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Antagonistic Effect of *Meloidogyne incognita* and *M. Javanica* on Pepper Veinal Mottle Virus (PVMV) (Genus: *Potyvirus*) Infecting Nigerian Pepper (*Capsicum* sp.) Lines

[1]I.A. Udo, [2]K.I. Ugwuoke and [1]D.A. Ukeh
[1]Department of Crop Science, University of Calabar, Calabar, Nigeria
[2]Department of Crop Science, University of Nigeria, Nsukka, Nigeria

Abstract: A greenhouse experiment was carried out to evaluate the relationship between 2 root-knot nematode species (*Meloidogyne incognita* and *M. Javanica*) and pepper veinal mottle virus (PVMV) (Genus: *Potyvirus*) infecting Nigerian pepper lines in Nsukka, Southeastern Nigeria. Twelve indigenous pepper lines were used as test plants. Individual plants were inoculated with 2,000 eggs, each of the nematode species. Uninoculated plants served as the control. The test plants were infected naturally with the PVMV by surrounding with green peach aphids (*Myzus persicae sulz*) infested PVMV source plants. The spread of the virus was aided by green peach aphids. Galling and egg production by the nematodes were scored on 0-5 rating scale, while viral infection was scored on 0-4 rating scale. The pepper lines differed significantly ($p<0.05$) in their severity of galling, egg production and viral infection. Only the nematode-free plants expressed the severe viral infection symptom rating 3. All the nematode infection ratings had negative correlation with the moderate infection rating of the virus as well as viral index. The results suggest an antagonistic interaction between the two pathogens. The biochemical or physiological basis of this interaction is yet to be ascertained.

Key words: Pepper, root-knot nematodes, virus and antagonism, *Capsicum* sp.

INTRODUCTION

Pepper (*Capsicum* sp.) belongs to the family of Solanaceae. There are both cultivated and wild species in the genus *Capsicum*. The 2 widely grown species are the bell or sweet peppers (*C. annum* L) and the pungent or "bird eye" peppers (*C. frutescens*). Pepper is an important spice crop, highly cherished for its pungent flavour. It is rich in vitamin A and C (Yayock *et al*., 1988).

The cultivation of pepper under field's culture, especially in the humid tropics is limited by pests and diseases (Williams *et al*., 1991). Root-knot nematodes (*Meloidogyne* sp.) are a major constraint to global production of pepper (Judy *et al*., 2002). Moreover, more than 20 viruses are implicated for the viral diseases of pepper (Smith, 1972). It is not uncommon either, for the two pathogens to induce disease complexes in pepper when they occur together. Many researchers have reported synergistic and antagonistic interactions between nematode infestation and virus infections in several plants (Weischer, 1975; Ismail *et al*., 1979; Huang and Chu, 1984; Jabri *et al*., 1985; Ali, 1988; Alam *et al*., 1990). In most of these studies, the development and multiplication of nematodes were enhanced or inhibited depending on the virus, nematode or plant species. *Tomato blackring nepovirus* enhanced the multiplication of *Ditylenchus dipsaci* but suppressed that of *Aphelenchoides ritzemabosi* (Weischer, 1975). According to Ali (1988), root-knot nematode, *M. incognita* produced five to ten times more individuals on Cardamon plants infected with Katte *mosaic virus* than on healthy plants. Similarly, plants of *Zinnia elegans* and *Solanum khasium* infected with *Zinnia mosaic virus* and *Tobacco mosaic tobamovirus*, respectively had higher root-knot index than their healthy counterparts (Ismail *et al*., 1979). Conversely, inhibitory effects on root-knot nematode, *M. javanica* were observed in Zucchini (*Cucurbita pepo*) infected with *Watermelon mosaic potyvirus*. Virus infection retarded the establishment of these nematodes in the roots as compared with healthy plants (Huang and Chu, 1984). Also, Alam *et al*. (1990) observed antagonistic effects between *Tomato mosaic tobamovirus* and *M. incognita*. The influence of the host on this type of interaction was demonstrated by Moura and Powell (1977). In 2 out of 3 tomato varieties, the egg production of *M. incognita* was significantly increased by the presence of *Tobacco mosaic tobamovirus* but in the third it was not.

Based on the above review and with the infestation of some indigenous pepper lines by *M. incognita*,

Corresponding Author: D.A. Ukeh, Department of Crop Science, University of Calabar, Calabar, Nigeria

M. javanica and Pepper Veinal Mottle Virus (PVMV) (Genus: *Potyvirus*) in the field of Crop Science Research farm, University of Nigeria Nsukka (Udo *et al*., 2005) these led the authors to evaluate statistically, the relationship between the two indigenous root-knot nematode species and Pepper Veinal Mottle Virus (PVMV) (Genus: *Potyvirus*) on Nigerian pepper lines.

