

RESPONSE OF TOMATO ACCESSIONS TO SINGLE AND MIXED INFECTION WITH CUCUMBER MOSAIC VIRUS AND CERCOSPORA JATROPHICOLA IN KWARA STATE, NIGERIA



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ABSTRACT

Experiment was carried out to determine the response of three tomato accessions to single and mixed infection with Cucumber mosaic virus (CMV) and Cercospora jatrophicola (CJ). The tomato accessions evaluated were NG/RM/JAN/10/001, NG/SA/07/10/002 and NG/AA/MAY/09/030. The tomato seeds were sown in plastic pots with sandy-loamy soil previously steam sterilized at 120° C for 6 hours. The treatments were combination of accession and inoculation regime. The results indicated higher percentage disease severity, reduced growth and yields in the pathogen inoculated plants compared with control. The percentage disease severity for the single and mixed pathogenic inoculations for accession NG/RM/JAN/10/001 at the 8th week after inoculation were: 55.7% (CMV), 38% (CJ) and 77.3% (CMV + CJ). Accession NG/AA/JAN/10/001 mixed inoculated with CMV + CJ were observed to have the significantly shortest plants (34.5cm). The yield parameters were also influenced by accessions and inoculation. The study suggests that the tomato accessions are susceptible to CMV and CJ either as single infections or in combination of CMV and CJ was more pathogenic. There is the need for farmers to adopt preventive measures against the occurrence of multiple viral and fungal infection on tomato fields to realize appreciably high yields and profitability.

Keywords: Cercospora jatrophicola, Cucumber mosaic virus, Infection, Multiple, Single, Tomato.

INTRODUCTION

Tomato (*Lycospersicon esculentum*) is a herbaceous plant in the Solanaceae family, that is grown widely for its edible fruits. It is one of the most important vegetables worldwide and the second-most consumed vegetable after potato. Its fruit is an essential component of human food for the supply of vitamins, minerals, certain types of hormones precursors' protein and energy (Naika *et al.*, 2005).

The nutritional attributes of tomatoes include folate, potassium (K), vitamins C and E, flavonoids, chlorophyll, β -carotene, and lycopene (Jones, 2008). Lycopene and β carotene are powerful antioxidants, which have been associated with the prevention of cardiovascular disease, and cancers of the prostrate and gastrointestinal tract (Perera and Yen, 2007). The average tomato yield in Nigeria is 10 t ha-¹, which is lower than 13.5 t ha-1 average yield in tropical Africa and world average of 22 t ha-1 (FAO, 2008). Tomato production under both field and greenhouse conditions is limited by pests and diseases (Atherton and Rudich, 1986).

In nature, many diseases of great economic importance to growers result from a mixture of different agents impinging the host at a given time (Balogun et al., 2002). Cucumber mosaic virus (CMV) and viruses of different taxa often have been found to act synergistically in solanaceous crops causing poor growth and low yields (Wege & Siegmund 2007). A "streak disease" often occurs in tomato when mixed infected with TMV and Potato virus X (PVX) genus Potexvirus (Matthews, 1991). Related and unrelated viruses can often replicate in the same cells and may interact synergistically or antagonistically (Otsuki and Takebe, 1976).

Cucumber mosaic virus (CMV) genus *Cucumovirus* is the type member of the *Cucumovirus* group, characterized by isometric particles of 28 nm diameter with a tripartite genome (Francki *et al.*, 1979). CMV is transmissible through seeds and by sap inoculation and vectored by several aphid species in a non-persistent manner (Fisher and Lockhart, 1976). The symptoms that are induced by CMV include mild to severe mottle, mosaic, distortion and reddish vein necrosis with yield reduction in crops (Pio-Riberio *et al.*, 1978).

Cercospora jatrophicola (Speg), is a monograph of the fungus genus *Cercospora* (Braun, 2000). The pathogen causes symptoms in the form of leaf spots that consist of well-delimited brown irregular necrotic spots (Dianese *et al.*. 2010). The genus Cercospora groups possess branched, septate hyphae that are colorless or near-colorous to pigmented (Crous and Brown, 2003).

