Original Research Article

Detection of viruses infecting tomatoes in Cross River State and its effect on selected growth parameters

ABSTRACT

Tomato (Solanum lycopersicum) is a crop grown for its fruits. It has been reported to play very important role in nutrition and medicine. However, the production of this crop is constrained by viral pathogens which can reduced the quality and quantity of yield produced. Virus like symptoms were observed in tomato fields during the 2024 planting season across tomato growing regions in Cross River State. This study was carried out to detected viruses infecting tomato and its effect on growth. The infected leaf samples were obtained and analysed using ACP ELISA and Tomato Mosaic Virus (ToMV), Tomato Spotted Wilt Virus (TSWV), Tomato Yellow Leaf Curl Virus (TYLCV) and Tomato Ring Spot Virus (ToRSV) were detected. Tomato Spotted Wilt Virus (TSWV) was further used to test the effect on selected growth parameters of tomatoes at 9 and 21 days after planting. The result revealed significant reduction on plant height, leaf number, fresh and dry left weight at 9 days after planting. There is need for strategic management efforts to control these viruses and ensure food security.

Keyword: ACP ELISA, tomatoes, Tomato Spotted Wilt Virus (TSWV), parameters.

INTRODUCTION

Tomato (Solanum lycopersicum) are a type of fruit commonly considered a vegetable, originating from South America and now cultivated worldwide, characterized by a green and

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hairy stem, dark green leaves, yellow flowers, and a juicy and tender fruit that comes in various shapes, sizes, and colors, typically requiring warm temperatures, well-draining soil, consistent moisture, and full sunlight to grow, and are used fresh, in cooking and sauces, canning, and processing, providing nutritional benefits rich in vitamins A and C, potassium, and antioxidants [9].

Report have revealed that tomatoes can be infected by several viruses which include Tomato Spotted Wilt Virus (TSWV), Causes yellowing, stunting, and spotting on leaves and fruits, Tomato Yellow Leaf Curl Virus (TYLCV) Leads to yellowing, curling, and stunting of leaves, reducing fruit production, Tomato Mosaic Virus (ToMV), Causes mottling, yellowing, and stunting of leaves, as well as reduced fruit quality, Tobacco Mosaic Virus (TMV) causing mottling, yellowing, and stunting, Tomato Ring Spot Virus (ToRSV) Results in yellowing, stunting, and ring-shaped spots on leaves and fruit, Pepino Mosaic Virus (PepMV) Causes yellowing, stunting, and mosaic patterns on leaves, as well as reduced fruit production, Tomato Bushy Stunt Virus (TBSV) leads to stunting, yellowing, and bushy growth, with reduced fruit production, Tomato Chlorosis Virus (ToCV) Causes yellowing, stunting, and (leaf blanching), reducing fruit production [1].

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Infection of tomatoes by these viruses has been reported to causes significant losses in quality and quantity of yield produces [5]. These viruses can be transmitted through Insect vectors such as whiteflies, aphids, thrips. Also through Contaminated tools and equipment, infected seeds or seedlings and direct contact with infected plants.

Include a paragraph regarding the statistics of tomato production and loss occurred due to the virus infestation.

It is important therefore to use resistant varieties, Implementing integrated pest management (IPM) strategies, Practicing good agricultural practices (GAPs), Controlling insect vectors,

Disinfecting tools and equipment and Avoiding infected seeds or seedlings as management strategies in controlling these viruses [9].

A survey conducted during the 2024 planting season across few tomatoes growing regions in Cross River State revealed severe virus infection of this crop. This study was therefore carried out to identity these viruses using ACP ELISA test as diagnostic tool [2].

MATERIALS AND METHODS

Field Survey and Sample Collection

Field survey was carried out in six tomato growing regions of Cross River State, Nigeria. (Odukpani, Akpabyo, Obubra, Ikom, Ogoja and Obudu) during the 2024 planting season. The infected leaf samples collected from the field were adequately labelled and preserved in vial bottles containing silica gel and later transferred to the laboratory of National Institute of Horticulture (NIHORT Ibadan for ELISA analyses.

