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SINGLE AND MIXED INFECTION EFFECTS OF *FUSARUIM OXYSPORUM* (SCHLECHT) HANSEN & SYNDER AND *MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD ON THE GROWTH COMPONENTS OF SUSCEPTIBLE AND RESISTANT TOMATO PLANTS

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ABSTRACT

Single and mixed infection of *Meloidogyne javanica* and *Fusarium oxysporum* on growth of susceptible and resistant tomato plants were investigated in pot experiments. The experiments were arranged in completely randomised design with 5 replications. Pie-pan technique was used for nematode extraction and the fungus was obtained from root rhizosphere soil. Data were subjected to one-way ANOVA. Results showed that single and mixed infections significantly reduced some growth components of the susceptible tomato. However, combined effects were not significantly different from that inflicted by either of the pathogens. Gall rating indicated no significant differences among single and mixed infections, although successive inoculation where fungus preceded that by nematode significantly reduced number of galls. For resistant tomato, single and combined infections did not impact significantly on the growth parameters except for shoot length, root length and dry weight. Number of galls showed significant differences in this order of decreasing magnitude N or N+F or N+f, F+n and C at $p \leq 0.05$. In both susceptible and resistant tomato, simultaneous infections caused the most reduction in the growth components and number of galls. That resistant tomato was not adversely impacted in single and combined infections underscores the need for its use in tomato production for improved yield.

Keywords: Fungus; *Meloidogyne javanica;* resistant and susceptible tomato; single and mixed infection
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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is consumed nearly in every household in Benue State and Nigeria at large, owing to its high nutritive value and diverse uses (Mucksood and Khan, 2011). It is grown world-wide because of its nutritional quality and in being an excellent source of vitamins A and C (Mucksood and Khan, 2011). According to Nkiru and Ifenkwe (2005), tomato is helpful in the development of rural agro-industries. In Benue State, tomato production is limited to wet season with attendant shortage and high price during dry season. Its vulnerability to various diseases including those of fungi, viruses, bacteria and nematodes constitutes a huge production challenge for the crop (Jaiteh *et al*., 2012).

Sedentary plant parasitic nematodes are microscopic round-worms that cause significant yield losses in the agricultural crops (Siddiqui *et al*., 2015). In Nigeria, *Meloidogyne* species constitute a major constraint to production of vegetables and other valuable crops (Adesiyan *et al*.,1990; Atungwu and Afolami, 2001; Iheukwumere and Orkpeh, 2007) and one of the major pathogens of tomatoes which limits its fruit production (Sikora and Fernandez, 2005). According to Siddiqui *et al*. (2015), successful parasitism is based on the formation of hyper metabolic feeding sites in the host. *Fusarium oxysporum* is a fungal pathogen that causes *Fusarium* wilt, a common vascular disease of plants (Louter and Wukasch, 2012) that is widely distributed both on the plant and in the soil. In tomato, there occurs two forms namely *Fusarium oxysporum* f.sp *lycopersici* (FOL) and *Fusarium oxysporum* f.sp *racidis-lycopersici* (Wojiciechszcechura *et al.,* 2013).

Plant parasitic nematodes interact with *Fusarium oxysporum*, resulting in increased damage to crops (Jaiteh *et al*., 2012). Nematode interaction with host plants most often results in wounds on the plants during penetration and feeding. These wounds become avenues for entry of other pathogenic agents that require aid to penetrate their host. In addition, they generally modify plant hosts in disease development such that tissues readily become susceptible to these infective agents and thus increase severity of the infection to the detriment of the host (Gourd *et al*., 1993; Agrios, 2005; Iheukwumere *et al*., 2009). Plants with combined infection often exhibit more severe symptoms which include wilting, foliage drooping, increased galling of the roots, raised number of second stage juveniles and eggs in the soil and in the root system; accompanied by significant losses in yield than is the case with individual pathogens alone (Iheukwumere *et al*., 2007). Preliminary surveys have shown that fungi and root-knot nematodes reduce growth and performance of susceptible tomato plants when they infect the plant singly. However, studies on mixed infection effects of these pathogens on the growth and performance of tomato plants in Nigeria and Benue State in particular is presently scanty. This necessitated this study, which was aimed at providing useful information on disease complexes in susceptible and resistant tomato. This will no doubt bridge the information gap on mixed infection involving a nematode and a soil fungus on susceptible and resistant crops.

