

Assessment of Single and Mixed Infections of *Meloidogyne enterolobii* and *Cucumber Mosaic Virus* on the Growth and Yield of Watermelon in Kwara State, Nigeria

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ABSTRACT

Watermelon is cultivated for both economic and nutritional benefits. However, pathogenic infections, threaten the production and utilization of watermelon. Screenhouse experiments were carried out to determine the effect of single and mixed infections of *Meloidogyne enterolobii* and *Cucumber mosaic virus* (CMV) on watermelon (cv.kaolak). The experiment was set out in a completely randomized design and replicated 15 times. Treatments were applied as follows: *Cucumber mosaic virus* alone (CMV), simultaneous inoculation of cucumber mosaic virus and *Meloidogyne enterolobii* (CMV&N), inoculation of *Cucumber mosaic virus* two weeks before *Meloidogyne enterolobii* (CMV-N), inoculation of *Meloidogyne enterolobii* two weeks before *Cucumber mosaic virus* (N-CMV), *Meloidogyne enterolobii* alone (N), no inoculation (control). Data were collected on vine length, number of leaves, fruit weight, shoot weight, nematode population and root gall index. Results showed that single and mixed inoculation of both pathogens reduced watermelon growth and yield. The fruit (3.30 kg) and shoot weight (62.24 g) was significantly higher ($p \leq 0.05$) in the control than those in inoculated plants. Higher nematode population was observed in single inoculation of nematode (8100) than in mixed infections ($p \leq 0.05$). This experiment showed that single and mixed infections of *M. enterolobii* and cucumber mosaic virus caused a reduction in growth and yield of watermelon.

Keywords: *Cucumber mosaic virus*, infection, *Meloidogyne enterolobii*, watermelon, yield.

INTRODUCTION

Citrullus lanatus (Thunb.) Matsum. and Nakai (Watermelon) originated from South Africa in the Kalahari Desert region, where it was first cultivated about 5,000 years ago (Dube *et al.*, 2020; Trinklein, 2020). Today, it is produced in many countries across the world. The plant is popular for its high

water content, from where it derived the name 'watermelon'. They are cultivated for nutritional, medicinal, economic and cultural purposes. It is also a source of revenue for farmers, especially in sub-Saharan Africa where farming is a major source of income (Shehu *et al.*, 2013). The production of watermelon in Nigeria is estimated around 139,223 tonnes (Abubakar *et al.*, 2020). This is low when

compared to other African countries like Algeria and Egypt with production estimates of 1.87 and 1.7 million respectively. Nevertheless, pests and pathogens which feed and reproduce on plants have severely reduced global watermelon production. The damage from these pathogens ranges from mild symptoms on growth to complete yield loss. Pathogens like bacteria, fungi, nematodes, and viruses have been reported to be associated with watermelon worldwide, infecting different plant parts (leaf, stem, root, and fruits) and causing diseases like fruit blotch, leaf blight, anthracnose, stem blight, and root distortion. Moreso, *Meloidogyne* spp. (root-knot nematodes) and *Cucumber mosaic virus* (*Cucumovirus*) have been identified as one of the main pathogens limiting watermelon production in Nigeria (Bello *et al.*, 2020; Apalowo *et al.*, 2022).

Generally, root-knot nematode (RKN) is a root-feeding nematode with an extensive host range that infects over 2000 plant species worldwide, including horticultural crops (Subedi *et al.*, 2020). More than 100 species of RKN has been described in which *M. incognita*, *M. javanica* and *M. enterolobii* are reported as the prevalent species in Nigeria (Bello *et al.*, 2020). Similarly, *Cucumber mosaic virus* (CMV) also has the widest host range among all plant viruses. CMV is a major virus affecting cucurbits, causing characteristic mosaic symptoms on infected leaves. Both pathogens are also biotrophic and apart from the ability of the two pathogens to cause infection on their own, reports of mixed infections have been documented in many plants (Leonetti *et al.*, 2018). However, unlike Nepovirus (NEPO) and Tobravirus (TOBRA) which are vectored by nematodes directly, *Cucumber mosaic virus* cannot be transmitted directly by root-knot or other nematodes. However, there are reports of indirect interactions between root-knot nematodes and many different viruses. The reproduction and feeding of root-knot nematodes could be affected by these interactions. For instance, more galls were observed in cucumber inoculated with only nematode than those inoculated with both RKN and CMV (Youseff *et al.*, 2011). Iheukwumere *et al.* (2008), also reported that the sequence of establishment of RKN and *Soybean mosaic virus*