MATERIALS AND METHODS

Two *Capsicum annum L.* lines (*Tatasai and Sombe*) and ten *C. frutescens* L. lines (*UNS2*, *UNS3*, *NSKY-LP*, *Atanukwu*, *NSKY-RW*, *NSKY-SE*, *Attaragu*, *Dangarawa*, *Oshosho* and a hybrid obtained from a cross between Sombe and *NSKY-RW*) were procured from the Department of Crops Science, University of Nigeria Nsukka pepper germplasm and used for the study. All the pepper lines were susceptible to the Pepper Veinal Mottle Potyvirus (PVMV) (Genus: *Potyvirus*). Thus, seeds were obtained from fruit of heavily infected plants. The seeds were not treated prior to planting. Seedlings were raised in steam sterilized soil mixture of sandy loam, compost soil and river sand mixed at the ratio of 3:2:1 by volume. Fifteen seedlings of each pepper lines, 4-week-old were transplanted on 22nd December 2003, one per 15 cm diameter clay pots containing 1 kg steam-sterilized soil mixture. Nsukka populations of *M. incognita* race I and *M. javanica* maintained on begonia plants *(Begonia rex-cultorum*) served as inocula sources. The population of each nematode species was increased on Indian Spinach (*Bassela rubra*) in the greenhouse. Root-knot nematode eggs were extracted from the heavily galled roots of the Indian Spinach using Sodium hypochlorite (NaOCI) technique (Hussey and Baker, 1973). Thirty milliliter of the inoculum suspension contained approximately 2,000 eggs by count. The seedlings in each pepper lines were divided into three sets with 5 seedlings in each line making a set One set were inoculated with *M. incognita*, the other set with *M. javanica* while the remaining set were not inoculated (control). A total of 180 test plants were used. Seedlings were inoculated with 2,000 eggs per pot by adding the inoculum in depression made in the soil round each young seedling. The pepper seedling were exposed to natural inoculation by placing them in an open pepper field made up of PVMV pepper infected and aphid-infested source plant. Green peach aphids (*Myzus persicae sulz)* localized on PVMV infected source plants were allowed inoculation access period of 3 weeks on the test plants. The test plants were naturally infected through the feeding of these viruliferous aphids. Pests and fungal diseases were not controlled with any chemical. Immediately after 3 weeks, the pots were taken to green house and arranged on benches in a Completely Randomized Design (CRD) fashion. Plants were grown at a mean day temperature of 29°C and mean night temperature of 20°C for 125 days. Data were collected on the number of galls per root system. For egg mass count, fresh root was stained with Phloxine B. (0.15 g L^{-1}) for 15 min (Daykin and Hussey, 1985). Root-gall or egg mass index was determined on a 0-5 scale rating used in the International Meloidogyne Project (IMP, 1978). 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = >100 galls or egg masses per root system. The severity of Pepper Veinal Mottle Virus (PVMV) (Genus: *Potyvirus*) disease was recoded at 15 weeks after transplanting by scoring using a 5 point scale (0-4): 0 = no symptoms (healthy), 1 = mottle (mild infection), 2 = mottle + puckring (Moderate infection), 3 = mottle + puckering+Necrotic lesions (severe infection) and 4 = death of plant (very severe infection). The percentage of leaves with each symptom was calculated per plant. At the termination of the experiment, random sample of leaf extracts containing the virus was serologically tested against an antiserum to PVMV in Protein-A Sandwich Enzyme Linked Immunosorbent Assay (PAS-ELISA). The antiserum used was supplied by the Virology Laboratory of International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. One hundred and eighty samples of PVMV infected pepper leaves tissue sap $^{1}/_{1000}$ (w/v) was prepared in ELISA extracting buffer (PBS-Tween + 2% PVP). The healthy and infected control used was supplied by the Department of Crop Science, University of Nigeria, Nsukka (UNN). One hundred mL per well of protein A (1 μL mL^{-1}) diluted in coating buffer was added and the plates were incubated for 2 h at 37°C. The plates were washed three times with PBS-Tween. Hundred microliter per well of polyclonal antiserum diluted $^{1}/_{1000}$ in PBS-Tween. Hundred milliter per well of the prepared 180 samples of PVMV infected leaves sap, the healthy and infected control were added three wells per sample in the plates and incubated overnight at 4°C. The plates were washed three times with PBS-Tween.Hundred milliter per well of polyclonal antiserum diluted $^{1}/_{1000}$ in PBS-Tween was added and the plate incubated for 2 h at 37°C. The plates were washed three times with PBS-Tween. Hundred milliter per well of protein A-alkaline phosphate conjugate diluted $^{1}/_{1000}$ in conjugate buffer was added and the plates incubated for 2 h at 37°C. Two hundred milliter per well of 1 mg mL^{-1} of P-nitro-phenol phosphate substrate buffer was added. The plates were read overnight.