MATERIALS AND METHODS

Study Area

The study was carried out in front of the Advanced Biology Laboratory of the Federal University of Agriculture, Makurdi, Benue State.

Soil Sterilisation

Soil for the experiment was sterilised for 3 hours at 100°C with fuel wood as the source of heat using the barrel steam sterilization method (Jaiteh *et al*., 2012).

Source of nematode, confirmation of identity and multiplication

The root-knot nematode (*Meloidogyne javanica*) used for the study was obtained from infected roots of Okra (*Abelmoschus esculentus*) on a farm land located at Shaminja village in Makurdi Local Government Area of Benue State. The okra roots were brought to the laboratory and washed free of soil and debris. The washed roots were divided into two equal portions. One portion was stored in the refrigerator at 4°C for multiplication of the nematode for further use. The other was used for preparing the perineal pattern morphology of the adult females. The portion for making perineal patterns was thoroughly washed and the roots plucked from their stumps and placed in a 30 cm diameter plastic tray containing water, just sufficient enough to cover the roots to avoid dehydration. The roots were carefully teased out in the tray, using a pair of forceps and a scapel, under a stereomicroscope, to remove the adult female nematodes needed for preparation of the perineal patterns according to the method of Hartman and Sasser (1985). One hundred adult females were extracted from the root tissues ensuring that every root was sampled in the process. The pattern made from each of these females was compared with the pictorial key of Eisenback *et al*. (1981) to determine the identity of the nematode and was confirmed to be *Meloidogyne javanica*.

Nematode Multiplication: The preserved portion of the okra roots were brought out of the refrigerator and cut into 1-2 cm fragments for the multiplication of the nematodes on tomato seedlings. The cut pieces were inoculated into fresh 2.4 kg of sterilised soil, that were measured into fifty, 20-21 cm diameter polyethylene bags placed on a concrete floor outdoors (Iheukwumere *et al*., 2009). The tomato seeds were thereafter planted in each of the polyethylene bags and allowed to grow for eight (8) weeks.

Nematode Extraction

Extensively galled roots of the tomato on which the nematode had been multiplied were carefully uprooted and washed free of soil and debris (Iheukwumere and Orkpeh, 2007). The roots were cut into 1-2 cm fragments and placed on modified Baermann's funnels (Whitehead and Hemming, 1965; Iheukwumere *et al*., 2009;

Iheukwumere, 2012) for 18 hours to extract the nematodes (second stage juveniles J2). An aliquot of 1ml extract of the nematodes obtained after 18 hours was placed in a counting dish and mounted under a stereomicroscope at a magnification of x40 and the number of juveniles (J2) diluted with water to give 500 larvae per millilitre (Iheukwumere *et al*., 2009). A repeated pipetting of the homogenous extract was used to deliver 1ml aliquot of 500 larvae into test tubes that were utilised for the inoculation of test plants.

Source of Fungus and Identification

Fusarium oxysporum (Schlecht) Snyder and Hansen used was isolated from 5 ml of soil collected from rhizosphere of plants using melon seeds (*Colocynthis citrullus* L.) as bait. The soil sample and the melon seeds were placed on Potato Dextrose Agar (Ataga and Ota-Ibe, 2006; Iheukwumere and Orkpeh, 2007) and incubated at room temperature (27±3°C) for 3 days (Iheukwumere *et al*., 2007). The plates were subsequently sub-cultured repeatedly on fresh PDA plates until pure cultures of the isolate were established (Chiejina**,** 2006; Iheukwumere *et al.,*2007). The isolate was identified as *Fusarium oxysporum* following the descriptions expressed by Barnette and Hunter (1999) and Alexopoulos *et al*. (2002). Discs were cut from 7-day old culture of the fungus with 0.5-cm-diameter sterile cork borer (Ataga and Ota-Ibe, 2006; Iheukwumere and Orkpeh, 2007) and transferred onto sterilised Whatman No.1 filter paper placed on a triple beam balance until 5 gm weight was obtained (Iheukwumere *et al*., 2007).

