

EFFECTS OF SINGLE AND MIXED INOCULATIONS OF TWO VIRUSES ON GROWTH PARAMETERS OF SOME TOMATO CULTIVARS

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SUMMARY

Worldwide, Tomato is an important vegetable in the diets of many consumers. However, its production is being hampered by many plant pathogens virus inclusive. To study the effect of two viruses on Tomato cultivars, *Cucumber mosaic virus* (CMV), genus *Cucumovirus* and *Tobacco mosaic virus* (TMV), genus *Tobamovirus* were mechanically inoculated separately and in mixed infections onto two tomato cultivars; F1 Mongal and Dankukumi D1 collected from the Tissue Culture Unit of Department of Crop Protection, Federal University of Agriculture, Abeokuta. Experimental design was a completely randomised design. There were four treatments namely: the buffer-inoculated controls, CMV-inoculated plants, TMV-inoculated plants and CMV+TMV-inoculated plants. For each treatment, there were two replicates with each replicate containing 4 plants. In all a population of 48 plants per cultivar were inoculated. Leaves of plants were inoculated at 4 weeks old. Scoring of inoculated plants was done between 2 and 5 weeks after inoculation (WAI). Parameters scored were disease incidence, severity of viral symptoms, plant height, Stem diameter and number of leaves per plant. Symptoms observed on inoculated tomato cultivars included mosaic, mottling and necrotic lesions. Viral disease incidence for CMV-inoculated plants ranged from 25.0 % at 2weeks after

inoculation (WAI) to 100.0 % at 5 WAI. Severity of viral symptoms expression for Tomato cultivar Dankukuimi D1 increased from 2.0 at 2WAI to 4.0 at 5WAI respectively. In TMV-inoculated Tomato cultivars, increase in plant height of both inoculated and buffer-inoculated controls was recorded from 2 to 5 WAI, however there was a significant($p<0.05$) increase in the plant height of the healthy control of Dankukumi D1 cultivar over inoculated plants. No significant difference was observed in the plant height of both control and inoculated plants of cultivar F1 Mongal.

Keywords: Tomato, virus, mosaic, mottling, Tobacco mosaic virus, inoculation

TOMATO is the edible, often red berry type fruit of *Solanum lycopersicum*, commonly known as a tomato plant, which belongs to the nightshade family, Solanaceae. In tropical Africa, vegetables are produced as components of prevailing farming systems including traditional shifting cultivation and associated fallow systems, home gardens, various uplands, systems, and specialized horticulture (9).

Tomato fruit can be bilocular or multilocular and is comprised of skin, pericarp, columella and locular contents. The locular cavities are filled with seeds that are surrounded with jelly parenchyma cells. Tomato dry matter normally varies between 5 and 10%, of which about 75% is soluble, and about 1 to 3% consist of skin and seeds (14).

On a worldwide scale tomato continues to increase the interest not only for the fresh market but also as component in a variety of processed

foods and pharmaceutical products (4, 12, 13).

Tomato species originated in Central and South America and consumed greatly in Mexico. Many of its varieties are widely grown, sometimes in greenhouse, in cooler climate. The plants typically grow 1-3metres and have a weak stem that often sprawls over the ground and vines over other plants. It is a perennial in its native habitat, and grown as an annual in temperate climates.

Tomato fruits are consumed fresh or incorporated in canned, frozen, preserved or dried foods. The most widely grown commercial tomatoes tend to be in the range of 5-7 cm in diameter (10).

In Nigeria, Tomato plays important role as a soup condiment as it is used in almost all homes for stew preparation, salad making and eggs. Its use is very diverse and varies

depending on occasions. However, the production of tomato in the world is being threatened by many pathogens among which are bacteria, fungi, nematodes and viruses.

The objectives of this study are to investigate the responses of two tomato cultivars to mechanical inoculations of *Cucumber mosaic virus* (CMV) genus *Cucumovirus* (cowpea strain) and *Tobacco mosaic virus* (TMV) genus *Tobamovirus* and check their levels of symptom severity and expression. Information obtained would be useful for Breeders for breeding improved Tomato varieties with durable resistance to TMV and CMV.

MATERIALS AND METHODS

The method of planting was a completely randomized design with Tomato plants sown as 5 pregerminated seedlings per pot but thinned to two seedlings before inoculation. Two pots (each of 2 L in capacity) were used per replicate and there were two replicates per treatment and tomato cultivar. In all, plant population was 48 tomato plants per cultivar. The two cultivars *Lycopersicon esculentum* used for this study were F1 Mongal and Dakunkunmi D1. Seeds of these cultivars were collected from the Tissue culture laboratory of the Department of Crop Production and

Protection, Federal University of Abeokuta, Ogun State, Nigeria.