Statistical analysis: The data were statistically analysed using the Analysis of Variance (ANOVA). Means were

tested using (F-LSD) at 5% level of probability. Before analysis, data expressed as percentages were transformed using the arcsine transformation method ($sine^{-1}/x$). The relationship between nematode infection ratings and viral infection ratings was established using simple linear correlation analysis according to Gomez and Gomez (1984).

RESULTS

The pepper plants showed the various symptoms of viral infection. The results of the serological test were positive to the PVMV antiserum in PAS-ELISA. The plant samples tested were positive. Root galling and egg production by *M. incognita* differed significantly ($p<0.05$) among the pepper lines (Table 1). Conversely, viral index was statistically similar among the pepper lines tested. However, the percentage of mottled leaves per plant and those with mottled plus puckered leaves differed significantly among the pepper lines. Table 2 shows the results of nematode (*M. javanica)* and viral infection ratings. The pepper lines differed significantly with respect to root galling and egg mass production by the nematode species. Also, there were significant differences among the pepper lines in viral index, percentage of mottled leaves per plant and in percentage mottled + puckered leaves. The nematode free plants (uninoculated control) also differed significantly in viral infection ratings when the pepper lines are considered (Table 3). Some lines namely: *UNS2*, *Tatasai* and *Dangarawa* had the viral disease severity score of 3 indicating severe infection. Comparing the viral indices of the nematode-infested plants on one hand, with that of nematode free plants on the other, generally, the latter had higher ratings than the former. However, cultivars *UNS3*, *Sombe*, *NSKY-SE* and *NSKY-RW* infested with *M. javanica* had slightly higher viral indices relative to the control. A similar result was obtained with *Attaragu* and *NSKY-RW* with *M. incognita* infestation (Table 1-3). Table 4 shows the correlation coefficients r between root-knot nematode and viral infection ratings among the tested plants. Although not statistically significant, all the nematode infection ratings correlated positively with the mild infection rating 1 of the virus. Conversely, there were negative correlations between all the nematode infection ratings and the moderate infection rating 2 of the virus. Gall index of *M. incognita* infested plants had a highly significant ($p<0.01$) negative relationship, while number of galls and egg masses were significant ($p<0.05$). In *M. javanica* infested plants the relationship was also negative but not statistically significant. Similarly, although not statistically significant, all the nematode

Table 1: Root-Knot nematode (*M. incognita)* infection ratings and viral infection ratings on Nigerian pepper lines

Pepper line	No of galls/ root system	Gall index	No. of egg masses /root system	Egg mass index	Mottled leaves /plant (%)	Mottled + puckered leaves/plant (%)	Viral index
UNS2	10.33	2.33	6.00	2.32	63.79	26.22	1.29
UNS3	83.33	4.67	40.66	4.00	81.05	0.00	0.86
NSKY-LP	8.00	2.00	2.68	1.68	60.45	21.47	1.18
Atanukwu	53.00	3.67	35.00	3.34	34.64	39.63	1.55
Sombe	31.67	3.10	20.68	3.00	90.00	0.00	0.67
NSKY-SE	4.32	1.35	1.35	1.00	56.24	33.76	1.40
Attaragu	8.00	2.00	2.00	1.00	53.51	36.49	1.37
Tatasai	69.32	4.00	45.34	3.34	80.00	8.04	1.31
Dangarawa	7.00	2.32	6.32	2.32	58.61	31.85	2.03
Oshosho	12.00	2.32	7.32	2.32	90.00	0.00	1.00
NSKY-RW	27.66	3.32	18.34	3.00	50.51	29.49	1.16
Hybrid	9.00	2.32	4.00	2.00	0.00	60.00	1.33
LSD (0.05)	40.81	1.34	24.73	1.19	43.38	11.15	Ns

Table 2: Root-knot nematode (*M. javanica)* infection ratings and viral infection ratings on Nigerian pepper lines

Pepper line	No of galls/ root system	Gall index	No. of egg masses /root system	Egg mass index	Mottled leaves/ plant (%)	Mottled + puckered leaves/plant (%)	Viral index
UNS2	27.67	3.32	19.68	3.00	74.81	0.00	0.81
UNS3	49.00	3.68	36.32	3.00	81.43	8.57	1.06
NSKY-LP	26.32	3.00	16.68	3.00	7.60	82.40	1.32
Atanukwu	90.00	4.32	73.68	4.00	65.13	26.04	1.24
Sombe	87.32	4.00	56.00	4.00	90.00	0.00	1.00
NSKY-SE	7.32	2.00	1.32	1.00	30.00	60.00	1.68
Attaragu	69.68	4.68	71.68	4.00	60.00	0.00	0.50
Tatasai	73.32	4.32	66.68	4.00	64.73	25.27	1.65
Dangarawa	86.68	4.68	70.68	4.00	25.29	64.73	2.08
Oshosho	67.68	3.68	54.32	3.68	90.00	0.00	1.00
NSKY-RW	67.32	4.00	49.00	4.00	60.00	30.00	1.32
Hybrid	100.00	5.00	86.68	4.32	77.80	0.00	0.94
LSD(0.05)	41.42	1.26	38.36	0.93	12.79	45.28	0.75