Arogundade *et al.* (2007) showed that dry season tomato plants at the three stages of growth were susceptible to both fungal and viral attacks. It was discovered that fungal and viral diseases are the most serious disease of tomato in the dry season under irrigation which causes reduction in yield parameters.

The objectives of this study therefore was to evaluate the responses of three tomato cultivars to single and mixed infection with *Cucumber mosaic virus* (CMV) and *Cercospora jatrophicola* (CJ).

MATERIALS AND METHODS

Collection of Tomato Accessions

The seeds of three tomato accessions (NG/RM/JAN/10/001-4F, G/SA/07/10/002-4F and NG/AA/MAY/09/030- 4F) used in the study were obtained from the National Centre for Genetic Resources and Biotechnological (NCGRB) Ibadan, Oyo state - Nigeria. The seeds (4 per pot) were sown in plastic pots with sandy-loamy soil that was previously steam sterilized at 120° C for 6 hours using the autoclave.

Experimental Design

The treatment combination of accessions and inoculations were as follows:

(1)	NG/RM/JAN/10/001-			
4F inoculated wi	th CMV (V1T ₁).			
(2)	NG/RM/JAN/10/001-			
4F inoculated wi	th CMV + CS (V_1T_2)			
(3)	NG/RM/JAN/10/001-			
4F inoculated wi	th CS (V_1T_3)			
(4)	NG/RM/JAN/10/001-			
4F buffer inocula	ution (V_1T_4)			
(5) NG/SA/07/10	0/002-4F inoculated			
with $CMV (V_2T_1)$)			
(6) NG/SA/07/10	0/002- 4F inoculated			
with $CMV + CS$	(V_2T_2)			
(7) NG/SA/07/10	0/002- 4F inoculated			
with CS (V_2T_3)				
(8) NG/SA/07/10/00	02- 4F buffer			
inoculation(V ₂ T ₄)				
(9) NG/AA	/MAY/09/030- 4F			
Inoculation with	$CMV (V_3T_1)$			
(10) NG/AA/N	MAY/09/030- 4F			
inoculation with	$CMV + CS (V_3T_2)$			
(11) NG/AA/N	MAY/09/030- 4F			
inoculation with	$n CS(V_3T_3)$			
(12) NG/A	A/MAY/09/030- 4F			
buffer inoculat	ion (V_3T_4) .			
Each treatment was replicated 4 times, thus				
giving a total of forty-eight observations				

Isolation of fungus from *Jatropha curcas* leaves

The causal organism Cercospora jatrophicola was isolated from Jatropha curcas leaves showing typical leaf spot symptoms. The diseased leaf pieces were sterilized in surface 0.5% Sodium hypochlorite for 30seconds and rinsed in several changes of sterile water and later blotted dry on clean sterile paper towel. The leaves were then transferred into aseptically onto Potato Dextrose Agar (PDA) amended with streptomycin in 9cm petri dishes to suppress the growth of bacteria. The plates were then incubated at 25^oC for 7 days. The fungal growth observed was sub-cultured to obtain pure culture. The morphological characteristics of the fungus were observed under the compound microscope and identified according to the method of Hashem and Farrag (2005).

Preparation of fungus and viral Inoculum

A modified method of Kimber (2011) was adopted for the fungal inoculum. Mycelia suspension was prepared by punching ten 5mm mycelial discs from ten day old cultures using 5mm cork-borer. The mycelia discs were homogenized in 200ml of sterile water for 10 seconds in warring blender. The suspension was strained through 2 layers of muslin cloth and the concentration of mycelial fragments was estimated using a haemocytometer. The final inoculum concentration was adjusted by dilution with water and surfactant (0.05% Tween 20). In the case of the viral inoculum, fresh pepper leaves infected with cucumber mosaic virus were obtained from the virology unit of International Institute for Tropical Agriculture (IITA) Ibadan, Oyo state, Nigeria. The pepper leaf tissues (1gm), was then placed in a mortar along with 0.05 M phosphate buffer, pH 7. Using a pestle, the tissue was crushed to produce a light green solution.

