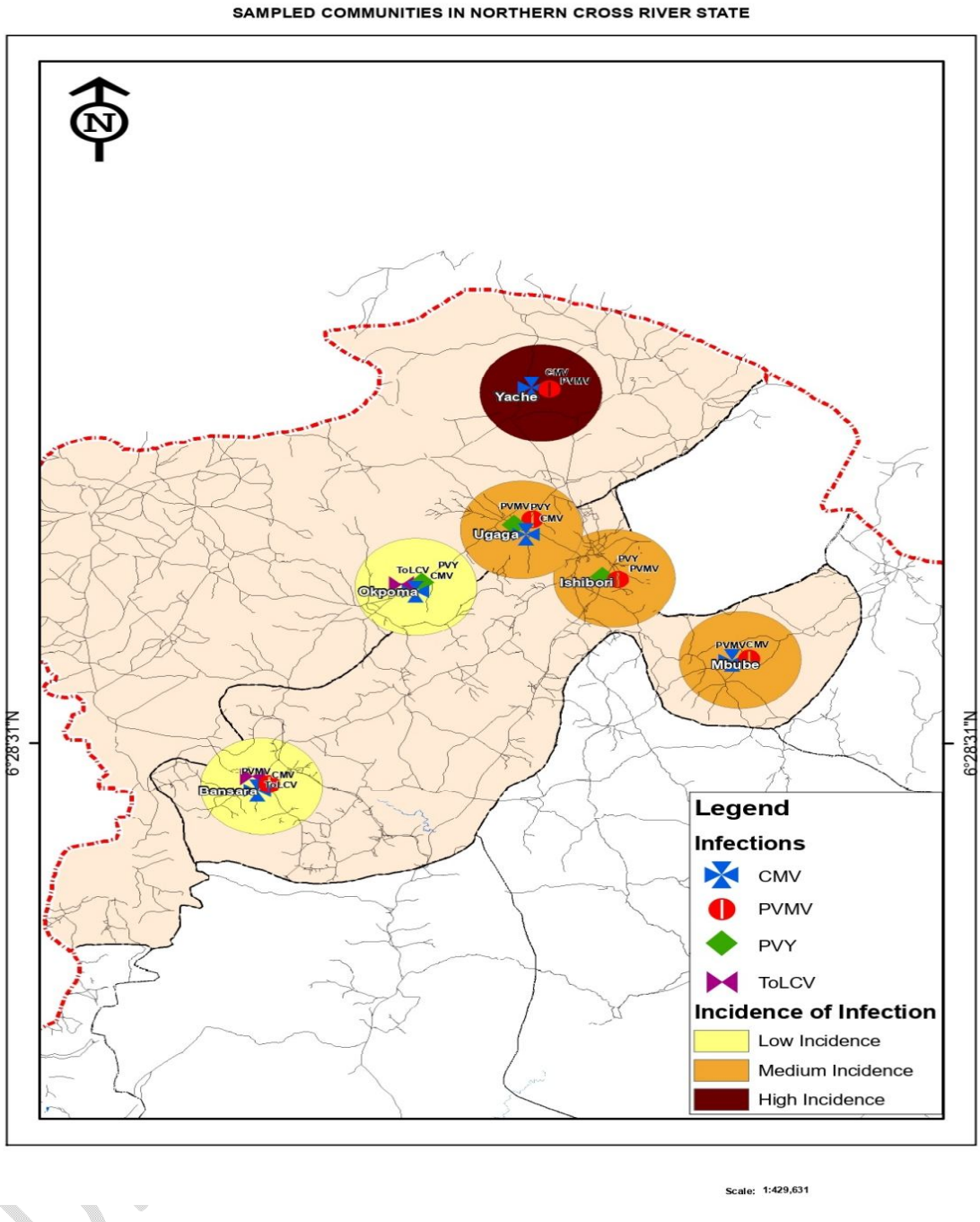

Figure 2: Sampling sites in two local governments in northern Cross River State.

In Ugaga, 46 samples were collected from backyard gardens and small farms, the viruses detected were PVY (4), CMV (11) and PVMV (7). 52.2 % of the samples returned negative.