3.2 Virus Detection by Enzyme-linked Immunosorbent Assay (ELISA)

The leaf samples were subjected to antigen coated plate enzyme-linked immunosorbent assay according to the protocol of [10]. Samples were ground in the sterilized mortar at the rate of 100 mg/mL using cold carbonate buffer pH 7.4 (0.015 M sodium carbonate plus 0.0349 M sodium bicarbonate per litre of distilled water). One hundred microlitres of the leaf extract were added to each well of the microtitre plates (Thermo Scientific "Nunc", Milford, MA). The plates were incubated at 370 C for 1 hour and washed three times with phosphate-buffered saline-Tween (8g NaCl, 1.1 g Na2HPO4, 0.2 g K2HPO4, 0.2 g KCl, 0.5 mL Tween – 20, 1 L distilled water, pH 7.4) (PBS-T). A blocking solution [3 % (w/v) dried non-fat skimmed milk in PBS – T] was applied at the rate of 200 µL per well. This was followed by incubation of the plates at 370C for 30 minutes. The plates were tap-dried on a paper towel.

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Polyclonal antibodies (PAbs) for Tomato Spotted Wilt Virus (TSWV), Tomato Yellow Leaf Curl Virus (TYLCV), Tomato Mosaic Virus (ToMV), Tobacco Mosaic Virus (TMV), Tomato Ring Spot Virus (ToRSV), Tomato Bushy Stunt Virus (TBSV) and Tomato Chlorosis Virus (ToCV) were diluted (1:2000; v/v) with conjugate buffer [half strength PBS-T containing 0.05 % (v/v) Tween-20, 0.02 % (w/v) egg albumin, 0.2 % (w/v) polyvinylpyrrolidone, and 100 µL each were tested against the extract of each sample. Plates were incubated again at 37oC for 1 hour, washed thrice and 100 µL of the goat antirabbit antibody diluted with conjugate buffer (1:15,000) was added to the wells. The plates were incubated at 370C for 1 hour and washed. Afterward, 100 µL of *p*-nitrophenyl phosphate dissolved in substrate buffer (97 mL diethanolamine, 1000 mL H2O, pH 9.8) was added to the well. The plates were finally incubated in the dark at room temperature (370 C) overnight. The absorbance of virus concentration was recorded at 405nm using a microplate reader (MRX, Dynex Technologies, Inc., USA). The values of the readings obtained were considered positive when the optical density reading at A405nm was twice the value of the healthy control.

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Effect of virus on selected growth parameters of tomato

Effect of virus on plant height and leaf number

The effect of the virus inocula on plant height was determined by measuring each of the plant from the soil level to the tip of the shoot using a metre rule. For the leaf number, the leaves were counted per plant. Data were also obtained for the controls.

Effect of virus strains on fresh and dry leaf shoot weights

The shoots were severed from the root system with a sharp knife and the fresh weight determined in situ. The samples were later oven-dried to constant weight at 70oC. They were then weighed using Blauscal weighing balance (DHB 9053A, Ocean Med, England).

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Give details about the treatment details mentioning number of treatments and replications and the statistical design and test used.

RESULTS AND DISCUSSION

Antigen coated plate (ACP) enzyme linked immunosorbent assay (ELISA) for detection of tomato viruses

The result revealed that the tomato sample obtained from Ikom and Obudu tested positive to Tomato Mosaic Virus (ToMV) antiserum with an optical density (OD) reading of 0.99. Similarly, tomato sample obtained from Odukpani and Obubra tested positive to Tomato Spotted Wilt Virus (TSWV) antiserum with an optical density (OD) reading of 0.97. The sample obtained from Akpabuyo and Ogoja recorded 1.10 and 1.21 as optical density (OD) reading which was a positive test for Tomato Yellow Leaf Curl Virus (TYLCV) and Tomato Ring Spot Virus (ToRSV) respectively. The reading for the healthy control is 0.405. Sample was considered virus positive when the optical density (OD) reading at *A*405nm was twice greater than the absorbance from healthy controls (Table 1).

The serological characterization of viruses infecting tomatoes in Cross River State has revealed a complex viral landscape, with multiple viruses detected in the crop. The prevalence of TYLCV, TSWV), ToMV, ToRSV in tomatoes highlights extend of spread of the virus

Serological techniques, such as ACP ELISA employed in this proved effective in identifying and differentiating the viral isolates. ACP-ELISA has been employed in the detection and identification of plant viruses into the both genus taxon and species [13].