Seed Source and Treatment

Seeds of a susceptible tomato UC 82 B and resistant Roma VFN varieties were obtained from the Agricultural Services Training and Marketing Centre (ASTC), Vom, Plateau State. Seeds of the different tomatoes were separately surface-sterilised for 5 minutes in 1.05% sodium hypochlorite solution and rinsed for 5 minutes in 6 changes of sterile distilled water prior to sowing (Koenning and McClure, 1981; Iheukwumere *et al.,* 2007).

Inoculation of Test Plants

Seedlings were not watered the day preceding inoculation to avoid over-wetting of soil. Inoculation was done with the aid of a plastic syringe with which 1ml aliquot of the suspension of 500 juveniles of *Meloidogyne javanica* was taken and introduced around the base of each plant using the trench method of Iheukwumere *et al*. (2007). Similarly, plants were inoculated with the fungus propagated and maintained on PDA by introducing 5 gm of *Fusarium oxysporum* into shallow holes made in the rhizosphere of the test plants according to the method of Iheukwumere and Orkpeh (2007).

Experimental Design and Data Analysis

The experiment was arranged in a completely randomised design (CRD) with 5 replications per treatment on a cemented platform. Treatments given to the 7-day old seedlings were:

i. The uninoculated control plants (C)
ii. Inoculation of test plants with 500 juveniles (J2) of the nematode only (N)
iii. Inoculation of test plants with 5 gm of fungus only (F)
iv. Simultaneous inoculation of test plants with 500 juveniles of the nematodes and 5 gm fungus (N+F)
v. Successive inoculation of test plants with 500 juveniles of the nematode followed by inoculation with 5 gm of the fungus 7 days later (N+f)
vi. Successive inoculation of test plants with 5gm of the fungus followed by inoculation with 500 juveniles of the nematode 7 days later (F+n)

Plants were watered on alternate days. Experiment was terminated 8 weeks after planting and the following growth and performance parameters were measured and subjected to a one-way ANOVA: shoot length, shoot fresh and dry weights, root length, root fresh and dry weights and number of leaves. The means were separated using the LSD ($p \leq 0.05$). Gall rating was assessed on a 0-5 scale according to the method described by Taylor and Sasser (1978) and Iheukwumere (2012) as follows: 0= No gall; 1 = 1-2 galls; 2=3-10 galls; 3=11-30 galls; 4=31-100 galls; 5 = greater than 100 galls.

RESULTS

For the susceptible tomato, there were no significant differences in the number of leaves per plant among single and mixed infections in which both pathogens were simultaneously inoculated or where the inoculation of one preceded the other successively by 7 days. However, the single and mixed infections significantly reduced the number of leaves, shoot length, shoot fresh and dry weights of inoculated plants in comparison with the control at $p \leq 0.05$ (Table 1). Furthermore, combined treatments in which the pathogens were simultaneously inoculated, had lower number of leaves, shoot length, shoot fresh and dry weights than in single and successive infections (Table 1).

For root length, root fresh and dry weights, there were no significant differences among the control, single and combined infections (Table 1). For number of galls, no significant differences were observed among single inoculation with only nematode, simultaneous and successive infections where nematode inoculation preceded that by fungus (Table 1). Each of them had significantly higher number of galls than those of successive inoculation where fungus preceded that by nematode and the control at $p \leq 0.05$ (Table 1). However, simultaneous inoculations reduced all the growth parameters and galls more than each of the other treatments (Table 1). Number of galls in successive infection in which fungus inoculation preceded that by nematode was significantly higher than that of fungus treatment alone or the control (Table 1).