has an effect on the growth and yield of soybean. These observations revealed that the presence of RKN and a virus on a plant may affect its growth and yield, as well as the development of the pathogen. This study aims to evaluate the effects of single and mixed infections of root-knot nematode and CMV on the growth and yield of watermelon, as well as the impact of these interactions on nematode reproduction.

MATERIALS AND METHODS

Experimental site and soil sterilization

The study was carried out at the Screen House of the Department of Crop Protection, Faculty of Agriculture, University of Ilorin, Kwara State, Nigeria (Longitude 4°40'39" and latitude 8°29'10" N). The average temperature inside the Screen house was 32° C. Sandy-loam topsoil was collected and the soil was steam sterilized in an iron drum. Sterilized soil was allowed to cool and later transferred to 5-litre plastic pots inside the screenhouse. Some of the sterilized soil were used for the main experiment, while part was kept aside for the establishment of inoculum.

Establishment and multiplication of virus inoculum

Susceptible seeds of cowpea (Ife brown) were planted in 5-litre plastic pots containing sterilized soil. Three seeds were planted per pot and seedlings were sap-inoculated with cowpea seedlings infected with cucumber mosaic virus two weeks after germination. The infected seedlings were collected from the International Institute of Tropical Agriculture (IITA), Ibadan and identified with enzyme-linked immunosorbent assay (ELISA) to confirm the presence of the virus. This was carried out to serve as reserve and multiply the virus inoculum required for this experiment.

Root-knot nematode identification and establishment of inoculum

Heavily galled roots of *Celosia argentea* infected with *Meloidogyne* spp. were collected from a vegetable farm inside Ilorin Stadium, Kwara State.

A single egg mass was collected from the galled root with a fine needle and transferred into a Petri dish containing sterile water, and incubated for five days to allow the juveniles emerge. The juveniles were later inoculated to a susceptible tomato cv. tropimech inside a screenhouse to establish a pure culture of the nematode. The remaining juveniles in the Petri dish were used to identify the nematode as *Meloidogyne enterolobii* by applying the mitochondrial DNA-based method as described by Kolombia *et al.* (2017).

RKN juvenile was transferred to 10 µl 0.05N NaOH, and 1 µl of 4.5% Tween was added. Mixture was heated for 15 mins at 95 °C and then allowed to cool at room temperature. Afterward, 40 µl of double-distilled water was added and stored at – 18°C to be used later (Kolombia *et al.*, 2020). PCR amplification of the mitochondrial was performed in 25 µL reaction volume which is made up of sterile 12.88 µl distilled water, 5 µl of 5x colorless GoTaq buffer, 1.5 µl of MgCl₂, 0.5 µl of dNTPs, 0.5 µl each of NAD5F2(5'-TATTTTTTGTGAGATATATTAG-3') and NAD5R1 (5'-CGTGAATCTTGATTTTCATTTTT-3') commercially available primers, 0.12 µl of polymerase and 4 µl of genomic DNA template. PCR conditions include a cycle of initial denaturation at 94 °C for 5 mins, followed by 35

cycles of each cycle comprised of 30secs denaturation at 94°C, 30secs annealing of primer at 55 °C, 1.5 min extension at 72 °C and a final extension for 7 mins at 72 °C. Purified PCR products of the RKN samples were analyzed in 1.5% agarose gel. The agarose gel was placed in an electrophoretic tank containing TAE buffer pH 7.4 and 4 µl of each stock nucleic acid sample which was pre-mixed with 3 µl loading dye and loaded into the wells of gel. A 100 base pair markers (Biolab, USA) was loaded in a separate well to calibrate sample movements and to serve as a control. The samples were run at 120 volts for 40 minutes and gel visualized under ultraviolet (UV) transilluminator (EZ imager, Bio-Rad, Inc, USA) to determine the banding patterns of the RKN DNA.