Tomato seedlings were inoculated when they were four weeks old. Viruses used in this study were *Cucumber mosaic virus* (CMV), genus *Cucumovirus* and *Tobacco mosaic virus* (TMV), genus *Tobamovirus*. The former was a Cowpea strain obtained from the Virology Unit of International Institute of Tropical Agriculture while the latter was collected from leaves of infected *Mucuna pruriens* collected from the wild at Kotopo area of Abeokuta, Ogun State, Nigeria.

Viruses were extracted by homogenizing the infected leaves in inoculation buffer (0.05M phosphate buffer pH 7.0) using sterilized mortar and pestle. Leaves of tomato plants were then rubbed with the extracted sap of CMV and TMV respectively. Equal volume of each of CMV and TMV inoculum were mixed to obtain mixed viral inocula. Inoculated leaves were rinsed afterwards with tap water using a wash bottle (11).

Plants were labeled and set aside for observation in screened cages. Data on viral disease incidence, severity, plant height, stem diameter and number of leaves was collected for

every plant in a replicate every week for 5 weeks.

Data Analysis

Data obtained for each parameter was subjected to analysis of variance (ANOVA) to determine level of significance (SAS version 9.0.1). Duncan multiple range test was used to separate the means so as to determine level of significance at $p \leq 0.05$.

RESULTS

Among various symptoms observed on leaves of Tomato cultivars, 'F1 Mongal' and 'Dankukumi D1' were yellowing, mosaic, necrosis and stunted growth. CMV induced leaf yellowing and necrotic lesions on leaves of 'Dankukumi D1', while necrotic lesions were observed on leaves of 'F1 Mongal' Tomato cultivars. When Tomato cultivars 'Dankukumi D1' were inoculated with TMV, symptoms such as chlorotic lesions and necrosis were observed.

Disease incidence observed on the inoculated cultivars steadily increased, for example CMV on Dankukumi D1 had an incidence of 25.0 % at 2WAI while at 5WAI, the incidence increased to 100.0 % (Table 1). Moreover, Dankukumi inoculated with mixed inocula of

CMV and TMV did not show any viral symptom at 2WAI but at 4 WAI, the incidence was 50.0 %. Throughout the period, the control plants did not show viral symptom, a disease incidence of 0.0 % was therefore recorded (Table 1).

On cultivar Dankukumi D1 inoculated solely with TMV, a disease incidence of 25.0 % was recorded at 2 WAI and this gradually increased to 100.0 % at 5 WAI. However, 50.0 % disease incidence was recorded for F1 Mongal at 2 WAI and 100.0 % at 5 WAI.

The severity of viral symptom expression for 'Dankukumi' tomato cultivars increased from 1.3 at 2WAI to approximately 4.0 at 5WAI when inoculated with mixed inocula of CMV and TMV. Tomato cultivar 'F1 Mongal' had its severity being constant at 2.0 from 2 WAI to 5WAI (Table 2).

Inoculation of tomato cultivars 'F1 Mongal' and 'Dankukumi D1' with CMV showed gradual increase in the plant height of both inoculated and control plants between 2 and 5 WAI. Plant height of CMV-inoculated 'F1 Mongal' and 'Dankukumi D1' cultivars were observed to be significantly higher than the control plants (Table 3).

Table 1: Effect of viral inoculation on Percentage disease incidence on Tomato plants.

Virus inoculated/Plant Cultivar		Percentage incidence (%):			
		2WAI	3WAI	4WAI	5WAI
CMV					
Dankukumi D1	Control (H)	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^b	0.0±0.0 ^b
	Diseased	25.0±0.0 ^a	62.5±47.8 ^a	100.0±0.0 ^a	100.0±0.0 ^a
F1 Mongal	Control (H)	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^b	0.0±0.0 ^b
	Diseased	12.5±0.0 ^b	25.0±0.0 ^b	100.0±0.0 ^a	100.0±0.0 ^a
TMV					
Dankukumi D1	Control(H)	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^b
	Diseased	25.0±0.0 ^b	37.5±21.7 ^a	87.5±12.5 ^a	100.0±0.0 ^a
F1 Mongal	Control (H)	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^b
	Diseased	50.0±20.4 ^a	50.0±0.0 ^a	75.0±14.5 ^b	100.0±0.0 ^a
CMV+TMV					
Dankukumi D1	Control (H)	0.0±0.0 ^b	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^b
	Diseased	0.0±0.0 ^b	25.0±10.0 ^b	50.0±0.0 ^b	100.0±0.0 ^a
F1 Mongal	Control (H)	0.0±0.0 ^b	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^b
	Diseased	25.0±0.0 ^a	50.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a

Means followed by the same letters along each column for each treatment are not significantly different at 5 % level of probability.