For the resistant tomato, data on number of leaves showed that there were no significant differences among all the treatments at $p \leq 0.05$ (Table 2). Data on shoot fresh and dry weights followed the same trend as observed for the number of leaves (Table 2). However, for shoot length, results showed that the control was significantly higher than any of the other treatments ($p \leq 0.05$), all of which did not differ significantly in plant height (Table 2).

For root length, results indicated that there were no significant differences among the control, single infections with only fungus or nematode. Similarly, there were no significant differences in root length among all the mixed infections; but root length in each of the mixed infection was significantly shorter than those of the control, and those singly treated with only nematode and fungus (Table 2). Simultaneous infections resulted in reduction in all the parameters when compared with the other treatments. Results of the number of galls showed no significant differences among single infection with nematode alone, simultaneous infection with both pathogens and successive infection where nematode inoculation preceded that by the fungus (Table 2). Each of them had a significantly higher number of galls than single infection with only fungus, control and successive infection where the fungus inoculation preceded that by the nematode. However, the number of galls in the treatment where fungus inoculation, preceded that by nematode, was significantly higher than those observed in single infection with only fungus and the control (Table 2).

Table 1: Single and mixed infection effects of *Fusarium oxysporum* and *Meloidogyne javanica* on growth components of a susceptible tomato plant.

	Shoot*				Root*			
Treatment**	Number of leaves	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Number of galls^{+}
C	123.80^{a}	39.99^{a}	65.60^{a}	32.80^{a}	28.00^{a}	2.05^{a}	1.04^{a}	0.00^{c}
N	97.00^{b}	26.88^{b}	29.90^{b}	13.95^{b}	16.90^{a}	1.80^{a}	0.90^{a}	68.40^{a}
F	102.00^{b}	30.99^{b}	34.70^{b}	17.00^{b}	25.30^{a}	1.30^{a}	0.75^{a}	0.00^{c}
N+F	83.20^{b}	23.29^{b}	27.00^{b}	13.90^{c}	16.30^{a}	1.20^{a}	0.61^{a}	41.40^{a}
N+f	100.00^{b}	27.59^{b}	30.18^{b}	14.05^{b}	17.19^{a}	1.92^{a}	0.96^{a}	49.60^{a}
F+n	99.60^{b}	32.49^{b}	38.80^{b}	20.05^{b}	22.70^{a}	1.78^{a}	0.90^{a}	16.30^{b}
LSD @ 5%	20.39	6.08	7.18	4.21	4.73	0.70	0.57	23.33

*****Each value is a mean of 5 replicates. Means with same superscript within the same column are not significantly different using the LSD ($p \leq 0.05$)

**C = uninoculated; N = nematode; F = Fungus; N+F = nematode and fungus inoculated simultaneously; N+f = nematode inoculation followed by that of fungus 7 days later; F+n = fungus inoculation followed by that of nematode 7 days later

0 = No galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; 5 = greater than 100 galls

Table 2: Single and mixed infection effects of *Fusarium oxysporum* and *Meloidogyne javanica* on growth parameters of a resistant tomato plant

	Shoot*				Root*			
Treatment**	Number of leaves	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Number of galls^{+}
C	106.60^{a}	35.90^{a}	23.18^{a}	10.18^{a}	22.00^{a}	0.68^{a}	0.35^{a}	0.00^{c}
N	41.60^{a}	17.90^{b}	9.24^{a}	2.66^{a}	10.92^{a}	0.82^{a}	0.34^{a}	3.00^{a}
F	74.00^{a}	24.52^{b}	13.94^{a}	6.42^{a}	16.90^{a}	0.66^{b}	0.23^{a}	0.00^{c}
N+F	30.80^{a}	13.16^{b}	7.80^{a}	3.23^{a}	9.70^{b}	0.28^{b}	0.14^{a}	2.00^{a}
N+f	51.60^{a}	14.44^{b}	7.94^{a}	3.41^{a}	10.70^{b}	0.50^{b}	0.25^{a}	2.00^{a}
F+n	68.40^{a}	19.98^{b}	14.82^{a}	7.74^{a}	16.26^{b}	0.52^{b}	0.20^{a}	1.00^{b}
LSD @ 5%	45.90	11.16	8.81	4.80	6.28	0.57	0.32	1.00