Sowing of seeds and experimental design

The experiment was set out in a completely randomized design. A watermelon cv. kaolak was selected based on its availability and being popularly cultivated among farmers in the state. Four seeds were sown per pot which was later thinned to one vigorous plant after germination in each pot. Treatments were applied two weeks after germination and the list of the treatments is shown in Table 1 below.

Table 1: List of treatments applied to watermelon seedlings

S/NO	Treatments
1	Cucumber <i>mosaic virus</i> (CMV)
2	simultaneous inoculation of <i>Cucumber mosaic virus</i> and <i>M. enterolobii</i> (CMV&N)
3	Inoculation of <i>Cucumber mosaic virus</i> two weeks before <i>Meloidogyne enterolobii</i> (CMV-N)
4	Inoculation of <i>M. enterolobii</i> two weeks before cucumber mosaic virus (N-CMV)
5	<i>M. enterolobii</i> (N)
6	No inoculation (control)

Each treatment was replicated 15 times, giving a total of 90 pots for the experiment. The experiment was repeated twice in order to validate the data.

Sap extraction and inoculation of *Cucumber mosaic virus* on watermelon seedlings

From the reserved inoculums, five grams of leaves with symptoms of virus infection was ground in a sterile mortar with 15 ml of Phosphate buffer (pH 7.0). A stabilizing agent, Ethylenediaminetetraacetic acid (EDTA), was added, and the suspension was gently rubbed with hand on three leaves of the watermelon seedlings, which had been dusted with carborundum (Aliyu *et al.*, 2011).

Extraction, estimation and inoculation of *M. enterolobii* on watermelon seedling

The Eggs and juveniles were extracted using Sodium Hypochlorite (NaOCl) as described by Riekert, (1995). Infected root samples were rinsed under a gentle stream of running water and blotted with tissue paper. The cleaned roots were chopped into 1 to 3 cm. The NaOCl solution was added to chopped infected roots in a beaker and agitated for four minutes. The resulting solution after four minutes was passed through a stack of sieves 250 µm, 70 µm, 45µm and 25µm mesh. Larger roots and other debris were collected by 250 µm, 70 µm and 45 µm sieves while nematode eggs and second stage juveniles (j2) were collected by 25 µm sieve after thoroughly washing with distilled water. An aliquots/ml containing 2,000 nematode eggs and j2 was determined by counting under a stereo microscope (Coyne *et al.*, 2007). This was taken as the initial nematode population. Four holes were made around the root of each watermelon seedling and the 2,000 suspension was poured into each plant root and covered immediately with topsoil.

Data collection and statistical analysis

Data were collected on vine length, number of leaves, fruit weight, shoot weight, days to 50% flowering,

nematode population and root gall index. At the end of the experiment (week 11), the total number of second-stage juveniles that were able to penetrate the roots was taken as the final nematode population. Data collected were subjected to analysis of variance (ANOVA) using SPSS statistical package version 21, and significant means were separated using the Duncan Multiple Range Test (DMRT) at $\alpha=0.05$.

Determination of reproductive factor and gall rating

The reproductive factor was determined by using the formula, $R=P_f/P_i$. The P_f represents the final and P_i initial population of nematode. Galls on the watermelon roots were scored using Bridge and Page, 1980 method as described below;

- 0 – No knots on roots
- 1 – Few small knots, difficult to find
- 2 – Small knots only but clearly visible, main roots clean
- 3 – Some larger knots visible, main root clean
- 4 – Large, knots predominant but main root clean
- 5 – 50% of roots affected knotting on some main roots.
- 6 – Knotting on main roots
- 7 – Majority of main roots knotted
- 8 – All main roots, including tap roots, knotted. Few clean roots visible
- 9 – All roots are severely knotted.
- 10 – All roots severely knotted no root system. Plant usually dead

RESULTS

Figure 1 shows the gel electrophoresis result from the amplification of the target mitochondria DNA of *M. enterolobii* using Polymerase chain reaction (PCR). This was also verified by analysis of Gel electrophoresis with the DNA fragment forming a band at 250 bp.

