INTERACTION EFFECTS OF *COWPEA MILD MOTTLE VIRUS* (CPMMV) AND ROOT-KNOT NEMATODE INFECTIONS ON GROWTH AND YIELD OF COWPEA (*Vigna unguiculata* L. Walp.)

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ABSTRACT

In order to ascertain the interactions between Cowpea mild mottle virus (CPMMV) and Meloidogyne incognita, screenhouse and field experiments were conducted in the early and late rainy seasons of 2014 in the tropical rainforest zone of Nigeria. The following treatments were applied 12 days after planting seeds of Ife-Brown and Ife-BPC cultivars of cowpea in the screenhouse and field on nematode-free soil: inoculation of plant roots with 5,000 eggs of M. incognita per plant; inoculation of primary leaves with crude CPMMV sap; inoculation of plant roots with 5,000 eggs of M. incognita two days before inoculating with CPMMV sap; inoculation of primary leaves with crude CPMMV sap two days before inoculating plant roots with 5,000 eggs of M. incognita; simultaneous inoculation of primary leaves with crude CPMMV sap and roots with 5,000 eggs of M. incognita; and uninoculated control. Treatments were replicated four times, and arranged in a 2 x 6 factorial using randomized complete block design. Inoculation of M. incognita before CPMMV inhibited the virus infection by reducing the number of leaves showing symptoms from 12 to 5, and 10 to 7 in the fifth week of inoculation in Ife Brown and Ife BPC respectively under screenhouse conditions; under screenhouse and field conditions, single and combined infections recorded no significant difference in biomass; under screenhouse conditions simultaneous inoculation of M. incognita and CPMMV recorded the nematode population; 2.39 (249) while those of M. incognita only that recorded 1.91 (83) in Ife-Brown. In Ife-BPC, M. incognita and CPMMV recorded 2.27 (188) while M. incognita only recorded 1.96 (90). The study concluded that combined infection was more damaging than either of the single infections, and either of the pathogens could have a suppressive effect on the other depending on the time of their establishment.

Keywords: Cowpea, Cowpea mild mottle virus, Meloidogyne incognita, Growth, Yield

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) belongs to the family Fabaceae. It is one of the most important food grain legumes in the tropics (Timko *et al*., 2008). According to Abate *et al*. (2011), about 7.56 million tonnes of cowpea are produced worldwide annually on about 12.76 million ha. in sub-Saharan Africa (SSA). Of the 20 million ha of land on which grain legumes were grown in 2006-08, 54% of the area harvested was under cowpea (Akibode, 2011). Nigeria, apart from being largest cowpea producer in the world, also records the highest level of consumption (Singh, 2007).

Viral diseases are recognized major constraints to cowpea production, and they reduce yield most drastically in tropical regions such as Africa, Asia and Latin America (Thottappilly and Rossel, 1992). *Cowpea mild mottle virus* (CPMMV) was first reported on cowpea in Ghana (Brunt and Kenten, 1973). Subsequently, it was reported from several tropical regions of Africa (Anno-Nyako, 1984), Asia (Reddy, 1991), Brazil and Argentina (Laguna *et al.*, 2006) and from Ivory Coast in a diverse range of plant species that include leguminous and Solanaceous food crops (Hartman *et al.*, 1999). *Cowpea mild mottle virus* can reduce yields of some cowpea varieties by 10 –15 % and, in mixed infections, could exacerbate the deleterious effects of other viruses and pathogens (Brunt and Kenten, 1973).

Root-knot nematode is a major problem in cowpea fields in most parts of the world (Trudgill and Blok, 2001). Iheukwumere *et al*. (1995) reported *Meloidogyne* spp. as one of the plant-parasitic nematodes of economic importance in legume production in Nigeria. When plants are infected with root-knot nematodes, the feeding activities of this parasite in the root tissues results in the formation of massive galls of different sizes on root system (Rahman, 2003). Severely affected plants can often wilt readily because galled roots have limited ability to absorb and transport water and nutrients to the rest parts of the plant (Coyne *et al.*, 2007). It is well known that *Meloidogyne* spp. are not vectors of plant viruses, but, under field conditions, they occur concomitantly with viruses (Youseff *et al.*, 2011). Root-knot nematodes can also 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 (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). *Cowpea mild mottle virus* is one of the most damaging viruses affecting cowpea production (Thottappily and Rossel, 1992), and when it is involved in a complex infection with another pathogen, it could be more destructive (Brunt and Kenten, 1973).