Inoculation procedure

The inoculation procedure for the fungal isolates was according to Dalong *et al.* (2011). This was done by injecting 30 ml of fungal liquid inoculum into each seedling root. The control seedlings received 30 ml of buffer solution with no fungal mycelium. The viral inoculation was carried out following the method of Balogun and Bakare (2007). The sap was applied on the surfaces of the two primary leaves previously dusted with carborundum. The sap was applied by rubbing the leaves gently with a cotton wool dipped in the sap. The control plants were only buffer inoculated. The inoculations were carried out at 21 days after planting.

Data collection and Statistical analysis

Days to appearance of first symptom, as well as some growth and yield parameters were taken. Disease severity was measured as the percentage of the leaf area covered by mosaic patterns and lesions on the top of three leaves of each plant, while fresh fruit weight was taken at harvest using a weighing balance. All collected data were subjected to analysis of variance and significant differences between them determined at P=0.05, using the New Duncan's Multiple Range test (1955).

Results and Discussion

Disease severity

Percentage disease severity in single and mixed infection with CMV and CJ at different times after inoculation is presented in Table 1. The result indicate that all the tomato accessions were susceptible to CMV and CJ either singly or in combination compared to the control plants which remained healthy. Indeed accession NG/RM/JAN/10/001 inoculated with CMV and CJ had the highest disease severity of 52.5%, 73%, 80% and 77.3% respectively from the 5th to the 8th week post inoculation. Conversely, lower disease severity was also recorded in accession NG/SA/07/10/002 inoculated with CJ (1.4%, 7.8%, 9.7% and 10.3% respectively). The result indicated that irrespective of accession, mixed infection of plants was more pathogenic tomato compared with single infections. Rentería-Canett et al. (2011) reported that mixed infections may result in additive, synergistic or antagonistic interactions. In the present experiment, the interaction of CMV and CJ resulted in higher levels of disease severity and could be described as synergistic, as it elicited symptoms more severe than the ones induced individually by the pathogens. This result also corroborates the evidence by van Baalen and Sabelis (1995) that mixed infections results in increased virulence and reduction in host fitness.

Plant height

The effect of the various treatments tested on plant height is presented in Table 2. The control plants were significantly taller from the 1st to the 7th week after inoculation, while 1^{st} week accessions in the a.i. NG/SA/07/10/002 and NG/RM/JAN/10/001 inoculated with CJ had the significantly taller plants (16.6 and 16.5 cm, respectively) compared with the other treatments. From the 4^{th} to the 6^{th} week a.i, the treatment with accession NG/AA/MAY/09/030 mixed inoculated with CMV and CJ produced the significantly shortest plants (32.2, 34.8 and 37.3 cm, respectively), while at the 7th week inoculation, the treatment after with NG/RM/JAN/10/001 accession mixed inoculated with CMV and CJ had the significantly shortest plants (34.5 cm) compared with the other treatment combinations..

It is apparent that accessions NG/AA/MAY/09/030 and NG/RM/JAN/10/001 mixed inoculated with CMV and CJ were the most severely affected. Murphy and Bowen (2005) had also shown that there were significant reductions in height of pepper in response to mixed infection. The reductions in plant growth can therefore be attributed to the occurrence of synergy due to interactions of the pathogens in vulnerable accessions (Kosman and Cohen, 1996).

Number of leaves

The effect of the treatment combination on average number of leaves per plant (Table 3), showed that the plants responded in various ways to the different inoculation regime. It appeared however, that the plants inoculated with mixed infection (CMV + CJ) were most severely affected. At the 2^{nd} week through the 6th week after inoculation accession NG/SA/07/10/002 inoculated with CMV and CJ significantly had the least average number of leaves per plant. While mock inoculated plants of the same accession had an average number of 10.5, 10.3, 10.8, 11.5 and 12.8 at the 2nd through the 6th week after inoculation respectively.

Aliyu *et al.* (2012) showed that mixed infection of Blackeye cowpea mosaic virus (BICMV) and Cowpea Yellow Mosaic Virus (CYMV) at different inoculation regimes resulted in lowest number of leaves while single virus inoculation with CYMV, resulted in significantly higher number of cowpea leaves. It was concluded that multiple viral infection caused significant leaf reductions compared with single virus infection.