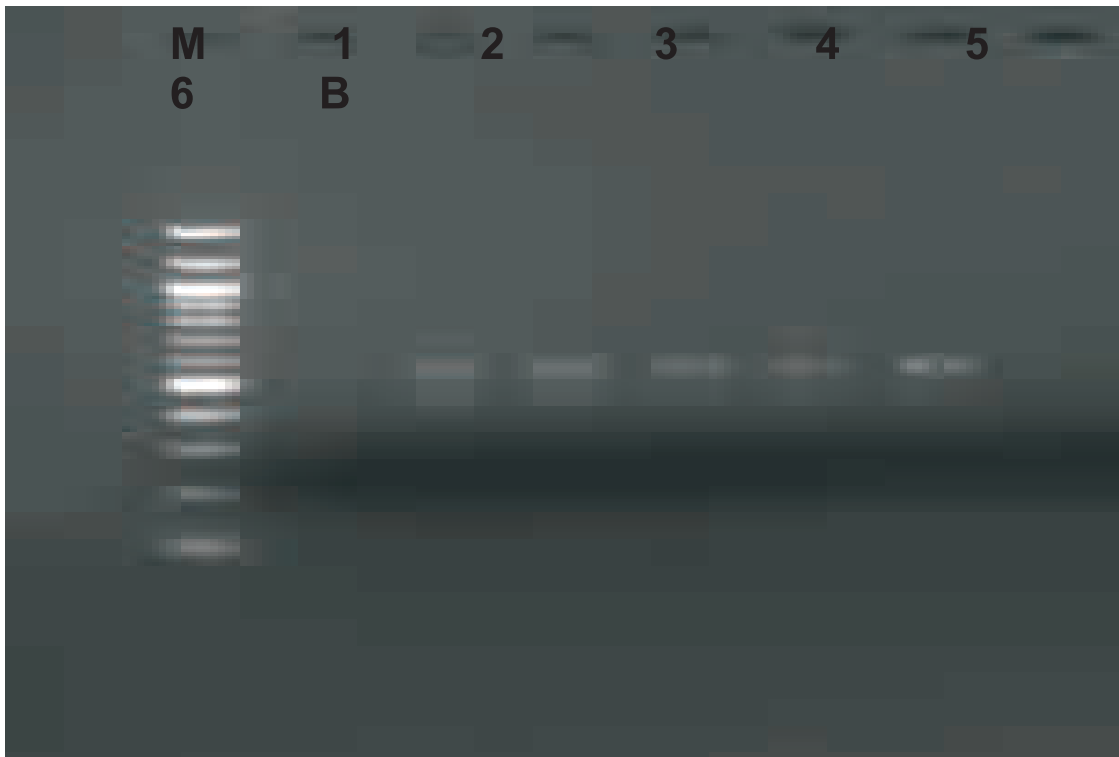


Figure 1: Gel analyses of polymerase chain reaction product targeting the mtDNA of root-knot nematode present in watermelon.

Table 2 shows the effect of inoculation of single and mixed infections of *Cucumber mosaic virus* and *M. enterolobii* on vine length of watermelon. At two weeks after planting (2 WAP), there were no significant differences between the plants inoculated with Cucumber mosaic virus, *M. enterolobii*, and the control. However, from 4 WAP to the end of the experiment, the vine length of the control was significantly higher than when inoculation was applied. Also, at 11 WAP, the vine

length of watermelon inoculated sequentially was significantly higher than when *Cucumber mosaic virus* and *M. enterolobii* were inoculated singly or simultaneously. Consequently, there were no significant differences in the vine length of single and simultaneous inoculation of Cucumber mosaic virus and *M. enterolobii*. Similar trend was observed in both first and second experiments (2018 and 2019).