Table 2: Effect of *Cucumber mosaic virus* and *Tobacco mosaic virus* on symptom severity of Tomato cultivars.

Virus inoculated/Plant Cultivar		Severity scores			
		2WAI	3WAI	4WAI	5WAI
CMV					
Dankukumi D1	Control (H)	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^b
	Diseased	2.0±0.18 ^a	2.0±0.27 ^a	2.0±0.2 ^a	2.0±0.1 ^a
F1 Mongal	Control (H)	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^b
	Diseased	2.0±0.16 ^a	2.0±0.27 ^a	2.0±0.2 ^a	2.0±0.2 ^a
TMV					
Dankukumi D1	Control (H)	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^c
	Diseased	2.0±0.2 ^a	1.4±0.2 ^b	2.3±0.3 ^a	2.5±0.2 ^a
F1 Mongal	Control (H)	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^b	1.0±0.0 ^c
	Diseased	2.0±0.1 ^a	2.0±0.3 ^a	2.3±0.2 ^a	2.3±0.2 ^b
CMV+TMV					
Dankukumi D1	Control (H)	1.0±0.0 ^c	1.0±0.0 ^b	1.0±0.0 ^c	1.0±0.0 ^c
	Diseased	1.3±0.3 ^a	2.3±0.3 ^a	3.0±0.1 ^a	3.6±0.1 ^a
F1 Mongal	Control (H)	1.0±0.0 ^c	1.0±0.0 ^b	1.0±0.0 ^c	1.0±0.0 ^c
	Diseased	2.0±0.0 ^b	2.0±0.2 ^a	2.0±0.1 ^b	2.3±0.2 ^b

Means followed by the same letters along each column per treatment are not significantly different at 5 % level of probability.

Table 3: Effect of viral inoculation on plant height of Tomato cultivars.

Virus inoculated/Plant Cultivar		Plant height (cm):			
		2WAI	3WAI	4WAI	5WAI
CMV					
Dankukumi D1	Control (H)	6.0±0.9 ^c	7.1±1.0 ^c	12.8±0.9 ^d	16.4±1.2 ^d
	Diseased	8.4±1.3 ^b	11.1±0.7 ^b	18.8±2.4 ^b	23.3±2.0 ^b
F1 Mongal	Control (H)	7.7±1.1 ^c	10.0±1.3 ^b	14.1±2.1 ^c	19.5±4.1 ^c
		14.2±3.0 ^a	18.7±4.3 ^a	28.5±5.8 ^a	36.5±8.0 ^a
TMV					
Dankukumi D1	Control(H)	6.0±0.9 ^c	7.1±1.0 ^d	12.8±0.9 ^c	16.4±1.2 ^b
	Diseased	6.5±0.5 ^c	8.3±1.3 ^c	11.1±2.1 ^d	11.8±2.3 ^c
F1 Mongal	Control (H)	7.7±1.1 ^b	9.9±1.3 ^b	14.2±2.1 ^b	19.5±4.1 ^a
	Diseased	10.0±2.1 ^a	12.8±3.1 ^a	17.6±4.2 ^a	19.5±3.9 ^a
CMV+TMV					
Dankukumi D1	Control (H)	6.0±0.9 ^b	7.1±1.0 ^c	12.8±0.9 ^c	16.4±1.2 ^c
	Diseased	8.9±0.5 ^a	12.2±3.0 ^a	15.9±1.7 ^b	19.5±1.7 ^b
F1 Mongal	Control (H)	7.7±1.1 ^{ab}	9.9±1.3 ^c	14.2±2.1 ^c	16.5±4.1 ^c
	Diseased	8.9±1.1 ^a	11.4±2.4 ^b	17.2±3.5 ^a	22.1±2.7 ^a

Means followed by the same letters along each column per treatment are not significantly different at 5 % level of probability.