Nematode interactions with viruses have been reported in some crops. For instance, it was observed that *Meloidogyne* spp. occur concurrently with viruses on cucurbits (Ozaslan *et al*., 2006). Varshney *et al.* (2005) and Ahmed *et al.* (2007) observed that more root-knot nematodes were recorded in mung bean plants inoculated only with root-knot nematode than in those inoculated with both root-knot nematode and *Mung bean yellow mosaic virus*. However, the relationship between *M. incognita* and CPMMV including the effect of the timing of the infection of individual pathogen in a combined infection has not been well reported in the literature. Hence the need to investigate and compare effects of single and combined infections of CPMMV and *M. incognita* on the growth and yield of cowpea.

MATERIALS AND METHODS

Experimental site

A screenhouse and two field trials were conducted in the early and late rainy reason of 2014 on the Teaching and Research Farm of 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 2cm pieces infected roots were placed in the laboratory trays. Eggs were extracted from the galled roots by chlorox method (Southey, 1986). The eggs in a counting dish were counted under a stereomicroscope (x 250). A standard CPMMV isolate maintained on soybean (*Glycine max*) cultivar TGx 1448-2E was obtained from Germplasm Health laboratory, Genetic Resource Unit (GRU) of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and homogenized in 0.01M, pH 7.7 phosphate buffer in the ratio 1:10 (w/v) as the virus inoculum source.

Screenhouse study

Forty-eight 150 mm diameter, perforated plastic pots were filled with hot air-sterilized sandy-loam top soil and were arranged on screenhouse benches. Two seeds each of *V. unguiculata* cultivars, Ife-Brown and Ife-BPC collected from the seed bank of Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria, were sown into each pot in the screenhouse, and seedlings were thinned to one stand, six days after emergence. The two cowpea cultivars are susceptible to *M. incognita* and CPMMV. Five thousand eggs of *M. incognita* were placed in a depression around the roots of each cowpea seedling 12 days after germination and covered with soil (Adekunle and Akinsanmi, 2003). The CPMMV inoculum source was inoculated onto young, tender leaves of cowpea plants with an absorbent cotton swab on carborundum-dusted leaves. The following treatments were applied two weeks after planting; inoculation of roots of each cowpea seedling with 5,000 eggs of *M. incognita*; mechanical inoculation of primary leaves of each cowpea seedling with crude CPMMV sap; inoculation of roots of a cowpea seedling with 5,000 eggs of *M. incognita* two days before inoculating primary leaves with CPMMV sap; inoculation of primary leaves of a cowpea seedling with crude CPMMV sap two days before inoculating plant roots with 5,000 eggs of *M. incognita*; simultaneous inoculation of primary leaves of cowpea seedlings with crude CPMMV sap; inoculating of plant roots with 5,000 eggs of *M. incognita*; and uninoculated control. Treatments were replicated four times for each cultivar, and arranged in a 2 x 6 factorial in randomized complete block design (RCBD). Twenty-four pots per cultivar were spaced 30 cm x 60 cm. The cowpea plants were watered every other day. The plants were observed one week after inoculation and every week thereafter for the number of leaves showing CPMMV symptoms for five weeks. Harvesting of mature pods per pot commenced eight weeks after planting and this continued at weekly intervals till all the pods were harvested. Pods were threshed and the grains were weighed. Data were also collected on biomass at harvest. The experiment was terminated after all the pods were harvested 90 days after planting. 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), 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 early (May to July) and late (August to November) rainy seasons of 2014. In the first trial, a land area of 22.4 m x 15.8 m was ploughed and harrowed and laid out in Randomized Complete Block with 2 x 6 factorial arrangements. Four blocks, each 15.8 m x 1.8 m, were marked out for each cultivar of cowpea. Each block was divided into six, 1.8 m x 1.8 m plots with a space of 1 m between plots, and 1 m between replicates. Seeds of Ife-Brown and Ife-BPC cultivars of cowpea (which were moderately susceptible to both pathogens) were sown at a spacing of 30 cm x 60 cm at the rate of one seed per hole. There were 30 plants per plot, or 180 plants per block, giving 720 plants per cultivar in the experimental field. Out of 30 plants in each plot, 10 plants in the inner rows were selected, inoculated and tagged for data collection, while the outer rows served as guard plants.

