EFFECT OF *Meloidogyne incognita* AND *Fusarium oxysporum* f. sp. *lycopersici* ON TOMATO VARIETAL GROWTH UNDER GHANAIAN FIELD CONDITIONS

H.D. VIGBEDOR, S.T. NYAKU and E.W. CORNELIUS

Department of Crop Science, College of Basic and Applied Sciences, University of Ghana, P. O. Box LG 44, Legon-Accra, Ghana

**Corresponding author:** stnyaku@ug.edu.gh, seloame.nyaku@gmail.com

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**ABSTRACT**

The excessive build-up of soil nematodes and uncontrolled spread of fungal diseases contribute to low yields in tomato (*Solanum lycopersicum* L.) production. The objective of this study was to investigate the pathogenicity of *Fusarium oxysporum* f.sp. *lycopersici* and *Meloidogyne incognita* on growth, yield and wilt severity in tomato varieties. A factorial experiment was laid out in a randomised complete block design (RCBD), with three replications, on two fields, namely University of Ghana, Legon farm and at the National Service Demonstration Farm at Papao both in Ghana, from July 2018 to June 2019. Two tomato varieties, namely Mongal F1 and Petomech were used for evaluation. *Fusarium* inoculum of \(1.3 \times 10^6\) cells per 5 mL suspension was inoculated in the experimental plots naturally infested with *Meloidogyne incognita*, at 7, 14, and 21 days (NF7, NF14, NF21), after transplanting tomato seedlings into the fields. Wilt severity was higher in the Petomech plants compared to Mongal plants, for all treatments. Wilt incidence was greater than 70% in the Petomech plants that received the treatments NF7, NF14, and NF21 in both experimental sites, respectively. Plants in the control plots had the least wilt incidence and severity among the two varieties in both sites. Mongal and Petomech plants without *Fusarium* inoculation had lower wilt incidence and severity compared to those that received only *Fusarium* inoculation. Yield was higher in the Mongal plants than in the Petomech plants. Galls scores were zero in the Mongal F1 plants for all treatments applied, in both experimental sites. *Fusarium oxysporum* f. sp. *lycopersici* and *Meloidogyne incognita* increase wilt severity in tomato plants when both pathogens coexist in the soil.

**Key Words:** *Fusarium oxysporum*, incidence, *Meloidogyne incognita*

**RÉSUMÉ**

L’accumulation excessive de nématodes du sol et la propagation incontrôlée de maladies fongiques contribuent au faible rendement de la production de tomates (*Solanum lycopersicum* L.). L’objectif de cette étude était d’étudier la pathogénicité de *Fusarium oxysporum* f.sp. *lycopersici* et *Meloidogyne incognita* sur la croissance, le rendement et la sévérité du flétrissement chez deux variétés de tomates. Une expérience factorielle a été mise en place dans un plan en blocs complets randomisés (RCBD),...
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INTRODUCTION

Tomato (Solanum lycopersicum L.) is cultivated widely throughout the world, but is produced seasonally in Ghana, due to differences in rainfall pattern and access to irrigation (Robinson et al., 2010). Over the past decade, however, production has been stagnant and to some extent, declining (Bortey and Osuman, 2016), hence the country is unable to meet the demand of consumers. This is largely attributed to excessive build-up of soil nematodes and uncontrolled spread of fungal, bacterial, viral diseases, a condition widely been referred to as the ‘Tomato Disease Complex’ (Tanzubil et al., 2004; Inusah, 2005).

Among these, Fusarium oxysporum and root-knot nematodes are of greatest importance. Fusarium oxysporum invades the root epidermis of the tomato plant and spreads into the vascular tissue, colonising the xylem vessels; and blocking it with mycelium and conidia resulting in wilt symptoms (Beckman, 1987). Root knot nematodes also feed on the roots of the plants, produce galls and eventually cause wilting of plants (Sasser, 1980; Sikora, 2005).

Fusarium and root- knot nematodes are both soil borne and cause wilting in tomatoes, which symptoms if not carefully examined, may be mistaken as phosphorus deficiency in the soil. It is possible that the co-existence of both pathogens in the soil which could cause interactions associated with severe consequence.