Number of fruits

Table 4 shows the effect of treatments on the number of fruits per plant. The plants with multiple infections were generally the most severely affected and also produced significantly lower number of fruits. In particular, accession NG/RM/JAN/10/001 inoculated with CMV and CJ produced no fruits at all.

Herranz *et al.*(2013), recently demonstrated that the simultaneous infection of a viroid and a plant virus synergistically affect the host transcriptome in infected peach fruits. Their work identified a novel synergistic effect upon single and double infection with Prunus necrotic ringspot virus (PNRSV) and Peach latent mosaic viroid (PLMVd).

Fresh fruit weight

The result of analysis of the fresh fruit weight per plant (Table 5) was similar to that of the number of fruits. It indicated that varietal influence and inoculation regime significantly affected the fresh fruit yields. The single inoculations were significantly less pathogenic than the mixed infections. The result further indicated that irrespective of the pathogens inoculated, accession NG/RM/JAN/10/001 is the most susceptible as this accession did not produce any fruit (31.5 g). The finding from this study supports the assertion of Balogun *et al.* (2000), that a mixed infection of tomato with *Potato virus* X (PVX) and *Tobacco mosaic virus* (TMV) normally results in alterations in the accumulation of PVX components and significantly reduced yield components than the singly infected one. Buete (1970) had also earlier observed that an infection with *Cucumber mosaic virus* or *Tobacco ring spot virus* increased the prevalence and severity of *Fusarium* and *stromatina* root rot disease of gladiolus, with attendant yield decreases.

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CONCLUSION

The study revealed differential responses to *Cucumber mosaic virus* and *Cercospora jatrophicola* disease in the tested tomato accessions. It also demonstrated that multiple infection of tomato with *Cucumber mosaic virus* and *Cercospora jatrophicola* rather than single infection with any of the pathogen, results in more severe disease symptoms and reduction in growth and yield attributes. It is suggested that plant resistance and cultural practices that could reduce infection should be explored.

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	Weeks after			
	Inoculation	_		
Inoc.	5wks	6wks	7wks	8wks
CMV	29.7 ^e	45.1 ^g	52.9 ^g	55.7 ^f
CMV	19.2 ^c	16.2 ^c	14.7 ^c	14.8 ^c
CMV	20.0 ^e	24.5 ^d	27.8 ^e	25.3 ^d
CJ	19.1 ^c	27.5 ^e	36.2 ^f	38.0 ^e
CJ	1.4 _b	7.8 ^b	9.7 ^b	10.3 ^b
CJ	10.8 ^e	16.6 ^c	23.4 ^d	23.3 ^d
CMV+CJ	52.5 ^f	73.0 ^g	80.0^{h}	77.3 ^g
CMV+CJ	27.6 ^d	34.4 ^f	26.6 ^e	26.6 ^d
CMV+CJ	29.0 ^d	35.7 ^f	26.9 ^e	27.9 ^d
Control	0.0 ^a	0.0 ^a	0.0^{a}	0.0^{a}
Control	0.0 ^a	0.0 ^a	0.0^{a}	0.0^{a}
Control	0.0 ^a	0.0 ^a	0.0^{a}	0.0^{a}
	7.8	5.9	5.4	4.8
	Inoc. CMV CMV CJ CJ CJ CJ CMV+CJ CMV+CJ CMV+CJ CMV+CJ Control Control Control	Weeks after InoculationInoc.SwksCMV 29.7^e CMV 19.2^c CMV 20.0^e CJ 19.1^c CJ 1.4_b CJ 10.8^e CMV+CJ 52.5^f CMV+CJ 27.6^d CMV+CJ 29.0^d Control 0.0^a Control 0.0^a Control 0.0^a 7.8	Weeks after InoculationInoc.Swks6wksCMV 29.7^{e} 45.1^{g} CMV 19.2^{c} 16.2^{c} CMV 20.0^{e} 24.5^{d} CJ 19.1^{c} 27.5^{e} CJ 1.4_{b} 7.8^{b} CJ 10.8^{e} 16.6^{c} CMV+CJ 52.5^{f} 73.0^{g} CMV+CJ 27.6^{d} 34.4^{f} CMV+CJ 29.0^{d} 35.7^{f} Control 0.0^{a} 0.0^{a} Control 0.0^{a} 0.0^{a} Control 0.0^{a} 0.0^{a} 7.8 5.9 5.9	Weeks after InoculationInoc.5wks6wks7wksCMV29.7° 45.1^g 52.9^g CMV19.2° 16.2^c 14.7^c CMV20.0° 24.5^d 27.8^e CJ19.1° 27.5^e 36.2^f CJ1.4b 7.8^b 9.7^b CJ10.8° 16.6^c 23.4^d CMV+CJ 52.5^f 73.0^g 80.0^h CMV+CJ 27.6^d 34.4^f 26.6^e CMV+CJ 29.0^d 35.7^f 26.9^e Control 0.0^a 0.0^a 0.0^a Control 0.0^a 0.0^a 0.0^a Control 0.0^a 0.0^a 0.0^a 7.8 5.9 5.4