Table 2: Effect of inoculation of single and mixed infections of *Cucumber mosaic virus* and *M. enterolobii* on vine length of watermelon

Treatment	Weeks After Planting									
	2	3	4	5	6	7	8	9	10	11
First experiment (2018)										
CMV	6.03 ^a	14.49 ^b	25.27 ^b	42.79 ^b	61.09 ^b	79.76 ^c	89.86 ^c	99.63 ^c	105.07 ^c	106.39 ^c
CMV&N	6.00 ^a	14.77 ^b	22.88 ^b	33.63 ^b	63.51 ^b	77.88 ^c	90.43 ^c	102.01 ^c	109.12 ^c	111.98 ^c
CMV-N	6.73 ^a	17.29 ^b	27.81 ^b	43.13 ^b	73.35 ^b	97.94 ^b	116.11 ^b	128.73 ^b	134.85 ^b	136.20 ^b
N-CMV	6.15 ^a	15.19 ^b	23.91 ^b	38.02 ^b	70.90 ^b	88.67 ^{bc}	105.82 ^b	119.01 ^b	126.41 ^b	128.83 ^b
N	6.69 ^a	15.19 ^b	22.11 ^b	36.81 ^b	63.05 ^b	78.21 ^c	87.63 ^c	97.05 ^c	104.11 ^c	107.09 ^c
Control	6.41 ^a	20.51 ^a	38.69 ^a	65.89 ^a	108.23 ^a	140.68 ^a	163.42 ^a	182.21 ^a	191.81 ^a	195.63 ^a
S.E	0.370	0.978	2.240	3.749	4.888	5.194	5.354	5.258	5.121	5.060
Second experiment (2019)										
CMV	7.24 ^a	11.20 ^b	18.39 ^b	38.26 ^{ab}	88.90 ^a	95.00 ^c	99.09 ^d	102.44 ^d	103.70 ^c	103.80 ^c
CMV&N	6.57 ^a	11.54 ^b	19.55 ^b	33.57 ^b	95.75 ^a	104.77 ^{bc}	110.42 ^{cd}	115.05 ^{cd}	117.47 ^c	117.48 ^c
CMV-N	7.89 ^a	13.16 ^{ab}	21.42 ^{ab}	40.8 ^{ab}	96.08 ^a	107.96 ^{bc}	120.97 ^{bc}	130.87 ^{bc}	134.57 ^b	134.67 ^b
N-CMV	6.57 ^{ab}	11.607 ^b	18.95 ^b	35.3 ^{ab}	93.33 ^a	113.71 ^{ab}	128.47 ^b	138.57 ^b	143.09 ^b	143.34 ^b
N	7.84 ^a	12.61 ^{ab}	20.23 ^b	36.50 ^{ab}	90.10 ^a	98.19 ^{bc}	103.4 ^c	107.72 ^d	109.62 ^c	109.74 ^c
Control	8.21 ^a	14.14 ^a	23.75 ^a	44.88 ^a	96.89 ^a	127.09 ^a	147.03 ^a	160.71 ^a	164.13 ^a	164.25 ^a
S.E	0.502	0.734	1.268	3.420	6.167	5.348	5.913	5.862	5.864	5.834

Means within a column followed by the same letter(s) are not significantly different $P=0.05$; S.E= Standard Error. Mean separated using DMRT. WAP= weeks after planting.

C=*Cucumber mosaic virus* alone, C+N= *Cucumber mosaic virus* and *M. enterolobii*, C-N=*Cucumber mosaic virus* before *M. enterolobii*, N-C=*M. enterolobii* before *Cucumber mosaic virus*, N=*M. enterolobii* alone

The effect of single and mixed infections of *Cucumber mosaic virus* and *M. enterolobii* on number of leaves produced by watermelon (cv. kaolak) is shown in Table 3. Results showed there were no significant differences in all the treatments at 2 to 4 WAP. Whereas, the control had a significantly higher number of leaves than other treatments at 5 to 11 WAP. At 11 WAP, the control had value of

45.67 which was the highest number of leaves. On the contrary, all the watermelon inoculated with *Cucumber mosaic virus* and *M. enterolobii* had no significant differences in number of leaves throughout the duration of the experiment (2 WAP to 11 WAP). Similar trend was observed during the second experiment.