Table 3: Viral infection ratings of Nigeria pepper lines uninoculated with root-knot nematodes (control)

Pepper line	Mottled leaves/plant (%)	Mottled + puckered leaves/plant (%)	Mottled +Puckered + necrotic lesion leaves/plant (%)	Viral index
UNS2	76.60	81.00	90.00	2.67
UNS3	15.00	86.00	0.00	1.00
NSKY-LP	46.05	71.00	0.00	1.83
Atanukwu	46.60	86.00	0.00	2.00
Sombe	69.92	61.00	0.00	0.77
NSKY-SE	86.60	66.00	0.00	1.66
Attaragu	90.00	73.30	0.00	1.00
Tatasai	88.30	56.60	60.00	2.32
Dangarawa	76.60	78.96	23.15	2.32
Oshosho	86.60	43.40	0.00	1.00
NSKY-RW	76.30	34.20	0.00	1.07
Hybrid	80.00	79.15	0.00	1.64
LSD(0.05)	28.53	22.49	-	0.83

Table 4: Correlation Coefficients (r) between root-knot nematode infection ratings and viral infection ratings

Viral infection ratings	Mottled leaves/plant (%)	Mottled + puckered leaves/plant (%)	Viral index
Nematode infection ratings			
M. incognita *(Gall index)*	0.298^{ns}	-0.761^{**}	-0.319^{ns}
M. incognita (No of galls/root system)	0.348^{ns}	-0.681^{*}	-0.323^{ns}
M. incognita *(No of egg masses /root system)*	0.333^{ns}	-0.702^{*}	-0.241^{ns}
M. javanica (Gall index)	0.331^{ns}	-0.021^{ns}	-0.144^{ns}
M. javanica (No of Galls/root system)	0.397^{ns}	-0.446^{ns}	-0.194^{ns}
M. javanica (No of Egg masses/root system)	0.371^{ns}	-0.408^{ns}	-0.117^{ns}

** = Significant at 1% level of probability; * = Significant at 5% level of probability; ns = Not significant

infection ratings of both species of root-knot nematode correlated negatively with the viral index (Table 4).

DISCUSSION

There is controversy in literature concerning nematode-virus interactions. Some authors reported synergistic relationship, while others are in support of antagonistic interaction. From this study, the bulk of evidence tends to corroborate the findings of the latter researchers. Higher viral indices of the nematode-free (uninoculated control) plants compared with the nematode-infested plants are an indication of antagonistic interaction. The results obtained showed that the highest viral disease severity score of 3 was recorded only in pepper plants without nematode infestation. The negative correlations between nematode infection ratings and the moderate viral infection rating index also support antagonistic relationship between the 2 pathogens. Statistically, it means that, in the presence of the nematode, viral infection is suppressed.

Some authors have argued that whether beneficial or detrimental to either pathogen, the basis of this interaction is influenced by the biochemical and/or physiological changes induced in the host by the pathogens (Weischer, 1975; Alam *et al*., 1990). However, little is known about the physiological or biochemical basis of enhancing or inhibiting virus development by a nematode infestation of the host. It has been suggested that changes in protein metabolism caused by nematode may interact with corresponding change caused by the virus, resulting in beneficial effects in one case and detrimental effects in another (Weischer, 1975). This view was supported by Shower *et al.* (1990). They showed that the changes in free amino acid levels in sugarcane caused by *sugarcane mosaic virus* were responsible for population changes in various nematodes on sugarcane. The relative occurrence of either pathogen determines to a greater extent the nature of interaction. Alam *et al.* (1990) observed that when virus infection preceded nematode inoculations, nematodes were suppressed and when nematode were the first agent, the virus was inhibited. The changes caused by one pathogen were detrimental to the other. However, our finding is in disagreement with their report as the pepper plants were infested with the nematodes at early stage prior to virus infection.

Conclusively, this study has statistically demonstrated antagonistic interaction between two root-knot nematode species (*M. incognita* and *M. javanica)* and Pepper Veinal Mottle Virus (PVMV) (Genus: *Potyvirus*) infecting Nigerian pepper lines. However, the biochemical or physiological basis of this interaction is yet to be ascertained.

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