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Interaction of Root-knot Nematode and *Cucumber mosaic virus* Infection on Growth and Yield of Tomato

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Abstract

The effect of single and combined infections of a root-knot nematode, *Meloidogyne incognita* and *Cucumber mosaic virus* (CMV) on the growth and yield of tomato (cv. Roma VF and UC82b) was investigated. This was with a view to determining the pattern of interaction of *M. incognita* and CMV on selected tomato cultivars. The study consists of one screenhouse experiment and two field trials with the following treatments applied for four days after transplanting of tomato seedlings: inoculation of plant roots with 5,000 eggs of *M. incognita* per plant only; mechanical inoculation of primary leaves with crude CMV sap only; inoculation of plant roots with 5,000 eggs of *M. incognita* two days before inoculating leaves with crude CMV; inoculation of CMV sap two days before inoculating the plant roots with 5,000 eggs of *M. incognita*; simultaneous inoculation of primary leaves with crude CMV sap and 5,000 eggs of *M. incognita*, and uninoculated control. Data collected on plant biomass, fruit yield and nematode population density at 90 days after planting were subjected to analysis of variance and treatment means separation using Least Significant Difference at 5% level of probability. Infection of *M. incognita* and CMV simultaneously performed better in plant growth parameters than single infection of CMV or *M. incognita* both in the screenhouse and in the field. Inoculation of *M. incognita* before CMV inhibited virus infection and *vice versa* both in the screenhouse and in the field. Combined infections of *M. incognita* and CMV resulted in a higher plant biomass and fruit yield than single infection. The study established that either of the two pathogens could be used to suppress the effect of the other on selected tomato plants.

Keywords: Interaction; *Meloidogyne incognita*; *Cucumber mosaic virus*; Tomato; Yield

Introduction

Tomato, *Lycopersicum esculentum* (Miller), belongs to the family Solanaceae (Van *et al.*, 2006). It is a staple fruit vegetable consumed all over the world. Its fresh fruits are very important sources of vitamins B and C, essential amino acids, sugars, dietary fibres and

minerals which are essential for human health (Babalola *et al*., 2010). Global production of tomato fruits tripled from 396 million MT in 1961 to 1.34 billion MT in 2003 (IITA, 2005). It is widely cultivated in most parts of sub-Sahara Africa (Adeolu and Taiwo, 2009). Nigeria ranked 16th on the global tomato production scale, accounting for 10.79% of Africa's and 1.2% of total world production of tomatoes (Weinberger and Lumpkin, 2007).
Root-knot nematodes are endoparasites which invade a diverse array of over 2000 plant species (Husseyand Janssen, 2002). *Meloidogyne* spp. are serious and economically most important pests of all the cultivated crops around the world (Hussain *et al*., 2011). When plants are infected with root-knot nematodes, their feeding activities in the root tissues result in the formation of massive galls of different sizes on root system (Rahman, 2003). Sikora and Fernandez (2005) reported that root-knot nematodes are particularly damaging vegetables in tropical and subtropical countries of the world and cause losses up to 80% in heavily infested fields. The overall yield loss averages 12.3% annually. This figures approaches 20% for some crops. In monetary terms, the worldwide figure exceeded $100 billion annually (Ozaslan *et al*., 2006).

Plant viruses can cause severe yield losses to tomato production amounting to about $1 billion globally (Parella *et al*., 2003). More than 100 viruses are known to infect tomato naturally and experimentally, and a number of these can cause significant yield losses, including *Cucumber mosaic virus* (CMV) (Brunt *et al*., 1990). *Cucumber mosaic virus* has a host range of over 1000 species of plants and it is transmitted by more than 60 species of aphids, which can acquire this virus within 5 to 10 seconds (Ozaslan *et al*., 2006). *Cucumber mosaic virus* is then spread from plant to plant within few hours.