Table 3: Effect of of single and mixed infections of *Cucumber mosaic virus* and *M. enterolobii* on number of leaves of watermelon (cv. kaolak)

Treatments	Weeks After Planting									
	2	3	4	5	6	7	8	9	10	11
First experiment (2018)										
CMV	2.93 ^a	6.27 ^{ab}	9.13 ^a	11.40 ^{bc}	21.07 ^b	23.33 ^b	25.73 ^b	27.73 ^b	29.40 ^b	30.00 ^b
CMV&N	3.00 ^a	5.87 ^{ab}	7.73 ^a	10.47 ^{bc}	20.33 ^b	22.93 ^b	25.13 ^b	27.53 ^b	28.80 ^b	29.73 ^b
CMV-N	3.40 ^a	6.27 ^{ab}	8.87 ^a	12.13 ^b	21.47 ^b	24.67 ^b	27.67 ^b	30.13 ^b	31.53 ^b	32.53 ^b
N-CMV	3.33 ^a	5.40 ^b	7.73 ^a	9.80 ^c	18.40 ^b	21.67 ^b	24.93 ^b	28.60 ^b	31.27 ^b	32.07 ^b
N	3.07 ^a	6.33 ^{ab}	8.60 ^a	11.27 ^{bc}	20.27 ^b	21.93 ^b	24.60 ^b	26.40 ^b	27.80 ^b	28.13 ^b
Control	3.40 ^a	6.93 ^a	9.33 ^a	14.00 ^a	28.48 ^a	33.73 ^a	38.33 ^a	41.93 ^a	44.33 ^a	45.67 ^a
S.E	0.208	0.385	0.505	0.659	1.37	1.182	1.206	1.227	1.253	1.267
Second experiment (2019)										
CMV	3.33 ^a	5.53 ^a	8.40 ^{ab}	11.13 ^{bc}	16.27 ^b	20.20 ^c	22.73 ^d	24.80 ^d	26.40 ^e	27.73 ^c
CMV&N	3.20 ^a	5.47 ^a	7.80 ^b	10.60 ^c	17.80 ^b	20.93 ^c	23.80 ^{cd}	25.60 ^d	27.60 ^e	28.60 ^c
CMV-N	3.47 ^a	5.87 ^a	10.67 ^a	14.60 ^{ab}	20.73 ^b	25.33 ^b	28.47 ^b	30.80 ^b	33.40 ^b	34.00 ^b
N-CMV	3.73 ^a	5.67 ^a	9.20 ^{ab}	12.47 ^{bc}	19.47 ^b	24.20 ^{bc}	27.00 ^{bc}	29.93 ^{bc}	32.80 ^b	33.60 ^b
N	3.53 ^a	6.33 ^a	10.20 ^{ab}	13.80 ^{abc}	19.27 ^b	21.73 ^{bc}	24.80 ^{cd}	27.40 ^{cd}	29.27 ^c	29.67 ^c
Control	3.67 ^a	7.00 ^a	11.00 ^a	16.60 ^a	26.07 ^a	32.07 ^a	37.67 ^a	41.80 ^a	44.73 ^a	46.53 ^a
S.E	0.293	0.570	0.828	1.147	1.434	1.379	1.217	1.138	1.151	1.229

Table 4 reveals the effect of treatments on the fruit and shoot weights of watermelon. In the year 2018 (first experiment), the control (73.02 g) performed significantly better than other treatments in respect to fruit weight. Also, N-C (25.92 g) performed significantly better than C-N (5.62 g) and N (9.74 g). Furthermore, C-N performed significantly better than C+N (5.62 g), while there were no significant differences between C, C+N, C-N, N-C and N.

The shoot weight of Control (62.24 g) was also significantly higher than other treatments. Moreover, C-N (32.54 g) and N-C (38.18 g) were also significantly higher than C (21.44 g), C+N (18.96 g) and N (20.12 g). Meanwhile, C, C+N and N were not significantly better than each other.