Disease complexes caused by interaction of nematodes and fungus causes changes in terms of mineral absorption, physiological and biochemical changes in infected plants (Lobna et al., 2017) to reduce vigour of the infected plants; and finally cause death. In a previous greenhouse study conducted in Ethiopia, there were significant (P<0.05) reductions in tomato growth, and biomass after simultaneous inoculation of Meloidogyne incognita and Fusarium oxysporum and inoculation of Fusarium oxysporum 10 days after M. incognita inoculation or the non-inoculated control. Additionally, mean number of root galls per plant was high (497) in simultaneous inoculations of M. incognita and F. oxysporum compared to tomato plants receiving nematode treatments 10 days prior to F. oxysporum inoculations (308) and control on the genotype Moneymaker (Kassie et al., 2020).

The objective of this study was to investigate the pathogenicity of the fungus, Fusarium oxysporum f.sp. lycopersici and nematode, Meloidogyne incognita on growth, yield and wilt severity in tomato in Ghana.
**Materials and Method**

**Experimental sites.** The experiment was carried out on two locations; University of Ghana, Legon farm (latitude: 5.65704 longitude: -0.192661); and at the National Service Demonstration Farm at Papao (latitude: 5.66433 longitude: -0.191885). Both fields had been used for cultivation of vegetables and had high population of root-knot nematodes. Soils at the two sites are classified under the Adentan series and are relatively light clayey soils with low fertility.

**Experimental design and data collection.** The experimental design on both fields was a 2 x 6 factorial experiment in a randomised complete block design (RCBD), with three replications. Two tomato varieties, Mongal F1 and Petomech, were used for this research with both varieties receiving a pathogenic treatment. The treatments used for both fields were:

- **NF7** = *Fusarium oxysporum* inoculated 7 days after transplanting in field naturally infested with *Meloidogyne incognita*;
- **NF14** = *Fusarium oxysporum* inoculated 14 days after transplanting in field naturally infested with *Meloidogyne incognita*;
- **NF21** = *Fusarium oxysporum* inoculated 21 days after transplanting in field naturally infested with *Meloidogyne incognita*;
- **N** = Plots naturally infested with *Meloidogyne incognita* but not inoculated with *Fusarium oxysporum* after transplanting (No *Fusarium* inoculation);
- **F** = *Fusarium oxysporum* inoculated on field naturally infested with *Meloidogyne incognita* and treated with nematicide; and
- **C** = *Fusarium oxysporum* not inoculated on field naturally infested with *Meloidogyne incognita* and treated with nematicide (Control).

The planting distance of tomato plants for both fields was 80 cm x 50 cm, with each plot comprising of 25 plants and an area of 4 m x 2.5 m. Each plot had 5 rows with 5 plants in each row. Distance between plots was 0.5 meters and 1 meter between blocks. Individual plots were demarcated and soil samples collected from the well labelled plots for nematode estimations before transplanting of tomato seedlings.

**Preparation and inoculation.** Two weeks old cultures of *Fusarium oxysporum* f. sp. *lycopersici* on PDA were scraped into a blender and topped up with 1000 cm$^3$ of distilled water. The mixture was blended and the suspension of fungal spores was determined using a haemocytometer as $1.3\times10^6$ cells per 5 mL of suspension. Roots of the five weeks old tomato transplants on the field were then exposed and the 5 mL of fungal spore suspension poured in the root zone. Inoculation was done after 7, 14, and 21 days to all 25 treatment plants.

**Nematicide application.** Three days after transplanting seedlings to the fields, a systemic nematicide (Velum Prime 400 SC) with active ingredient, Fluopyram was applied to treatments which required only *Fusarium* to be within the soil and also in the control plots. The nematicide (100 mL) was applied per plant to all 25 plants within each plot. The rate of application was 8 mL of the nematicide in 15 litres of water.

**Data analysis.** Agronomic data on plant height, plant girth, and chlorophyll content were taken at 4, 6, 8 and 10 weeks after transplanting tomato seedlings to the field. The chlorophyll content index (CCI) was measured using a MC-100 chlorophyll concentration meter (Apogee Instruments, North Logan, USA). Yield as well as data on biomass (fresh
and dry shoot and root weight) were taken at the end of the experiment.