It is well known that *Meloidogyne* spp. are not vectors of plant viruses, but under field conditions, it was observed that *Meloidogyne* spp. occur concomitantly with viruses on the same plant, as is the case with tomato (Ozaslan *et al.*, 2006). Root-knot nematodes can form synergy with plant pathogenic fungi, and bacteria causing greater yield losses (Rivera and Aballay, 2008). Nematode interactions with fungi, bacteria and viruses have been documented to synergistically elicit certain responses in some crops. For example, *Meloidogyne* and *Fusarium* can cause wilts of cotton and tobacco (Starr *et al*., 1989; LaMondia, 1992; Devay *et al*., 1997), and Pineapple mealybug wilt associated virus-I infection has a greater reduction in crop yield in the presence of environmental stress such as drought (Sether and Hu, 2001). Often, a plant stressed by a pathogen is more susceptible to attack by another parasite. There are also several reports of interactions of viruses and *M.*

incognita on tomato (Goswani and Chenulu, 1974). However, whether this would occur with CMV and *M. incongnita* was not known (Ozaslan *et al.*, 2006). Varshney *et al*. (2005) and Ahmed *et al*. (2007) observed that more root-knot nematodes were recorded in mungbean plants inoculated only with root-knot nematode than in those inoculated with both root-knot nematode and *Mungbean yellow mosaic virus*. The effects of simultaneous infection of *M. incognita* and CMV on tomato plants have not been reported in literature hence this study. The objectives of this study were therefore to investigate the effect of single and combined infections of CMV and *M. incognita* on disease symptom severity, growth and yield of tomato.

Materials and Methods

Experimental site

One screenhouse and two field trials were conducted in the rainy season (July- October) of 2014 and in the dry season (January- April) of 2015 on the Teaching and Research Farms of the Obafemi Awolowo University, Ile-Ife, located on latitude 7°28'N and longitude 4°33'E at 244 m above sea level. This site was free of plant–parasitic nematodes. This was determined by taking soil samples from different plots on the experimental field and subjecting them to nematode analysis.

Production of nematode and virus inocula

Meloidogyne incognita-infected *Celosia argentea* was harvested from nematode culture plot. The aerial portion was chopped off while 2 cm pieces of infected roots were placed in the laboratory trays. Eggs were extracted from the galled roots using chlorox method (Southey, 1986). The eggs in a counting dish were counted under a stereomicroscope (x 250). A standard CMV isolate previously detected on tomato (Kayode *et al*., 2014) maintained on cowpea (*Vigna unguiculata*) cultivar Ife brown was obtained from Virology laboratory, Department of Crop Production and Protection, Ile-Ife, Nigeria, and homogenized in 0.01M, pH 7.7 phosphate buffer in the ratio 1:10 (w/v) as the virus inoculum source.

Nursery practices

Two cultivars of tomato, Roma VF and UC82b, which have been reported to be susceptible to both *Cucumber mosaic virus* and *Meloidogyne incognita* were collected from National Horticultural Research Institute (NIHORT), Ibadan Nigeria, and used in this study. Top soil and rivers sand in the ratio 20:1 were thoroughly mixed, steam sterilized

and allowed to cool for 48 hrs. The soil was poured into planting trays, and each of the tomato cultivars was planted separately.