However, in TMV-inoculated tomato cultivars, there was an increase in plant height of both inoculated and control plants from 2 to 5 WAI. At 5 WAI, there was a significant increase ($p<0.5$) in the plant height of the healthy control of Dankukumi D1 with mean height of 16.4±1.2 centimetres (cm) over the inoculated

plants with mean height 11.8±2.3 cm while no significant difference was observed in the control and inoculated plants of F1 Mongal at 5 WAI.(Table 3). Similar trends such as obtained on effect of virus inoculations on plant heights of tomato cultivars were also observed on the stem diameter parameters of

inoculated and healthy controls of ‘Dankukumi D1’ and ‘F1 Mongal’. There was a steady increase in the stem diameter of both inoculated and healthy controls of both Tomato cultivars from 2 to 5 WAI (Table 4). However, at 5WAI there was a

significant increase in the stem diameter of CMV inoculated F1 Mongal over that of healthy control ($p<0.05$) (Table 4). This was also observed for cultivar ‘Dankukumi’ inoculated with mixed inocula.

Table 4: Effect of virus inoculation on Stem diameter of two tomato cultivars

Virus inoculated/Plant Cultivar		Stem diameter (cm)			
		2WAI	3WAI	4WAI	5WAI
CMV					
Dankukumi D1	Control (H)	0.71±0.0 ^c	0.89±0.1 ^{ab}	1.06±0.1 ^b	1.18±0.1 ^{ab}
	Diseased	0.84±0.0 ^b	0.96±0.1 ^{ab}	1.15±0.1 ^{ab}	1.45±0.1 ^{ab}
F1 Mongal	Control (H)	0.64±0.0 ^c	0.79±0.1 ^b	0.96±0.1 ^b	1.00±0.1 ^b
		0.94±0.1 ^a	1.01±0.1 ^a	1.36±0.2 ^a	1.60±0.3 ^a
TMV					
Dankukumi D1	Control(H)	0.71±0.0 ^a	0.88±0.1 ^{ab}	1.06±0.1 ^{ab}	1.18±0.1 ^a
	Diseased	0.66±0.1 ^a	0.80±0.1 ^b	1.06±0.1 ^{ab}	1.21±0.1 ^a
F1 Mongal	Control (H)	0.64±0.0 ^a	0.79±0.1 ^b	0.96±0.1 ^b	1.00±0.1 ^b
	Diseased	0.66±0.1 ^a	1.01±0.1 ^a	1.08±0.1 ^a	1.02±0.1 ^b
CMV+TMV					
Dankukumi D1	Control (H)	0.71±0.0 ^b	0.89±0.1 ^b	1.06±0.1 ^{ab}	1.18±0.1 ^b
	Diseased	0.89±0.1 ^a	1.00±0.1 ^a	1.10±0.1 ^a	1.33±0.1 ^a
F1 Mongal	Control (H)	0.64±0.1 ^c	0.79±0.1 ^c	0.96±0.1 ^b	1.00±0.1 ^b
	Diseased	0.76±0.1 ^b	0.85±0.1 ^b	1.11±0.1 ^a	1.26±0.1 ^b

Means followed by the same letters along each column per treatment are not significantly different at 5 % level of probability.

Effect of viruses on number of leaves per plant showed that leaves of tomato cultivar ‘F1 Mongal’ inoculated with CMV at 5WAI

increased significantly ($p \leq 0.05$) with mean number of leaves per plant of 12.38 ± 1.0^a over that of healthy controls (10.38 ± 1.0^b). Mean number of leaves of CMV-inoculated

‘Dankukumi D1’ (7.87 ± 0.4^c) were not significantly different from that of healthy controls which had 8.25 ± 1.0^c (Table 5).

Table 5: Mean number of leaves observed on Tomato cultivars inoculated with the two viruses.

Virus inoculated/Plant Cultivar		No. of leaves per plant			
		2WAI	3WAI	4WAI	5WAI
CMV					
Dankukumi D1	Control (H)	4.00±0.2 ^b	4.13±0.4 ^b	7.13±0.7 ^b	8.25±1.0 ^c
	Diseased	4.75±0.5 ^b	5.75±0.5 ^b	6.63±0.3 ^c	7.87±0.4 ^c
F1 Mongal	Control (H)	4.50±0.4 ^b	5.88±0.8 ^b	8.63±0.7 ^b	10.38±1.0 ^b
		7.38±0.5 ^a	9.00±0.6 ^a	10.75±0.7 ^a	12.38±1.0 ^a
TMV					
Dankukumi D1	Control(H)	4.00±0.2 ^b	4.13±0.4 ^c	7.13±0.7 ^{bc}	8.25±1.0 ^b
	Diseased	4.00±0.2 ^b	5.00±0.4 ^b	6.38±0.3 ^c	8.25±0.3 ^b
F1 Mongal	Control (H)	4.50±0.4 ^{ab}	5.88±0.8 ^{ab}	8.63±0.7 ^a	10.38±1.0 ^a
	Diseased	5.25±0.3 ^a	6.00±0.5 ^a	7.88±0.4 ^{ab}	10.13±1.0 ^a
CMV+TMV					
Dankukumi D1	Control (H)	4.00±0.2 ^b	4.13±0.4 ^c	7.13±0.7 ^c	8.25±1.0 ^c
	Diseased	5.65±0.4 ^a	6.40±0.3 ^b	8.25±0.5 ^b	10.00±0.2 ^b
F1 Mongal	Control (H)	4.50±0.4 ^b	5.88±0.8 ^b	8.63±0.7 ^{ab}	10.38±1.0 ^b
	Diseased	5.65±0.6 ^a	7.05±0.4 ^a	9.63±0.4 ^a	10.90±0.8 ^a