Table 1: Percentage disease severity in healthy and diseased tomato plants under single and mixed infection with CMV and CJ

Means followed by the same letter (s) are not significantly different at p=0.05 using the new Duncan's multiple range test.

Key: Inoc.= inoculum, CMV = *Cucumber mosaic virus*, CJ = *Cercospora jatrophicola*, CMV+CJ = Mixed inocula of *Cucumber mosaic virus* and *Cercospora jatrophicola*.

		weeks	After	Inoc.	_			
Accession	Inoc.	1wk	2wk	3wk	4wk	5wk	бwk	7wk
NG/RM/JAN/10/001	CMV	13.8 ^d	25.3 ^d	35.8 ^d	44.0 ^b	55.4 ^b	60.9 ^b	58.5 ^b
NG/SA/07/10/002	CMV	17.2 ^b	29.3 ^b	37.4 ^c	44.6 ^b	47.1 ^d	53.2 ^c	58.3 ^b
NG/AA/MAY/09/030	CMV	20.4 ^a	26.0 ^c	38.9 ^b	45.3 ^b	49.2 ^c	53.9 ^c	59.0 ^b
NG/RM/JAN/10/001	CJ	16.5 ^b	30.8 ^a	33.8 ^e	42.3 ^c	46.0 ^d	43.5 ^e	46.5 ^d
NG/SA/07/10/002	CJ	16.6 ^b	24.7 ^e	32.1 ^e	40.1 ^d	42.5 ^e	54.8 ^c	59.2 ^b
NG/AA/MAY/09/030	CJ	15.6 ^c	19.3 ^f	27.0 ^g	35.6 ^f	32.5 ^g	42.0^{f}	42.9 ^e
NG/RM/JAN/10/001	CMV+CJ	15.4 ^c	24.5 ^e	31.8 ^f	40.0 ^d	43.6 ^e	40.5 ^g	34.5 ^h
NG/SA/07/10/002	CMV+CJ	14.5 ^d	16.9 ^g	30.0 ^f	37.3 ^e	40.4^{f}	52.0 ^d	56.1 ^c
NG/AA/MAY/09/030	CMV+CJ	10.4 ^e	11.9 ^h	23.3 ^h	32.2 ^g	34.8 ^h	37.3 ^h	41.3 ^f
NG/RM/JAN/10/001	Control	19.8 ^a	32.3 ^a	41.1 ^a	49.7 ^a	58.6 ^a	62.1 ^a	72.0 ^a
NG/SA/07/10/002	Control	21.3 ^a	33.1 ^a	39.9 ^b	48.1 ^a	57.8 ^a	62.3 ^a	71.0 ^a
NG/AA/MAY/09/030	Control	21.2 ^a	31.0 ^a	43.0 ^a	48.9 ^a	58.7 ^a	62.2 ^a	71.5 ^a

Table 2: Plant height (cm) in healthy and diseased tomato plants under single and mixed infection with CMV and CJ

Means followed by the same letter (s) are not significantly different at p=0.05 using the new Duncan's multiple range test