Screenhouse study

Forty-eight, 150 mm diameter, plastic pots were filled with steam-sterilized sandy-loam top soil and were arranged on benches in two batches of 24 in an insect-proof screenhouse. One 10-days seedling each of the planted tomato cultivars Roma VF and UC82b, were transplanted into each pot, with each cultivar occupying a batch of 24 pots in the screenhouse. The following treatments were applied one week after transplanting; inoculation of roots of each tomato seedling with 5,000 eggs of *M. incognita*; mechanical inoculation of primary leaves of each tomato seedling with crude CMV sap; inoculation of roots of a tomato seedling with 5,000 eggs of *M. incognita* two days before inoculating primary leaves with CMV sap; inoculation of primary leaves of a tomato seedling with crude CMV sap two days before inoculating plant roots with 5,000 eggs of *M. incognita*; simultaneous inoculation of primary leaves of tomato seedlings with crude CMV sap; inoculating of plant roots with 5,000 eggs of *M. incognita*; and uninoculated control. The 5,000 eggs of *M. incognita* were placed in a depression around the roots of each tomato seedling and covered with soil (Adekunle and Akinsanmi, 2003). The CMV inoculum source was inoculated onto young, tender leaves of tomato plants with an absorbent cotton swab on carborundum-dusted leaves. Treatments were replicated four times for each cultivar, and arranged in a 2 x 6 factorial in Randomized Complete Block Design (RCBD). The tomato plants were staked and watered every other day. The plants were observed one week after application of treatments and every week thereafter for the number of leaves showing CMV symptoms for five weeks. Harvesting of mature fruits per pot commenced eight weeks after transplanting and this continued at weekly intervals till the termination of the experiment. Data were also collected on growth parameters and biomass at harvest. The experiment was terminated at 12 weeks after transplanting, after all the fruits were harvested. At termination of experiment, vermiform nematodes were extracted from soil samples collected from each pot using modified Baermann funnel method after 24 hrs (Whitehead and Hemming, 1965; Coyne *et al.,* 2007), and counted under stereomicroscope (x250). Cultures of nematodes species were confirmed by perineal pattern of adult female and identified as *M. incognita* (Eisenback *et al.,* 1981).

Field study

Two identical field trials were conducted in the rainy season (July - October) of 2014 and the dry season (January to April) of 2015. Carbofuran 3G was applied to each of the plots at

the rate of 3.0 kg ai/ha four weeks before transplanting, so as to kill the nematodes in the soil. The soil was well watered after the application of carbofuran in the dry season trial. In the first trial (July - October), a land area of 26 m x 14 m was ploughed and harrowed and laid out in Randomised Complete Block with 2 x 6 factorial arrangements. Four blocks, 15.8 m x 1.8 m each, were marked out for each tomato cultivar. Each block was divided into six, 2.25 m x 1.5 m plots with a space of 1 m between plots, and 1 m between blocks (replicates). Two week-old tomato seedlings of both cultivars (cv. Roma VF and UC82b) were transplanted at the spacing of 50 cm × 75 cm at one plant per stand. There were 16 plants per plot (96 plants per block, 384 plants per cultivar) out of which 10 plants were inoculated per plot while other plants served as guard plants. Six treatments similar to those of the screenhouse study described above were applied in this trial 16 days after transplanting of the tomato cultivars Roma VF and UC82b. Weeds were manually controlled from the third week of planting and every two weeks thereafter. Data were collected weekly on the number of leaves showing disease symptoms and on growth parameters during the active growth of the plants, and fruit yields and biomass of the plants on a plot by plot basis at termination of the experiment. Nematodes were extracted from soil samples collected around the roots of the 10 selected plants in each plot using the modified Baermann funnel method (Whitehead and Hemming, 1965), and they were counted under a stereomicroscope (x250) and identified under a light microscope (x400) to species level (Adekunle, 2011).

The second trial (dry season) was conducted between January to April, 2015. A separate field from the first trial was used for the second field experiment. A land area of 26 m x 14 m was cleared, ploughed and harrowed. This trial was carried out following the procedure used in the rainy season trial without any modifications.

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) using SAS software (SAS, 2002) package. Treatment means were separated using Fisher's Least Significance Difference at 5% level of probability. Data on population density of nematode were logarithm transformed before analysis because the values were high.