Means followed by the same letters along each column per treatment are not significantly different at 5 % level of probability.

DISCUSSION

Viruses are important plant pathogens causing diseases and loss in crop yield. Plant viruses upon infection replicate at the site of infection and move to the other plants making use of the host factors. Therefore, viruses are completely depending upon their host plants to complete their cycle. Various efforts have been made to investigate the mechanisms of virus infection and their systemic spread in plants. This research work had been carried out to investigate effect of inoculation of two different viruses on growth parameters of two tomato cultivars 'Dankukumi D1' and F1 Mongal. The results obtained in this study are in agreement with the studies of Agrios *et al.* (1) on effects of *Cucumber mosaic virus* (CMV) inoculations on growth and yield of pepper.

Similar symptoms such as necrosis on leaves and mosaic as earlier reported by Mohammed (8) were observed. Observation of these symptoms on leaves of Dankukumi and F1 Mongal showed the susceptibilities of these cultivars to *Cucumber mosaic virus* and *Tobacco mosaic virus* (TMV-Mucuna strain). Incidence of CMV on tomato crops and others plants such as pepper and melon had been shown to be very

high (between 30 and 100 %) in the Mediterranean Basin (7). This virus had been shown to cause a decrease in plant height by 0.97 to 30.19% in tested Tomato genotypes. The decrease in height varies with Tomato cultivars (2).

Furthermore, CMV infection on some Tomato plants had been shown to decrease relative water content of Tomato (5). A combination of *Tomato mosaic virus* (ToMV) and CMV on Tomato plants caused slight symptoms of mosaic in some tomato plants studied by Mohammed (8). The virus combinations decreased the effect of ToMV on Tomato plants. Infection with ToMV supported the symptoms of subsequent infection with CMV (8). Infection of Tomato plants by CMV and *Tomato yellow leaf curl virus* (TYLCV) was shown to significantly caused a reduction in plant height of Tomato (8).

Ability of some plants to express necrotic lesions during infection by CMV is possible due to the presence of of satellite ribonucleic acid (RNA) (14) which is the fifth RNA satellite component designated CARNA 5 (CMV-associated RNA 5) which modulate symptom severity in infected plants.

Another plant's pathogenic virus of Tomato plants includes TMV. The

virus particles are rigid rods measuring about 200 x 18 nm, and the virus has a positive sense RNA genome that encodes at least four proteins (6). In Brazil, TMV had been found to infect Tomato plants and symptoms such as mosaic and leaf narrowing were recorded (3). Transmission of TMV is possible through rubbing and use of contaminated planting materials.

Mixed infection of CMV and TMV is possible on the field especially when an infected seed stocks or seedlings had been planted and also by mechanical rubbing during rainfall. Insects also play major roles in the transmission of CMV.

The reports on the increase in stem diameter of inoculated plants over buffer-inoculated healthy controls is in correlation with the study of Parzarlar *et al.* (11) who observed increase in stem diameter in TMV-infected pepper plants than the controls in Turkey. The reason for the increment in stem girth in inoculated plants could be probably because of genetic make-up of the two tomato cultivars used in this study. They are hybrid crops obtained from the Tissue Culture unit. They might have been able to become tolerant to the inoculated viruses to some extent, hence the mild severity obtained in CMV and

TMV-inoculated varieties. Therefore, the responses of these Tomato plants depend on their resistant abilities to inoculated viruses.

CONCLUSION

The two tomato cultivars (F1 Mongal and Dankukumi D1) inoculated singly and in mixed infection were observed to be infected with varying degrees of symptoms and severities. Infection with single inoculations of these viruses caused increase in stem length and diameter of the two Tomato varieties. Though no spectacular losses or damage of plants were recorded, more researches are still recommended to check for the effect of these viruses on fruit yield.

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