To determine the wilt incidence, a wilt incidence scale (Nene et al., 1981) was used with wilt percentage and wilt severity computed as:

\[
\text{Number of plants wilted} \times 100\% \\
\text{Total number of plants}
\]

\[
\text{Sum (rating number \times number of plants in the rating)} \times 100\% \\
\text{Total number of plants \times highest rating}
\]

All data collected were subjected to analysis of variance (ANOVA), using GenStat12th edition software (VSN International Limited), and means separated, using least significant difference (LSD 5%). Where necessary values were transformed using square root (SQRT) for normality.

**RESULTS**

**Plant height and girth.** Plant height (A1, A2) and plant girth (B1, B2) were generally significantly different, as weeks progressed for both tomato varieties (Fig. 1). Mongal F1 had taller plants and wider girths than Petomech on both fields.

**Chlorophyll contents.** There were no significant differences (P>0.05) among the inoculated treatments and between both varieties on the two study sites (Fig. 2). Within the treatment applied in the University of Ghana farm, the chlorophyll content increased from the fourth week after transplanting to the eight’s week, after which it decreased till the tenth week. However, there was a general decrease in the chlorophyll content of both Mongal F1 and Petomech variety as the weeks progressed for the National Service farm.

**Fresh and dry shoot and root weights.** There were no significant differences (P> 0.05) in fresh shoot and dry shoot weights among the inoculated pathogen treatments of Mongal F1 and Petomech. However, there were significant differences (P<0.05) between the varieties; with Mongal F1 having higher values compared to Petomech on both fields.

On the university of Ghana farm, the fresh shoot weight plants in the control treatment had the highest weights in both Mongal F1(169.0 g) and Petomech (60.0 g) varieties, respectively (Table 1). Treatment NF14 had the highest weights in of 34.6 and 30.9 g in Mongal F1 and Petomech tomato varieties, respectively (Table 1). The dry root weight of Mongal F1 was significantly higher (Pde 0.05) from Petomech variety, ranging from 5.5 g to 11.6 g and 1.3 g to 4.8 g for Mongal F1 and Petomech varieties respectively (Table 1).

On the National service farm, Mongal F1 had 285.0 g as the highest fresh shoot weight from treatment NF14 and the least as 163.0 g from treatment N (Table 1). Petomech also, had the highest fresh shoot weight as 220.0 g and least fresh shoot weights as 137.0 g in treatments NF14 and N, respectively (Table 1). Generally, Mongal F1 had higher dry root weights than Petomech; except for treatment C where Petomech had 10.2 g and Mongal F1 had 8.0 g (Table 1).

**Incidence and severity.** There were significant differences (P<0.05) in wilt incidence between varieties and among the treatments imposed upon Mongal F1 and Petomech, varieties for both wilt incidence and severity (Table 2). Within Mongal F1 plants grown at the University of Ghana (UG) farm, the treatment, NF21 gave a wilt incidence of (73.3%), which was significantly higher than all other treatments. The control plants had the least wilt incidence (0.0%). Petomech plants that received the treatments NF7, NF14, and NF21 were not significantly different from each other, and had wilt incidences of 92.8, 72.8 and 86.1%, respectively. However, these were significantly different from the other treatments. Mongal F1 plants that received the treatments NF14 and NF21, had wilt severities of 57.3 and 66.7% and these were
Figure 1. Plant height and girth of Mongal F1 and Petomech plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* on University of Ghana (UG) and National Service (NS) farms 4 to 10 weeks after transplanting.
Figure 2. Chlorophyll contents of Mongal F1 and Petomech plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* on University of Ghana (UG) and National Service (NS) farms 4 to 10 weeks after transplanting.
TABLE 1. Fresh and dry shoot and root weights for Mongal F1 and Petomech tomato varieties after *Fusarium* inoculations on both fields