Results and Discussion

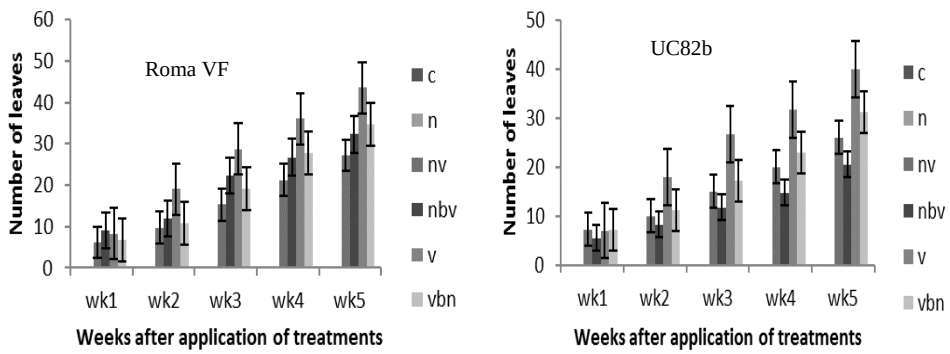
Knowledge of the relationships that exist between two or more pathogens infecting a plant is important for proper diagnosis and management of the resultant disease complex(es) in the field. Both *M. incognita* and CMV have been reported to cause significant damage in yield loss and growth of tomato plants (Parella *et al.*, 2003; Sikora and Fernandez, 2005), and these have been established in this study. This study demonstrates that there exist clear

and obvious effects on growth and yield of tomato when *M. incognita* and CMV are present individually in the plant than when simultaneously inoculated.

Screenhouse study

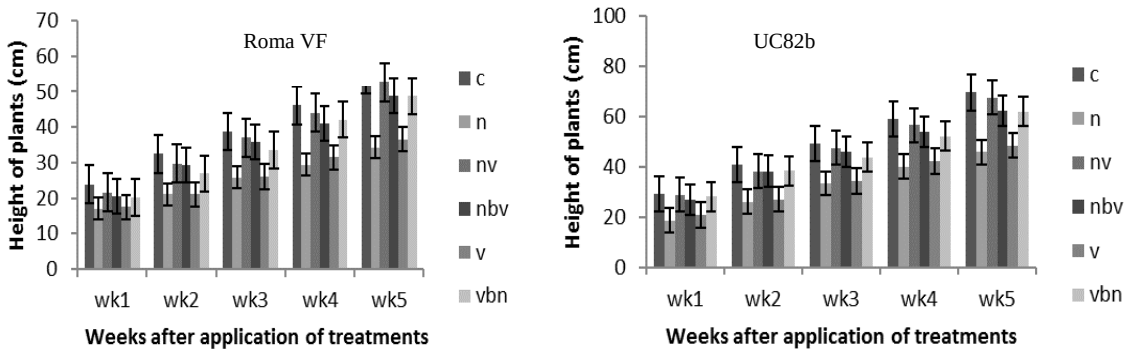
In actively growing tomato plants cultivar Roma VF in the screenhouse trial (Figure 1), no leaf showed virus-like symptoms in the control and nematodes only in treatments. From the third week after application of the treatments, virus only (v) was significantly higher ($p < 0.05$) than those with simultaneous inoculation with nematode and virus (nv). The number of leaves showing symptoms of virus infection was significantly higher in the treatment with virus only (v) than in the simultaneous inoculation of both the nematode and virus (nv) together.

The same trend was observed in actively growing plants of tomato cultivar UC82b in the screenhouse trial (Figure 2); no leaf showed symptoms in the control and nematodes only treatments. From the third week after application of the treatments, virus only (v) was significantly higher ($p < 0.05$) than those with simultaneous inoculation with nematode and virus (nv). The number of leaves showing symptoms of virus infection was significantly higher ($p < 0.05$) in the treatment with virus only (v) than in the inoculation of both the nematode and virus (nv) together.



Figures 1 and 2: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* on the number of leaves showing virus-like symptoms of two tomato varieties (Roma VF – Figure 1 and UC82b – Figure 2) in the screenhouse experiment.

Also, from the third week after application of the different treatments on tomato variety Roma VF in the screenhouse, there was no significant ($P \geq 0.05$) difference between the heights of the plants treated with virus (v) and those with nematode (n). The tomato plants from both virus and nematode were significantly shorter ($p < 0.05$) than those of the other treatments (Figure 3). The same trend was observed on tomato variety UC82b in the screenhouse (Figure 4).