Six treatments were applied in these trials in Ife-Brown and Ife-BPC similar to those of the screenhouse study. Fresh eggs of *M. incognita* were placed in a depression around the roots of each cowpea seedlings 12 days after germination and covered with soil. Young leaves of CPMMV-infected soybean plants were used as CPMMV inoculum source. The treatments were applied 12 days after germination similar to those of screenhouse trial. Insects were controlled with profenofos 40% and cypermethrin 4% at the rate of 0.2 kg a.i. per hectare, three weeks after planting and weekly thereafter. Weeds were manually controlled from the third week of planting and every two weeks thereafter with a hoe. Harvesting of mature pods in each plot commenced eight weeks after planting and this continued at weekly intervals till all the pods were harvested. Pods were threshed, while, grain yield per plot was recorded. The experiment was terminated after all the pods were fully harvested, 90 days after planting. Data were collected on plant biomass per plot at harvest. 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 late rainy season trial was conducted in the month of August. A separate field from the first trial was used for the second field experiment. A land area of 22.4 m x 15.8 m was cleared, ploughed and harrowed. This trial was carried out following the procedure used in the early 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

Results presented in Figure 1 shows that after the first week of inoculation, the number of leaves showing CPMMV symptoms recorded in plants inoculated with *M. incognita* before CPMMV was significantly lower than what was recorded in other plants. In the second and fifth week after inoculation, number of leaves showing viral symptoms recorded in plants inoculated with *M. incognita* before CPMMV was significantly lower ($P \leq 0.05$) than what was recorded in other plants. In the third and fourth week after inoculation, number of leaves showing viral symptoms recorded in plants inoculated with *M. incognita* before CPMMV was significantly lower ($P \leq 0.05$) than what was recorded in other plants. Figure 2 shows that in the first and fifth week after inoculation, the number of leaves showing CPMMV symptoms recorded in plants inoculated with *M. incognita* before CPMMV was lower than that in other treatments. In the second, third and fourth week after inoculation, the number of leaves showing viral symptoms recorded in plants inoculated CPMMV alone was significantly higher ($P \leq 0.05$) than what was recorded in plants inoculated with *M. incognita* before CPMMV. In Ife-Brown and Ife-BPC, the single and combined infections recorded biomasses that were not significantly different from each other.