In 2019, Control (68.38 g) performed significantly better than other treatments in respect to fruit weight. Meanwhile, there were no significant differences in the performance of treatments C (13.52 g), C+N (9.88 g), C-N (19.66 g), N-C (21.92 g) and N (10.4 g).

The shoot weight of Control (64.24 g) was significantly higher than other treatments. Although, N-C (28.56 g) was not significantly higher than treatments C-N (25.38 g) and N (22.46 g), however, it was significantly better than treatments C (21.14 g) and C+N (19.78 g). In the meantime, C, C+N, C+N and N were not significantly different from each other.

Table 4: Effect of treatments on fruit and shoot weights of watermelon

Treatment	First experiment (2018)		Second experiment (2019)	
	Fruit weight (kg)	Shoot weight (g)	Fruit weight (kg)	Shoot weight (g)
CMV	0.84 ^{bcd}	21.44 ^c	1.08 ^b	21.14 ^c
CMV&N	0.51 ^d	18.96 ^c	0.99 ^b	19.78 ^c
CMV-N	1.00 ^{bc}	32.54 ^b	1.10 ^b	25.38 ^{bc}
N-CMV	1.24 ^b	38.18 ^b	1.05 ^b	28.56 ^b
N	0.71 ^{cd}	20.12 ^c	1.00 ^b	22.46 ^{bc}
Control	3.30 ^a	62.24 ^a	3.64 ^a	64.24 ^a
S.E	4.963	3.133	3.93	2.191

Table 5 shows the initial nematode population, final nematode population, reproductive rate and gall rating of watermelon (cv. kaolak) infected with single and mixed inoculations of *Cucumber mosaic virus* and *M. enterolobii*. In the experiment, the final population of *M. enterolobii* recovered from single inoculation of *M. enterolobii* (8100 nematodes) was significantly ($p \leq 0.05$) higher than the population of simultaneous inoculation of CMV and *M. enterolobii* (5500 nematodes), CMV two weeks before *M. enterolobii* (7200 nematodes) and *M. enterolobii* two weeks before CMV (7700 nematodes). The *M. enterolobii* recovered from sequential inoculation of CMV two weeks before *M. enterolobii* was not significantly higher than *M. enterolobii* two weeks before CMV but significantly higher than simultaneous inoculation of CMV and *M. enterolobii*. Consequently, simultaneous inoculation

of CMV and *M. enterolobii* had the lowest final nematode population (5500).

Results also showed that single inoculation of *M. enterolobii* (4.05) had the highest reproductive rate. This was followed by the inoculation of *M. enterolobii* two weeks before CMV (3.85), and CMV two weeks before *M. enterolobii* (3.60) respectively. The simultaneous inoculation of CMV and *M. enterolobii* (2.75) had the lowest reproductive rate.

The gall rating of the infected watermelon (cv. kaolak) at harvest revealed that single inoculation of *M. enterolobii* (7) had the highest rating while simultaneous inoculation of CMV and *M. enterolobii*, CMV two weeks before *M. enterolobii*, and *M. enterolobii* two weeks before CMV, all had a rating of 6. Similar trend was observed in 2019 experiment.

Table 5: Effect of treatments on initial and final nematode population, reproductive rate and root gall index of pots inoculated with root-knot nematode

Treatment	Initial population	Final population	Reproductive rate	Gall rating
CMV&N	2000	5500.00 ^a	2.75	6
CMV-N	2000	7200.00 ^c	3.6	6
N-CMV	2000	7700.00 ^{bc}	3.85	6
N	2000	8100.00 ^d	4.05	7
SE		299.779		

Plates 1 and 2 revealed the effect of inoculation and uninoculation of *Cucumber mosaic virus* and *M. enterolobii* on the shape of watermelon fruit. The seedling inoculated was deformed (Plate 1), while the control had a normal round shape.