Figures 3 and 4: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* on the height of two tomato varieties (Roma VF – Figure 3 and UC82b – Figure 4) in the screenhouse experiment

The application of the single treatment of the two pathogens, nematode only (n) and virus only (v), caused significant reductions ($p < 0.05$) in the fruit yields and biomasses of both tomato varieties in the screenhouse trial (Table 1). The introduction of either of the two pathogens two days before the other did not have any significant effect, while simultaneous application (nv) of the two pathogens had significantly higher ($p < 0.05$) fruit yields and biomasses.

Table 1: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* (CMV) on Biomass (g) and cumulative fruit yields of tomato plants at termination of experiment under screenhouse conditions

Treatment	Fruit yield (g)		Biomass (g)	
	Roma VF	UC82b	Roma VF	UC82b
M. incognita only	8.03	9.81	9.5	11.58
CMV only	7.78	8.62	10.41	14.13
CMV two days before *M. incognita*	12.08	14.52	17.63	21.72
M. incognita two days before CMV	11.8	13.35	18.98	22.44
M. incognita + CMV simultaneously	14.33	17.58	22.63	24.81
Uninoculated control	17.65	21.63	26.07	30.11
LSD ($P \leq 0.05$)	1.99	2.081	4.39	4.57

Each value is a mean of four replicates.

The development of the *M. incognita* second juvenile population in the screenhouse trial was greatly inhibited by the presence of the CMV (Table 2). There was significant reduction in the population of *M. incognita* second juvenile population when CMV was simultaneously inoculated with the nematode (nv), and also when the virus was applied two days before the nametode (vbn), but not *vice versa*.

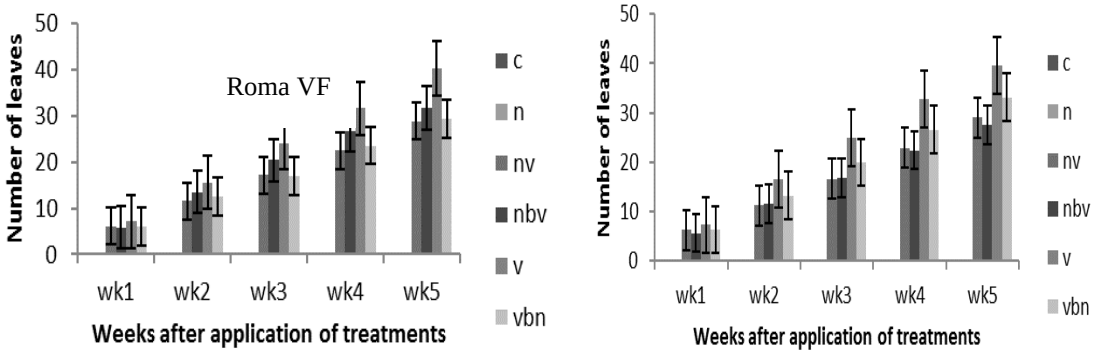
Table 2: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* on *Meloidogyne incognita* population (J_2) (number/200ml soil) at termination of experiment in the Screenhouse

Treatment	Tomato varieties	
	Roma VF	UC82b
M. incognita only	2.00 (101)	2.14 (138)
CMV only	0.0	0.0
CMV two days before *M. incognita*	1.85 (73)	2.00 (100)
M. incognita two days before CMV	1.94 (89)	2.08 (119)
M. incognita + CMV simultaneously	1.77 (60)	1.92 (82)
Uninoculated control	0.0	0.0
LSD ($P \leq 0.05$)	0.08	0.10

Each value is a mean of four replicates. Analysis of variance is based on logarithm transformed data. Figures in parenthesis are means of original values