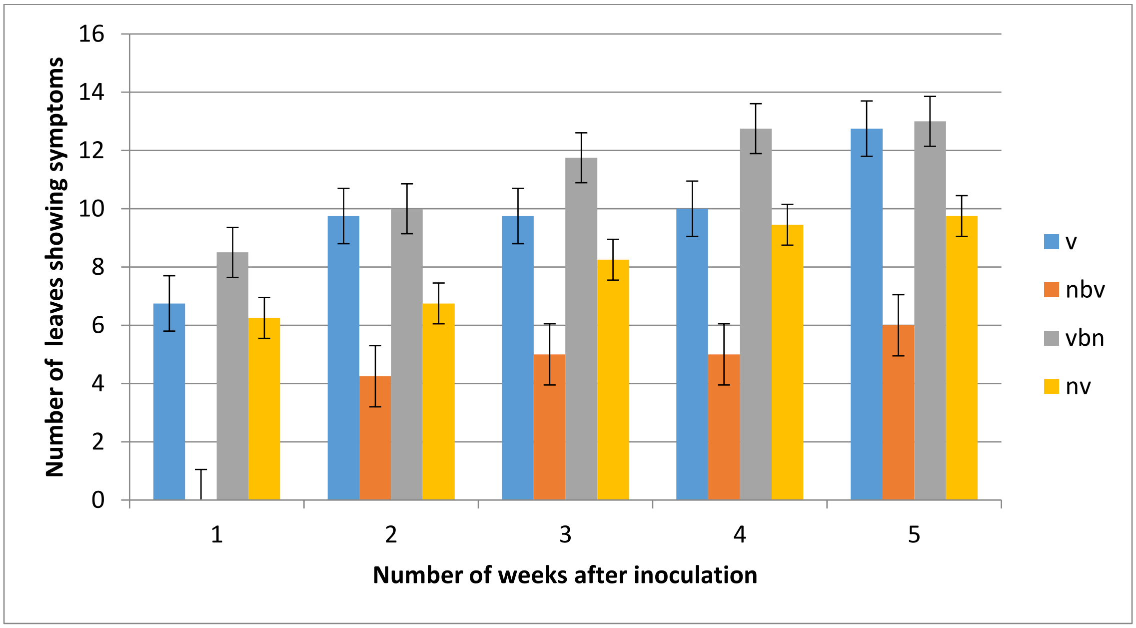

Figure 1. Effects of *Meloidogyne incognita* on the rate of spread of *Cowpea mild mottle virus* infection in cowpea cv. Ife-Brown

v= CPMMV only
nbv= *M. incognita* before CPMMV
vbn= CPMMV before *M. incognita*
nv= *M. incognita* + CPMMV simultaneously

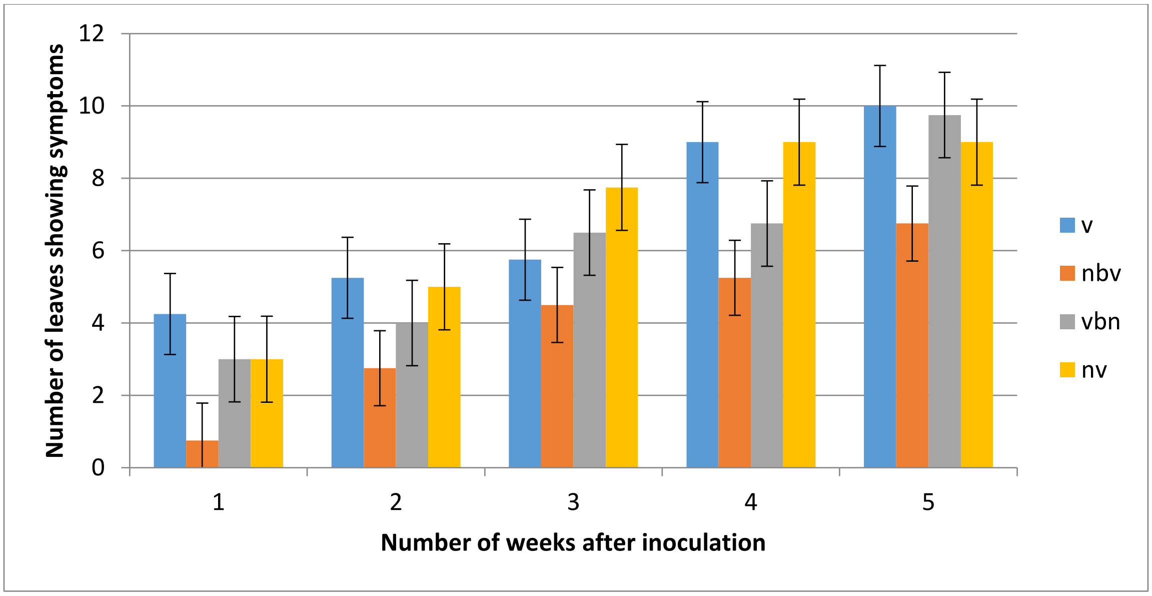

Figure 2. Effects of *Meloidogyne incognita* on the rate of spread of *Cowpea mild mottle virus* infection in cowpea cv. Ife-BPC

v= CPMMV only
nbv= *M. incognita* before CPMMV
vbn= CPMMV before *M. incognita*
nv= *M. incognita* + CPMMV simultaneously