Accession		weeks	after	Inoc.	_		
	Inoc.	1wk	2wk	3wk	4wk	5wk	6wk
NG/RM/JAN/10/001	CMV	5.3°	8.0 ^c	8.5 ^c	10.8 ^b	12.3 ^a	12.5 ^b
NG/SA/07/10/002	CMV	6.8 ^b	8.0 ^c	9.3 ^b	8.3 ^d	9.3 ^d	10.5 ^c
NG/AA/MAY/09/030	CMV	5.8 ^c	7.3 ^d	7.3 ^d	9.8 ^c	9.5 ^d	10.8 ^c
NG/RM/JAN/10/001	CJ	4.3 ^d	9.3 ^b	10.5 ^a	10.5 ^b	11.5 ^b	10.0 ^c
NG/SA/07/10/002	CJ	5.8 ^c	9.3 ^b	9.3 ^b	10.5 ^b	11.3 ^b	14.0 ^a
NG/AA/MAY/09/030	CJ	7.0^{a}	7.5 ^d	8.0 ^c	7.8 ^c	8.0 ^e	10.5 ^c
NG/RM/JAN/10/001	CMV+CJ	4.0 ^d	8.5°	8.5 ^c	10.3 ^b	10.5 ^c	10.8 ^c
NG/SA/07/10/002	CMV+CJ	5.3°	7.3 ^d	5.8 ^c	7.5 ^c	6.5 ^f	7.8 ^d
NG/AA/MAY/09/030	CMV+CJ	5.3°	9.3 ^b	7.8 ^d	8.8 ^d	8.0 ^e	10.8 ^c
NG/RM/JAN/10/001	Control	6.0 ^b	10.3 ^a	11.3ª	12.0 ^a	13.5 ^a	14.0 ^a
NG/SA/07/10/002	Control	7.5 ^a	10.5 ^a	10.3 ^a	10.8 ^b	11.5 ^b	12.8 ^b
NG/AA/MAY/09/030	Control	7.8^{a}	7.8 ^d	8.5 ^c	11.0 ^a	10.0 ^c	10.0 ^c
S.E		0.6	0.6	0.6	0.7	0.8	0.9

 Table 3: Average number of leaves (per plant) in healthy and diseased tomato

 plants under single and mixed infection with CMV and CJ

Means followed by the same letter (s) are not significantly different at p=0.05 using the new Duncan's multiple range test

Table 4: Number of fruits per plant in healthy and diseased tomato plants under single and mixed infection with CMV and CJ Accession Mean number of Inoc fruits/plt NG/RM/JAN/10/001 0.0^f CMV 5.8^c NG/SA/07/10/002 CMV 4.3^d NG/AA/MAY/09/030 CMV 0.0^{f} NG/RM/JAN/10/001 CJ 5.5^c NG/SA/07/10/002 CJ CJ 3.0^d NG/AA/MAY/09/030 NG/RM/JAN/10/001 CMV+CJ 0.0^{f} 3.8^d NG/SA/07/10/002 CMV+CJ 2.3^e NG/AA/MAY/09/030 CMV+CJ NG/RM/JAN/10/001 Control 6.0^c 9.3^a NG/SA/07/10/002 Control 7.3^b NG/AA/MAY/09/030 Control S.E 1.3

S.E

Means followed by the same letter (s) are not significantly different at p=0.05 using the new Duncan's multiple range test

Accession	Inoc.	Mean fresh wt of fruits/plt
		(g)
NG/RM/JAN/10/001	CMV	0.0 ^k
NG/SA/07/10/002	CMV	19.8 ^g
NG/AA/MAY/09/030	CMV	20.8 ^{ef}
NG/RM/JAN/10/001	CJ	0.0^{k}
NG/SA/07/10/002	CJ	23.0 ^d
NG/AA/MAY/09/030	CJ	21.9 ^e
NG/RM/JAN/10/001	CMV+CJ	0.0^{k}
NG/SA/07/10/002	CMV+CJ	17.3 ^h
NG/AA/MAY/09/030	CMV+CJ	16.9 ^{ij}
NG/RM/JAN/10/001	Control	31.5 ^b
NG/SA/07/10/002	Control	32.9 ^a
NG/AA/MAY/09/030	Control	30.2 ^{bc}
S.E		2.6

Table 5: Weight of fresh fruit	per plant in healthy and diseased t	tomato plants
under single and mixed infect	ion with CMV and CJ	

Means followed by the same letter (s) are not significantly different at p=0.05 using the new Duncan's multiple range test