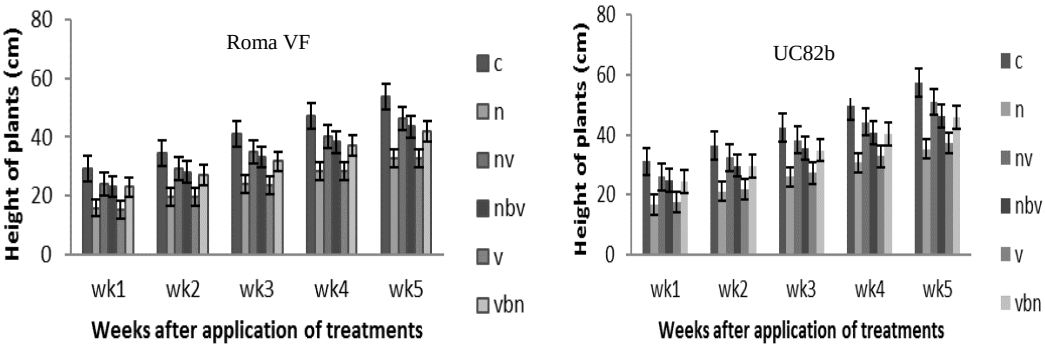
Field trials

Figures 5-8 show the numbers of symptomatic leaves and height of the tomato varieties (Roma VF and UC82b) with the different treatments in the field trials. The trends observed on the tomato plants of the two varieties (Roma VF and UC82b) in the field trials starting from the first week after application of the different treatments were similar with the only difference in the height of the control (c) which was significantly taller than both nbv and vbn, and not with nv (Figures 7 and 8).



UC82b

Figures 5 and 6: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* on the number of leaves showing virus-like symptoms of two tomato varieties (Roma VF – Figure 5 and UC82b – Figure 6) in the field experiments for two planting seasons.



Figures 7 and 8: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* on the height of two tomato varieties (Roma VF – Figure 7 and UC82b – Figure 8) in the field experiments for two planting seasons.

The interaction between *M. incognita* and CMV had significant effects on the cumulative fruit yield and biomass of tomato variety Roma VF but not on UC82b (Tables 3 and 4). The cumulative fruit yield and biomass recovered from Roma VF at the expiration of the field trials for the two seasons were more when both pathogens were inoculated, especially when they were simultaneously inoculated (nv). Results of effects of the interaction between the two pathogens on *M. incognita* second juvenile population recovered from the soil after expiration of the field trials in two planting seasons were similar to those of the screenhouse experiment (Table 5). The presence of the CMV significantly reduced the developmental

ability of *M. incognita*.

Table 3: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* on weight of biomass (g) of tomato plants at harvest in the rainy season of 2014 and dry season of 2015 under field conditions

Treatment	Rainy season (2014)		Dry season (2015)	
	Roma VF	UC82b	Roma VF	UC82b
M. incognita only	200.5	313.38	189.34	321
CMV only	198.8	292.5	176.9	278
M. incognita two days before CMV	308.8	432.63	291.6	423.6
CMV two days before *M. incognita*	326.88	458.25	304.76	427
M. incognita + CMV simultaneously	439.7	541.75	421	517
Uninoculated control	516	633.56	502	597
LSD. (P=0.05)	105.6	135	93.27	119

Each value is a mean of four replicates.

Table 4: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* on cumulative fruit yield of tomato plants (tons/ha) in the rainy season of 2014 and dry season of 2015 under field conditions

Treatment	Rainy season (2014)		Dry season (2015)	
	Roma VF	UC82b	Roma VF	UC82b
M. incognita only	12.07	13.24	10.44	12.81
CMV only	13.45	15.35	12.13	14.83
M. incognita two days before CMV	33.18	31.42	25	28.44
CMV two days before *M. incognita*	29.18	37.19	24.34	26
M. incognita + CMV simultaneously	42.68	43.73	27.38	37.16
Uninoculated control	47.67	50.20	40.30	46.01
LSD. (P=0.05)	8.85	11.02	11.92	10.43

Data are means of four replicates

Table 5: Effects of interaction between *Meloidogyne incognita* and *Cucumber mosaic virus* on soil *M. incognita* population densities (J_2) (number/200ml soil) in tomato plants in rainy season of 2014 and dry season of 2015 under field conditions