Under screenhouse conditions, Ife-Brown plants inoculated with *M. incognita* before CPMMV, CPMMV before *M. incognita*, and *M. incognita* and CPMMV simultaneously recorded nematode population densities that were significantly higher ($P \leq 0.05$) than what was recorded in plants inoculated with *M. incognita* alone (Table 1). Similarly, in Ife-BPC, the plants inoculated with *M. incognita* alone recorded nematode population density that was significantly lower ($P \leq 0.05$) than what was recorded in combined infections. Results presented in Table 2 show that under screenhouse conditions, the biomasses recorded in Ife-Brown plants inoculated with *M. incognita* alone, *M. incognita* before CPMMV, and *M. incognita* and CPMMV simultaneously were significantly lower ($P \leq 0.05$) than what was recorded in other plants. In Ife-BPC, all inoculated plants recorded biomasses that were not significantly different. Under screenhouse conditions, in Ife-Brown, the grain yield recorded in the plants inoculated with *M. incognita* alone, *M. incognita* before CPMMV, and *M. incognita* and CPMMV simultaneously were significantly lower than what was recorded in other plants (Table 2).

TABLE 1. EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON SOIL NEMATODE POPULATION (J_2) (NUMBER/200ML SOIL) OF IFE-BROWN AND IFE-BPC CULTIVARS OF COWPEA PLANTS AT HARVEST UNDER SCREENHOUSE CONDITIONS

Treatments	**Ife-Brown**	**Ife-BPC**	**LSD (P= 0.05)**
M. incognita only	1.91 (83)	1.96 (90)	**0.16**
CPMMV only	0.00	0.00	**0.00**
M. incognita before CPMMV only	2.19 (182)	2.08 (119)	**0.29**
CPMMV before *M. incognita*	2.16 (153)	2.10 (127)	**0.23**
M. incognita + CPMMV simultaneously	2.39 (249)	2.27 (188)	**0.14**
Uninoculated control	0.00	0.00	**0.00**
LSD (P= 0.05)	**0.24**	**0.09**	

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 while figures outside parenthesis are means of transformed values

TABLE 2. EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON BIOMASS (G) AND GRAIN WEIGHT (G) OF IFE-BROWN AND IFE-BPC CULTIVARS OF COWPEA PLANTS AT HARVEST UNDER SCREENHOUSE CONDITIONS

Treatments	**Biomass (g)**			**Grain weight (g)**		
	Ife-Brown	**Ife-BPC**	**LSD (P= 0.05)**	**Ife-Brown**	**Ife-BPC**	**LSD (P= 0.05)**
M. incognita only	36.9	35.4	**18.3**	10.9	8.9	**4.7**
CPMMV only	69.8	50.0	**28.7**	22.0	20.0	**9.9**
M. incognita before CPMMV only	40.1	42.6	**18.9**	12.0	9.5	**5.2**
CPMMV before *M. incognita*	70.6	43.8	**16.0**	28.8	17.0	**10.8**
M. incognita + CPMMV simultaneously	36.3	44.3	**22.1**	7.7	7.5	**6.5**
Uninoculated control	68.9	70.4	**12.6**	36.9	25	**14.2**
LSD (P= 0.05)	**16.8**	**17.1**		**8.8**	**7.0**	

Each value is a mean of four replicates

Plants inoculated with *M. incognita* and CPMMV simultaneously, and *M. incognita* before CPMMV recorded yields that were not significantly different but these were significantly lower ($P \leq 0.05$) than that in plants inoculated with CPMMV only. For Ife-BPC, plants inoculated with *M. incognita* and CPMMV simultaneously, and *M. incognita* before CPMMV recorded a significantly lower yield than what was recorded in plants inoculated with CPMMV only.