Plate 1: Deformed watermelon fruit infected with CMV and RKN



Plate 2: Uninfected watermelon fruit showing no sign of deformation

DISCUSSION

Effect of inoculations on growth and yield of watermelon

The study shows there were disparities in response of watermelon (cv. kaolak) to *Cucumber mosaic virus* and *Meloidogyne enterolobii*, in single and mixed infections. The disparity could be attributed to differences in the way watermelon responded to the invasion of the two different pathogens as well as the sequence of establishment of each pathogen. Generally, the growth and yield of the control were notably better than other treatments in both single and mixed infections during the two trials. This was possible as a result of non-interference of the phytopathogenic agents in the tissues of the plant. *Cucumber mosaic virus* and *Meloidogyne enterolobii* have been reported to be parasitic to plants, interfering in the cell metabolic process and ultimately reducing nutrient availability in plants and plant parts as a result of their parasitic activities (Escobar *et al.*, 2015). Similar study by Ahmed *et al.* (2021), confirmed that the presence of root-knot nematode and *Zucchini yellow mosaic virus* caused accelerated reduction in the growth and yield of different cucumber genotypes.

Effect of mixed infections on growth and yield of watermelon

The plant growth and yield were more reduced in simultaneous inoculation of both pathogens (C and N) than when one pathogen was established before the other, which revealed there was a positive synergistic effect between both pathogens that is detrimental to watermelon. This could also be the cause of fruit deformation in the early fruiting stage of the plant (Plates 1 and 2). Perhaps, this was the reason yields of plants treated simultaneously with virus and nematode (C+N) have the lowest fruit yield (0.51 kg) among all the treatments tested. It was also observed that sequential inoculation of the pathogens was not as effective in suppressing plant growth when compared with simultaneous inoculation. However, all pathogen treatments caused a considerable reduction in plant growth and yield. Although, there is not much evidence to support a direct relationship between CMV and *Meloidogyne enterolobii* in plants, however, it could be reasonably inferred from this experiment that the presence of both pathogens enhanced the disease etiology of watermelon. It is not clear though if the presence of one stimulated the activity of the other, or that the presence of both at the same time caused severe stress to the plant metabolic mechanisms.

Effect of mixed infections on reproduction of *Meloidogyne enterolobii*

There was a relationship of direct proportionality between mixed infections and inhibition of nematode reproduction. A higher nematode population was observed in plants inoculated with nematode alone than in those with mixed infections. This implies there was reduction in the number of egg mass produced in mixed inoculation with CMV. This might be a result of the reduction in the nutrients available for the nematodes in the root cells of virus-infected plants. Youssef *et al.* (2011) suggested that the presence of Cucumber mosaic virus in cucumber induces changes in the plant physiology that suppress nematode development. There is also a possibility that the virus inclusion body present reduced useful nutrients that would have been available for the nematodes (Koenning and McClure, 1981). This study contrasts with the observation of Adekunle and Owa (2008), where they recovered more nematodes from cowpea with mixed infections of aphid-borne mosaic virus and *Meloidogyne incognita* compared to cowpea plants where only *Meloidogyne incognita* was inoculated. This experiment is also at variance with the observation of Alam *et al.* (1990), that inoculation of *Meloidogyne incognita* before the Tomato mosaic virus had a damaging effect on growth and yield of tomato. In this case, there was no significant difference in the sequence of inoculation between the two pathogens. It should however, be noted that the difference in viruses tested, the type of crop and experimental conditions by the authors mentioned above were not exactly the same as this experiment which could have influenced the experimental outcome. Also, this experiment did not take into account environmental data like humidity and soil temperature which could have influence the nematode reproduction and disease development. Notwithstanding, results from this experiment showed that the performance of both pathogens was affected by the presence of the other and the presence of both had a deleterious effect on watermelon variety tested. Going forward, there is a need for further experiments on the field to see if these effects hold under natural conditions.

CONCLUSION

The screenhouse experiment established that both Cucumber mosaic virus and *Meloidogyne enterolobii* have a damaging effect on the growth and yield of watermelons. Simultaneous inoculation of both pathogens (CMV+N) also had a more severe effect on watermelon than single or sequential inoculation. The study also revealed that the presence of CMV in watermelon caused a reduction in the reproduction of nematodes. There is a need to carry out more studies on the mechanisms that make the presence of CMV inhibit reproduction in nematodes and explore the possibility of using particles of the virus as biological control.

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