Treatment	Rainy season (2014)		Dry season (2015)	
	Roma VF	UC82b	Roma VF	UC82b
M. incognita only	2.54 (352)	2.61 (408)	2.65 (449)	2.59 (393)
CMV only	0.001 (0)	0.002(0)	0.002(0)	0.003(0)
M. incognita two days before CMV	2.39 (252)	2.48 (305)	2.52 (331)	2.48 (305)

CMV two days before *M. incognita*	2.35 (231)	2.45 (283)	2.45 (287)	2.45 (280)
M. incognita + CMV simultaneously	2.03 (218)	2.01 (227)	2.11 (269)	2.14(273)
Uninoculated control	0.002(0)	0.001(0)	0.001(0)	0.002(0)
LSD. (P=0.05)	0.033	0.16	0.05	0.04

Each value is a mean of four replicates.

Single inoculation of the two pathogens resulted in significant changes in symptomatology, height, biomass and fruit yields of the two tomato cultivars both in the screenhouse and the two field trials. The screenhouse experiment conducted only once and the field trials which were carried out over two planting seasons gave similar results in all the parameters measured. The screenhouse and field study showed that simultaneous inoculation of *M. incognita* and CMV on the two tomato cultivars significantly reduced the number of leaves showing symptoms consistent with the virus infection compared with plants inoculated with CMV alone from the third week of treatments application (Figures 1, 2, 5 and 6). Also, the screenhouse and the two field trials showed that simultaneous application of the two pathogens produced significantly taller plants compared with either of the single inoculation of either of the two pathogens from the second week of treatment application (Figures 3, 4, 7 and 8). This is indicative of the regulatory or inhibitory effect either of the two pathogens might be imposing over one another when applied simultaneously. This agrees with the findings of Goswami and Chenulu (1974), who reported that the numbers of local lesions on leaves of the virus infected plants in simultaneous inoculations of *Tobacco mosaic virus* (TMV) and *M. incognita* were less than in single infection by TMV. This may be explained by a negative interaction between the virus and the nematode. Also, there were significant reductions in the population densities of second-stage juvenile *M. incognita* recovered from the soil planted with the two tomato cultivars simultaneously inoculated with the two pathogens compared with the other treatments (Tables 2 and 5). The population densities of second-stage juvenile *M. incognita* was higher in single infection compared to simultaneous inoculation of *M. incognita* and CMV. This is in agreement with findings of Varshney *et al.* (2005) who reported interaction between *M. incognita* and *Mungbean yellow mosaic virus* (MbYMV).

Combined infection of *M. incognita* and CMV resulted in a higher biomass than single infection both in sreenhouse and under field conditions. This is at variance with the findings of Varshney *et al.* (2005) who reported that fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight were reduced more, when mungbean plants were inoculated with both *M. incognita* and MbYMV than with individual pathogen. Similarly, Alam *et al.* (1990) reported that combined infection of *M. incognita* at 100, 500 and 1000 J_2 per plant

and *Tomato mosaic virus* resulted in a greater reduction in tomato plant weight than single infection of *M. incognita*. Inoculation of *M. incognita* before CMV resulted in a significant reduction in fruit yield compared to the simultaneous inoculation of the two pathogens and the inoculation of CMV before *M. incognita*. This result is in agreement with the reports of Adekunle and Owa (2008), that inoculation of *M. incognita* before CMV had a greater detrimental effect on grain yield than when CMV was inoculated before *M. incognita*. The authors reported that the combined effects of the pathogen were limited on nematode-resistant cultivars of cowpea.

Conclusion

This study established that the presence of either of the two pathogens before the other does not have any significant effects on any of the parameters measured. A further study on the physiological processes on the effect of CMV on the penetration and multiplication/reproduction capability of *M. incognita* in tomato plant is necessary. Also, the effect *M. incognita* has on the multiplication and symptom initiation/induction in the cells of CMV infected tomato plant should be investigated so as to have a clear understanding of the effects either of the pathogens has on the other.

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Authors' Contributions

OOT conducted the study as a Postgraduate student, while BOO and OKA supervised it.
BOO wrote the manuscript with editorial assistance from OKA.

Conflict of Interest

The authors do not have any conflict of interest to declare.

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