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fresh shoot weight (g)</th>
<th>Dry shoot weights (g)</th>
<th>Fresh root weight (g)</th>
<th>Dry root weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mongal F1</td>
<td>Petomech</td>
<td>Mongal F1</td>
<td>Petomech</td>
</tr>
<tr>
<td>UG FARM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF7</td>
<td>135.0a</td>
<td>49.0a</td>
<td>32.9a</td>
<td>12.6a</td>
</tr>
<tr>
<td>NF14</td>
<td>155.0a</td>
<td>42.0a</td>
<td>31.5a</td>
<td>17.9a</td>
</tr>
<tr>
<td>NF21</td>
<td>121.0a</td>
<td>48.0a</td>
<td>29.8a</td>
<td>10.6a</td>
</tr>
<tr>
<td>F</td>
<td>159.0a</td>
<td>91.0a</td>
<td>30.2a</td>
<td>21.2a</td>
</tr>
<tr>
<td>N</td>
<td>157.0a</td>
<td>18.0a</td>
<td>26.8a</td>
<td>6.6a</td>
</tr>
<tr>
<td>C</td>
<td>169.0a</td>
<td>60.0a</td>
<td>29.5a</td>
<td>18.9a</td>
</tr>
<tr>
<td>NS FARM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF7</td>
<td>181.0a</td>
<td>149.0a</td>
<td>37.1a</td>
<td>35.8a</td>
</tr>
<tr>
<td>NF14</td>
<td>285.0a</td>
<td>156.0a</td>
<td>37.2a</td>
<td>30.9a</td>
</tr>
<tr>
<td>NF21</td>
<td>227.0a</td>
<td>108.0a</td>
<td>54.2a</td>
<td>26.4a</td>
</tr>
<tr>
<td>F</td>
<td>233.0a</td>
<td>72.0a</td>
<td>41.7a</td>
<td>17.8a</td>
</tr>
<tr>
<td>N</td>
<td>163.0a</td>
<td>112.0a</td>
<td>38.4a</td>
<td>28.3a</td>
</tr>
<tr>
<td>C</td>
<td>203.0a</td>
<td>198.0a</td>
<td>33.8a</td>
<td>47.3a</td>
</tr>
</tbody>
</table>

NF7 = fungus inoculated 7 days after transplanting to naturally infested nematode infested, NF14 = fungus inoculated in 14 days after transplanting to naturally infested nematode field, NF21 = fungus inoculated 21 days after transplanting to naturally infested nematode field, F = fungus inoculated on naturally infested nematode field treated with nematicide, N = *Fusarium oxysporum* not inoculated on tomato plants after transplanting in *Meloidogyne incognita* infested field, C = Control (*Fusarium oxysporum* not inoculated on tomato plants, soil treated with nematicide), UG = University of Ghana and NS = National Service
### TABLE 2. Incidence and Severity of Tomato Wilt Disease in University of Ghana (UG) and National Service (NS) farms

<table>
<thead>
<tr>
<th>Treatments</th>
<th>University of Ghana Farm</th>
<th>National Service Farm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wilt incidence (%)</td>
<td>Wilt severity (%)</td>
</tr>
<tr>
<td></td>
<td>Treatment mean (%)</td>
<td>Treatment mean (%)</td>
</tr>
<tr>
<td>Mongal F1</td>
<td>Petomech</td>
<td>Mongal F1</td>
</tr>
<tr>
<td>NF7</td>
<td>26.7ab 92.8b 60</td>
<td>42.7ab 52ab 47.4</td>
</tr>
<tr>
<td></td>
<td>46.7bc 72.8b 60</td>
<td>57.3b 56.7ab 57</td>
</tr>
<tr>
<td>NF21</td>
<td>73.3c 86.1b 80</td>
<td>66.7b 82.7b 74.7</td>
</tr>
<tr>
<td>F</td>
<td>60.0bc 26.1a 43.4</td>
<td>48.7ab 54.7ab 51.7</td>
</tr>
<tr>
<td>N</td>
<td>20.0ab 34.1a 19.2</td>
<td>14.0a 25.3a 19.6</td>
</tr>
<tr>
<td>C</td>
<td>0.0a 12.8a 16.7</td>
<td>14.0a 28.0a 21</td>
</tr>
<tr>
<td>Variety mean (%)</td>
<td>41.2 54.1</td>
<td>40.6 49.9</td>
</tr>
</tbody>
</table>