In the early rainy season, the nematode population densities recorded in single and combined inoculations for Ife-Brown and Ife-BPC were not significantly different in the early rainy season (Table 3). In contrast, during the late rainy season, Ife-Brown plants inoculated with *M. incognita* and CPMMV simultaneously recorded *M. incognita* population densities that were significantly higher (P ≤ 0.05) than what was recorded for plants inoculated with *M. incognita* only. Ife-Brown recorded a significantly higher nematode population density with inoculation of CPMMV before *M. incognita* in comparison to Ife-BPC (Table 3). In both seasons, for both cultivars of cowpea, all inoculated plants recorded biomasses that were not significantly different, but were significantly lower (P ≤ 0.05) than biomasses recorded in uninoculated plants (Table 4).

Results presented in Table 5 show that in the early rainy season, in Ife-Brown, inoculation of CPMMV before *M. incognita* produced grain yield that was significantly lower (P ≤ 0.05) than that in plants inoculated with *M. incognita* only; *M. incognita* before CPMMV; and simultaneous inoculation of *M. incognita* and CPMMV while inoculation with CPMMV only produced grain yield that was significantly lower (P ≤ 0.05) than that in plants inoculated with *M. incognita* only and plants inoculated with *M. incognita* and CPMMV simultaneously. In Ife-BPC, inoculation of CPMMV before *M. incognita* produced yield that was significantly lower (P ≤ 0.05) than that in plants inoculated with *M. incognita* and CPMMV simultaneously, but was not significantly different from yield in other inoculated plants. In the late rainy season, in Ife-Brown, the grain yield recorded in plants inoculated with *M. incognita* only; and CPMMV only were significantly lower (P ≤ 0.05) than what was recorded in plants inoculated with *M. incognita* before CPMMV. In Ife-BPC, all inoculated plants recorded grain yields that were not significantly different.

TABLE 3. EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON SOIL NEMATODE POPULATION (J_2) (NUMBER/200ML SOIL) IN COWPEA PLANTS IN EARLY AND LATE RAINY SEASONS OF 2014 UNDER FIELD CONDITIONS

	Early rainy season			**Late rainy season**		
	Cowpea cultivar			**Cowpea cultivar**		
Treatments	**Ife-Brown**	**Ife-BPC**	**LSD (P= 0.05)**	**Ife-Brown**	**Ife-BPC**	**LSD (P= 0.05)**
M. incognita only	2.25 (178)	2.15 (145)	**0.20**	2.17 (156)	2.22 (172)	**0.24**
CPMMV only	0.00	0.00	**0.00**	0.00	0.00	**0.00**
M. incognita before CPMMV only	2.05 (110)	1.96 (97)	**0.23**	2.32 (211)	2.24 (174)	**0.11**
CPMMV before *M. incognita*	2.14 (142)	2.15 (145)	**0.22**	2.31 (218)	1.95 (98)	**0.34**
M. incognita + CPMMV simultaneously	2.45 (339)	2.31 (206)	**0.35**	2.40 (256)	2.35 (226)	**0.15**
Uninoculated control	0.00	0.00	**0.00**	0.00	0.00	**0.00**
LSD (P=0.05)	**0.24**	**0.24**		**0.22**	**0.21**	

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 while figures outside parenthesis are means of transformed values

TABLE 4. EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON BIOMASS OF COWPEA PLANTS (TONS/HA) UNDER FIELD CONDITIONS IN EARLY RAINY AND LATE RAINY SEASONS OF 2014 UNDER FIELD CONDITIONS

	Early rainy season			Late rainy season		
	Cowpea cultivar			Cowpea cultivar		
Treatments	**Ife-Brown**	**Ife-BPC**	**LSD (P= 0.05)**	**Ife-Brown**	**Ife-BPC**	**LSD (P= 0.05)**
M. incognita only	13.2	10.9	**9.8**	11.5	11.3	**5.2**
CPMMV only	14.9	11.2	**6.7**	12.3	11.8	**4.6**
M. incognita before CPMMV only	11.9	10.5	**5.8**	13.3	10.5	**4.5**
CPMMV before *M. incognita*	15.0	7.81	**10.0**	11.6	10.1	**3.9**
M. incognita + CPMMV simultaneously	14.3	10.5	**5.3**	11.3	9.5	**3.2**
Uninoculated control	20.5	17.6	**11.6**	19.9	15.4	**2.0**
LSD (P=0.05)	**4.0**	**4.1**		**3.8**	**2.8**	

Each value is a mean of four replicates.