Table 1: Prevalence and severity of pepper viruses in Yala and Ogoja local government areas

LGA	Location	Mean incidence (%)	Mean severity
Yala	Okpoma	44 (22)	2.8
	Bansara	40 (20)	2.6
	Yache	90 (45)	4.6
Ogoja	Mbube	68 (34)	3.6
	Ishibori	60 (30)	3.2
	Ugaga	54 (27)	3.0

At Yache the last location in Yala, three viruses were detected out of the 68 samples collected. The viruses were ToLCV (8), PVY (12) and CMV (16). In Ogoja local government area, of the 60 samples considered atIshibori, three viruses were detected namely ToLCV (9), CMV (7) and PVMV (12). AtBansara, 30 samples were examined with a detection of PVY (5), CMV (2) and PVMV (5). In the last location of Mbube, out of the 60 samples that were considered, two viruses were detected namely CMV (20) and PVMV (8) (Table 2).

Table 2.Pepper viruses identified in leaf samples obtained from the six locations.

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	68	32 (53.3)
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)

*Detected viruses by ACP-ELISA: 1, virus positive; X, virus negative; PVY, potato virus Y; PVMV, pepper veinal mottle virus; CMV, cucumber mosaic virus; ToLCV, tomato leaf curl virus.

Majority of the samples investigated were either infected by any of the four viruses or none at all. The current investigation also found mixed viral infections in the sick pepper plants. Infections between ToLCV and PVY occurred in Yache, as well as between PVMV and CMV in Bansara. Three (3) samples were evaluated for double infections in Yache, and two (2) in Bansara.

Discussions

This study employed ACP-ELISA for the detection of viruses in the northern zone of Cross River State. The findings confirmed the presence of four viruses in pepper in the six

locations. CMV and PVMV were found to be the most prevalent plant virusesof pepper in northern Cross River state, followed by PVY, and ToLCV. Previous studies by various authors confirmed the presence of these viruses as infecting pepper in Nigeria[18, 13, 6, 12].
Leaf deformity, vein clearing, fruit malformation, leaf mosaic, leaf mottling, leaf curl, and, in extreme cases, stunted development were typical symptoms seen in the pepper crops. Similar observation has been made on pepper plants in Nigeria [12, 6] and such symptoms on diseased plants have been attributed to be as a result of the toxic effect of some component of the virus [2]. With Ogoja and Yala local governments lying on the fringes of the rain forest and bordering the southern guinea savannah, the mean incidence and severity was highest in Yache (90 % and 4.6) followed by Mbube (68 % and 3.6). The location from where the samples were collected and the micro climate of these local government provides a conducive environment for the spread of the viruses by insect vectors andand the human factor[1, 19]. The occurrence of CMV and PVMV as the most prevalent of the viruses detected in 5/6 (83 %) of the locations can be attributed to the unique nature of both CMV and PVMV which are readily transmitted by seeds [3, 5]. CMV and PVMV have been implicated in cultivated pepper plants in several countries such as Iran, Afghanistan, Colombia and India [23] and in Colombia[21].The higher cumulative occurrence of CMV (94.6 %) over PVMV (77.4 %) is at variance with the findings of [4] who posited that pepper plants are mostly infected by PVMV than CMV (Table 2).

A mixed infection of ToLCV and PVY in Yache and between PVMV and CMV in Bansarawas detected in the study using ACP-ELISA. This corroborates with the findings of [1] and [18]who have reported about the prevalent nature of mixed infections on solanaceous crops.
Frequent surveillance is necessary to prevent viruses from destroying pepper crops. This is due to factors such as the seeds of pepper being able to transmit the disease and unwanted plants the field acting as reservoirs for the virus

Conclusion

The current study sought to investigate the viral incidence and severity among pepper plants in the northern part of Cross River State. Results showed that four viruses were prevalent with CMV and PVMV being dominant occurring in five of the six locations where sampling were made. Others were PVY and a begomovirus, ToLCV. Majority of the viruses which infected the plant in this study occurred as a single infection whilea fewoccurred as a double infecton.

At the risk of a complete loss of harvest which could be due toseed transmissibility of the virus, aphidand mechanical transmission, These viruses can only be controlled by using healthy seeds, handling pepper plants carefully before planting them in the field, and sanitation of infected plants as soon as symptoms appear.

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