Eyong, [8] have employed ELISA test in the detection of several viruses in Cross River State. The detection of different tomato viruses in this study could be an indication widespread virus infection of this disease in within tomato planting fields in Cross River State

The detection of the virus species in this study using virus specific antiserum further supports the effectiveness of ELISA test in plant virus diagnosis by [6]. This result shows a strong

link between the virus species and feeding pattern of the agent of transmission. For examples all the viruses detected were tomatoes viruses.

The study's findings have significant implications for tomato production in Cross River State, emphasizing the need for improved seed quality: ensuring virus-free seeds to prevent primary infection, enhanced agricultural practices by implementing best practices to minimize virus transmission, virus monitoring and surveillance which can be a regular monitoring to detect and respond to emerging viral threats, development of resistant varieties by breeding tomato varieties with built-in resistance to prevalent viruses.

Understanding the role of serological in plant virus detection it will help in the development of targeted management strategies, ultimately enhancing tomato productivity and food security in the region.

Table 1: Antigen coated plate (ACP) enzyme linked immunosorbent assay (ELISA) for detection of tomato viruses

S/N	Samples	Location	OD reading at A_{405nm} against Virus Polyclonal Antibodies	Virus detected
1	Tomato	Odukpani	0.97	TSWV
2	Tomato	Akpabuyo	1.10	TYLCV
3	Tomato	Obubra	0,97	TSWV
4	Tomato	Ikom	0.99	ToMV
5	Tomato	Ogoja	1.21	ToRSV
6	Tomato	Obudu	0,99	ToMV
7	Healthy Control		0.45	
8	Infected Control		1.894	

***Sample was considered virus positive when the optical density (OD) reading at *A*405nm was 2x greater than the absorbance from healthy controls**

Effect of TSWV on growth parameters of tomatoes

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The results showed that **the virus** significantly reduced ($p<0.05$) plant height, leaf number, fresh and dry shoot weight, fresh and dry leaf weight of tomatoes when inoculated at 9 DAP by 12%, 25%, 49.1% and 84.5% respectively compared to the values obtained for the corresponding controls (Table 2).

For 21 DAP, values obtained for leaf number, dry shoot weight, fresh leaf weight and dry leaf weight were also significantly lower with percentage reduction ranging from 8% for Plant height to 53.0% for dry leaf weight compared to the corresponding controls. However, values obtained for plant height and leaf number, though reduced, were statistically insignificant ($p<0.05$). The results also showed that values obtained for all the growth parameters at 9 DAP were lower and differed significantly from those obtained when plants were inoculated at 21 DAP

Tomato Spotted Wilt Virus (TSWV) used in this aspect of study was detected by serological testing as presented in the result above. This virus was further subjected to test the physiological parameters of tomato. The result agrees with previous studies by [7] who reported significant reduction in growth and yield of cucumber using Morroccan watermelon mosaic virus. The results obtained in this study confirm previous reports that virus infection reduces growth performance of crops [4] with greater loses associated with early infections [5]. Previous have also revealed significant reduction and growth parameters when crop were inoculated few days after planting than later [12].

Table 2: Effect of two strains of Moroccan water melon mosaic virus on growth parameters of tomatoes

Treatments	Plant height (cm)	Reduction (%)	Leaf number	Reduction (%)	Fresh shoot weight (g)	Reduction (%)	Dry leaf weight (g)	Reduction (%)
TSWV								

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9	39a	12	11a	25	49.47a	49.1	5.48a	84.5
21	43b	8	20b	16	73.89b	24.0	16.56b	53.0
Control	51b		36c		97.24b		35.26c	

¥Each value is a mean of 4 replicates. In each column of specific treatment, means followed by the same letter are not significantly different according to Duncan multiple range test. Percentage reduction was calculated by expressing the difference between the control and the treatment as a percentage of the value for the control.

CONCLUSION

Tomato field were surveyed during the 2024 planting season in Cross River State, Nigeria with the view of identifying virus infection using ELISA test. Viruses detected included TYLCV, TSWV, ToMV and ToRSV. After which one the viruses TSWV was used to further investigate its effect on the growth pararmeters of tomatoes. The result revealed a more significant difference in almost the growth parameters tested at 9 days after planting than those inoculated 21 days after planting.

Growers of this crop are at the risk of incurring significant losses if infection occurs early during the vegetative growth which could come through transmission by the ubiquitous Aphids spiraecola which has been established as an efficient vector of the virus strains [11]. It is recommended that sourcing for and planting of resistance varieties of cucumber, monitoring and control of aphid vectors may be helpful to stem this.

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