TABLE 5. EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON GRAIN YIELD OF COWPEA PLANTS (TONS/HA) IN EARLY AND LATE RAINY SEASONS OF 2014 UNDER FIELD CONDITIONS

	Early rainy season			Late rainy season		
	Cowpea cultivar			Cowpea cultivar		
Treatments	**Ife-Brown**	**Ife-BPC**	**LSD (P= 0.05)**	**Ife-Brown**	**Ife-BPC**	**LSD (P= 0.05)**
M. incognita only	1.38	1.60	**0.94**	0.76	0.91	**0.46**
CPMMV only	0.62	1.42	**0.42**	0.67	1.92	**0.47**
M. incognita before CPMMV only	1.03	1.45	**0.99**	1.34	1.39	**1.28**
CPMMV before *M. incognita*	0.53	0.94	**0.62**	0.59	1.04	**0.69**
M. incognita + CPMMV simultaneously	1.16	1.99	**1.27**	0.70	1.35	**1.07**
Uninoculated control	1.19	3.32	**1.17**	2.04	3.84	**1.54**
LSD (P=0.05)	**0.48**	**0.97**		**0.47**	**1.01**	

Each value is a mean of four replicates

Ife-Brown recorded a significantly lower ($P \leq 0.05$) yield with CPMMV alone inoculation in comparison to Ife-BPC in both seasons.

DISCUSSION

Inoculation of *M. incognita* before CPMMV reduced virus infection under screenhouse conditions. This agrees with the findings of Alam *et al.* (1990) who reported that the dominance of *M. incognita* and *Tomato mosaic virus* (TMV) over each other in tomato plant was dependent on the time of establishment of each pathogen. This finding is however at variance with those of Iheukwumere *et al.* (2008) who reported that the combined infections in which *Soybean mosaic virus* inoculation preceded *M. incognita* (10,000 J_2 per plant) by 7 days in soybean cultivar TGm 80 caused significant inhibition of nematode growth and development. In the screenhouse study simultaneous infection recorded higher nematode population densities than single infection. This indicated that *M. incognita* reproduced more readily in the presence of CPMMV. This finding agrees with those of Adekunle and Owa (2008) who reported that the interaction of *Cowpea aphid-borne virus* and *M. incognita* at inoculum level of 5,000 J_2 per cowpea plant resulted in a significantly higher population density of the nematode at harvest in comparison to inoculation of either pathogen alone. This finding is also at variance with that of Youssef *et al.* (2011) who reported that number of galls, and egg masses of *M. incognita* inoculated at inoculum levels of 100 and 1000 J_2 per plant were greater on the roots of cucumber infected with the nematode alone than those of plants inoculated with both the nematode and *Cucumber mosaic virus* (CMV). In the same vein, Iheukwumere *et al*. (2008) reported that combined infection of *Soybean mosaic virus* and *M. incognita* at inoculum level of 10,000 J_2 in soybean cultivar TGm 80 significantly reduced the number of galls, egg masses and second stage juveniles of the root-knot nematode compared to the number found with single infection of the nematode. In the current study, single and combined infections of *M. incognita* and CPMMV recorded no significant difference in biomass under screenhouse and field conditions. This suggests that the nature or degree of infection did not correlate with the weight of infected cowpea plants. Alam *et al.* (1990) reported that combined infection of *M. incognita* at 100, 500 and 1000 J_2 per plant and *Tomato mosaic virus* caused a greater reduction in tomato plant weight than single infection of nematode.