Means having different letters in a column differ significantly (P<0.05). NF7 = fungus inoculated 7 days after transplanting to naturally infested nematode infested, NF14 = fungus inoculated in 14 days after transplanting to naturally infested nematode field, NF21 = fungus inoculated 21 days after transplanting to naturally infested nematode field, F = fungus inoculated on naturally infested nematode field treated with nematicide, N = *Fusarium oxysporum* not inoculated on tomato plants after transplanting in *Meloidogyne incognita* infested field, C = Control (*Fusarium oxysporum* not inoculated onto tomato plants and soil treated with nematicide) *Wilt incidence was scored using wilt incidence scale (Nene et al., 1981)
significantly different from those that received the treatments N and C, with wilt severities of 14% each. However, within the Petomech plants, only those that received the treatment NF21 had wilt severity (82.7%) which was significantly different from those that received the N and C treatments, with wilt severities of 25.3 and 28.0%, respectively.

Plants grown in the National Service (NS) farm did not show any significant differences (P>0.05) among the treatments for Mongal F1 plants for wilt incidence. However, Petomech plants that received the treatment N, had the least wilt incidence (60.7%), and this was significantly different (P<0.05) from the other treatments imposed. Petomech plants which received the treatments NF7, NF14, NF21, and F had wilt incidences of up to 100%. Mongal F1 plants that received the treatment C, had a wilt severity of 6.7%, which was the least and was significantly different (P>0.05) from plants that received the treatments NF14, NF21, and N with wilt severities of 29.3, 20.7 and 22.0%, respectively. Petomech plants that received the treatment C, had the least severity of 12.7%, and this was significantly different (P<0.05) from plants that received the treatments NF21 and F with wilt severities of 52.7 and 54.0%, respectively.

**Yield for Mongal F1 and Petomech plants.**
There were significant differences (P<0.05) between the two tomato varieties on both fields with Mongal F1 plants having relatively higher yields compared to Petomech (Table 3). Significant differences (P<0.05) existed among treatments for Mongal F1 plants grown in the UG farm. Mongal F1 plants within the UG farm, that received the treatment C, had the least yield of 557.0 kg ha⁻¹ and this was significantly different (P<0.05) from plants that received the treatment NF14 with yields of 1655.0 kg ha⁻¹. Significant differences (P>0.05) did not exist among Petomech plants for the various treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>UG farm yield (kg ha⁻¹)</th>
<th>NS farm yield (kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mongal F1</td>
<td>Petomech</td>
</tr>
<tr>
<td>NF7</td>
<td>1,027.0ab</td>
<td>434.0a</td>
</tr>
<tr>
<td>NF14</td>
<td>1,655.0b</td>
<td>682.0a</td>
</tr>
<tr>
<td>NF21</td>
<td>1,273.0ab</td>
<td>477.0a</td>
</tr>
<tr>
<td>F</td>
<td>914.0ab</td>
<td>373.0a</td>
</tr>
<tr>
<td>N</td>
<td>1,172.0ab</td>
<td>352.0a</td>
</tr>
<tr>
<td>C</td>
<td>557.0a</td>
<td>220.0a</td>
</tr>
</tbody>
</table>

Means having different letters in a column differ significantly (P<0.05). Values in parenthesis were square root (SQRT) transformed, and mean separation was by Duncan Multiple Range Test. NF7 = fungus inoculated 7 days after transplanting to naturally infested nematode infested, NF14 = fungus inoculated in 14 days after transplanting to naturally infested nematode field, NF21 = fungus inoculated 21 days after transplanting to naturally infested nematode field, F = fungus inoculated on naturally infested nematode field treated with nematicide, N = Fusarium oxysporum not inoculated on tomato plants after transplanting in Meloidogyne incognita infested field, C = Control (Fusarium oxysporum not inoculated onto tomato plants and soil treated with nematicide)
Mongal F1 were above 1,000.0 kg ha\(^{-1}\) for all treatments and these were generally higher than Petomech yields (Table 3).