*****Each value is a mean of 5 replicates. Means with same superscript within the same column are not significantly different using the LSD ($p \leq 0.05$)

**C = uninoculated; N = nematode only; F = Fungus only; N+F = nematode and fungus inoculated simultaneously; N+f = nematode inoculation followed by that of fungus 7 days later; F+n = fungus inoculation followed by that of nematode 7 days later

0= No galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; 5 = greater than 100 galls

DISCUSSION

The studies clearly showed the vulnerability of susceptible tomato to attack by *Fusarium oxysporum* and *Meloidogyne javanica* both in mono and co-infection complexes. The finding aligns with the report of others who showed the damaging effects of these pathogens on some vegetable crops (Kemble *et al*., 2007; Iheukwumere *et al*., 2009). The severity of effects in single and multi- infections were more on the above ground parts of the plants as observed on shoot length, shoot fresh and dry weights than on the underground parts as the roots. Both infections did not significantly affect the root systems of the susceptible tomato plant. The differences observed in the impact of the treatment on the above and below ground parts of the susceptible tomato may be due to the fact that different parts of a plant can respond differently to the activities of a given pathogen or pathogens (Matthews, 1981; Iheukwumere *et al*., 2009). This could explain why the above-ground parts of the susceptible tomato were significantly reduced while the underground part was not.

In the resistant tomato plant, it was observed that with the exception of shoot and root lengths and root dry weight that were significantly lower than the control, all other growth components of this plant were not adversely impacted by the single and co-infection with both pathogens. This could be due to resistant factors in the plant, while those impacted negatively might probably be due to the fact that different parts of a plant respond differently to a given pathogen (Matthews, 1981) as earlier explained. Furthermore, resistance measurement by visual inspection and scoring may not give complete information on the genetic status of a plant and might in fact fail to highlight certain susceptible factors in "resistant" lines that can be expressed in any part of the plant as has been shown in this study (Bookbinder and Bloom. 1980; Iheukwumere *et al*., 2009).

It was generally observed that in all the treatments, mixed infections in which fungus and the nematode were inoculated simultaneously led to the highest and significant reductions in the above-ground parts of the susceptible tomato plant than those of other treatments. This effect was similarly established for the resistant tomato plants, although among the treatments the impacts on most of the plant parts were not statistically significant. This could be due to the fact that simultaneous inoculation of the plants with both pathogens resulted in intense competition for nutrients and space such that severe physiological upset was initiated in the plants which probably led to such adverse effects.

The simultaneous and successive infections caused no significant reductions in the number of galls in the susceptible tomato plant roots except in that where fungus inoculation preceded that by nematode which significantly resulted in the least number of galls noted. This observation could be due to the fact that feeding sites of sedentary endoparasitic nematodes are preferred substrates for plant parasitic fungi (Sakhuja and Sethi, 1986; Iheukwumere *et al.,* 2009). In addition, giant cells in nematode-infected root tissues are reported to be disrupted and damaged by fungal colonisation (Khan and Dasgupta, 1993; Iheukwumere *et al*., 2009). Furthermore, the fungal inhibition of the nematode may be the result of competition for nutrients (Iheukwumere *et al*., 2009). The observations made on number of galls of the susceptible tomato roots were similarly so for those on the roots of the resistant variety, although fewer number of galls were observed on its roots. However, the least number of galls were observed on successive infections where fungal inoculation preceded that by nematode as was the case with the susceptible tomato variety. This observation could probably have resulted from antagonistic disposition of the fungus to the nematode development and growth. In general, the resistant tomato plant seemed to have performed better in mono and co-infections with the nematode and fungus, which should justify the use of such resistant cultivars in our cropping systems to enhance food productivity and sustainability.

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