Under field conditions, infection of *M. incognita* before CPMMV resulted in lower grain yield than single infection of the nematode, in Ife-Brown. This is at variance with the findings of Iheukwumere *et al*. (2009) who reported that combined inoculation of *M. javanica* at inoculum level of 500 J_2 per okra seedling and *Fusarium oxysporum* resulted in a greater reduction in yield than those of single infection with either pathogen. Husain *et al.* (1985) reported that combined inoculation of *M. incognita* at inoculum level of 2,000 J_2 and *Pea mosaic virus* was more devastating on *Pisum sativum* than single infection with either pathogen. *M. incognita* and *Cowpea mild mottle virus* interaction in which the nematode preceded virus by two days reduced grain yield more in Ife-Brown than Ife-BPC in the screenhouse and vice versa in the early season. This suggests that Ife-Brown was more susceptible to combined infection of *M. incognita* preceding CPMMV than Ife-BPC in the screenhouse while Ife-BPC was more susceptible in the early season. At variance with this study, Adekunle and Owa (2008) reported that concomitant inoculation of *M. incognita* and *Cowpea aphid-borne mosaic virus* in TVU 2657 cultivar of cowpea known to be nematode resistant had no effect on grain yield, but in Ife-Brown known to be susceptible to both pathogens, grain yield was reduced.

CONCLUSION

This study showed that combined infection was more damaging than either of the single infections, and either of the pathogens could have a suppressive effect on the other depending on the time of their establishment. Further study is necessary to understand the physiological processes involved in the symptom inhibition of CPMMV infected cowpea earlier infected by *M. incognita.*

TABLE 6: MEANS SQUARES FROM THE COMBINED ANALYSIS OF VARIANCE ON THE EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON GRAIN YIELD AND PLANT BIOMASS UNDER SCREENHOUSE CONDITIONS

Source	DF	Grain yield (t/ha)	Plant biomass (t/ha)
Replicate	3	35.948	143.336
Variety	1	292.004**	430.621
Pathogen	5	693.668**	1427.942**
Pathogen x Variety	5	53.350	388.023*
Error	33	974.234	4428.807
R^2		80.9	69.2
CV (%)		31.53	22.8

TABLE 7: MEANS SQUARES FROM THE COMBINED ANALYSIS OF VARIANCE ON THE EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON *MELOIDOGYNE INCOGNITA* POPULATION DENSITIES UNDER SCREENHOUSE CONDITIONS

Source	DF	*Meloidogyne incognita* population
Replicate	3	0.00495
Variety	1	0.0269
Pathogen	3	0.2170**
Pathogen x Variety	3	0.01197
Error	12	0.00907
R^2		89
CV (%)		4.47

TABLE 8: MEANS SQUARES FROM THE COMBINED ANALYSIS OF VARIANCE ON THE EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON GRAIN YIELD AND PLANT BIOMASS UNDER FIELD CONDITIONS

Source	DF	Grain yield (t/ha)	Plant biomass (t/ha)
Replicate	3	0.9175	6.2798
Season	1	0.1207	16.4360
Variety	1	12.0832**	176.2925**
Pathogen	5	7.9378**	121.9647**
Pathogen x Variety	5	0.9847**	5.0447
Pathogen x Season	5	0.7646*	3.3106
Variety x Season	1	0.1400	17.5262
Pathogen x Variety x Season	5	0.0341	8.2272
Error	54	0.0341	8.1300
R^2		85.7	68.9
CV (%)		33.4	22.3

TABLE 9: MEANS SQUARES FROM THE COMBINED ANALYSIS OF VARIANCE ON THE EFFECTS OF INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *COWPEA MILD MOTTLE VIRUS* ON *MELOIDOGYNE INCOGNITA* POPULATION DENSITIES UNDER FIELD CONDITIONS

Source	DF	*Meloidogyne incognita* population
Replicate	3	0.2977
Season	1	0.0620
Variety	1	0.1559**
Pathogen	3	0.2114**
Pathogen x Variety	3	0.0166
Pathogen x Season	3	0.0825
Variety x Season	1	0.0033
Pathogen x Variety x Season	3	0.0569*
Error	36	0.0164
R^2		73.8
CV (%)		5.8

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