**DISCUSSION**

*Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* are both soil borne plant pathogens, they initially infect root epidermis after which the fungi invades the vascular tissue of the plant. The simultaneous presence of both pathogens in roots resulted in lower dry root weights compared to the individual presence of either nematode or fungi for Mongal F1 tomato variety. In a previous study conducted, there was reduced vigour in tomato growth when both *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* infected the roots simultaneously (Bhagawati and Goswami, 2000). Simultaneous inoculation of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* (NF) and *Meloidogyne javanica* inoculated ten days prior to *Fusarium oxysporum* f. sp. *lycopersici* (NIF2) treatments, had the highest severity based on a rating scale developed (Song *et al*., 2004). Tomato genotypes Assila, Cochoro, and Marmande had severity values of 2.5 and 2.8; 3.0 and 3.5; and 4.0 and 3.5 for NF and N1F2 treatments, respectively, compared to uninoculated control with the least wilt severity (Beyan *et al*., 2019). In the present study, however, the highest wilt severity occurred when the University of Ghana field naturally infected with nematode were inoculated with fungi, 21 days (NF21) after transplanting in both tomato varieties.

Synergy between *Meloidogyne* spp. and other pathogens have been known to cause more damage worldwide (Rivera and Aballay, 2008). Increased damage by both organisms might have been caused by prior wounds created by *Meloidogyne incognita* favouring the fungi infection by creating rich metabolic substrates (Bhabesh *et al*., 2007). The synergism by both pathogens could have caused malfunction in the roots that weaken the tomato making it more susceptible to the fungi (Ganaie and Khan, 2011). The plants exhibit wilt as a result of root damage, eventual stunting and leading to death. In another study, the interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* was investigated using a susceptible tomato cultivar, Pusa Ruby (Kumar *et al*., 2017). The highest number of galls per plant (45.67) and egg masses per plant (31.33) were observed in plots with nematode inoculum only. This observation may be because of the absence of fungi in disrupting feeding sites of the nematodes for egg production. In our study, the highest number of eggs per gramme of root in Petomech variety were 13.0 and 47.0 on plots naturally infested with nematode only at the University of Ghana farm and National service farm, respectively. The interaction between *Fusarium oxysporum* f. sp. *lycopersici* and *Meloidogyne incognita* reduces galling and nematode reproduction on tomato plants (Ganaie and Khan, 2011; Nagesh *et al*., 2006). This is probably because feeding sites of the nematodes may be destroyed by the fungus, thus nematodes are unable to also complete their life cycles.

The low number of nematode eggs in roots of Mongal F1 variety, might be because some plants died; while others had early senescence, hence the nematodes were not able to complete their life cycles, thus reducing the number of second stage juveniles, as well as their reproductive factor. In other studies, low reproductive factors in chickpea and coleus have been attributed to low numbers of second stage juveniles of nematode (Senthamarai *et al*., 2006). In the present study, no galls were observed on the roots of Mongal F1 tomato plant (data not shown). This may be because Mongal F1 have some level of tolerance to *Fusarium oxysporum* f. sp. *lycopersici* (https://greenseeds.net/product/tomato-f1-mongal/) and *Meloidogyne incognita* (Okorley *et al*., 2006).
Meloidogyne incognita and Fusarium oxysporum f. sp. lycopersici on tomato growth

2018). Susceptibility of tomato to Fusarium wilt is as a result of physiological changes and root exudates that prevent the fungi from penetrating resistant cultivars (Juliatti et al., 1994). Additionally, nematode parasitism on roots create openings for entry of secondary micro-organisms such as Fusarium. Resistant varieties may compensate for loss of carbohydrates associated with Fusarium infections. Also, the ability of plants to resist root-knot nematodes, depends on their ability to allow second stage juvenile nematodes to penetrate roots and even after penetrating, cause juveniles to die before females are able to reproduce or even form galls (Gharabadiyan et al., 2012). This therefore leads to reduced nematode population densities in soils cropped with these tolerant varieties.

CONCLUSION

The presence of Meloidogyne incognita and Fusarium oxysporum f sp. lycopersici in the soil either as individuals or combined had similar effect on plant height, girth, fresh root weight and yield of tomato. When both Meloidogyne incognita and Fusarium oxysporum f.sp. lycopersici were together, their interaction resulted in reduced root weight and severe wilt in both tomato varieties.

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