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Response of tomato genotypes to *Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *lycopersici* co-infestation under glasshouse conditions

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Abstract

Tomato (*Solanum lycopersicum*) is an economically and nutritionally important vegetable crop grown worldwide. However, its yield in Ethiopia is very low as compared to the world and African average yield. Root-knot nematodes and Fusarium wilt are among the most economically important pathogens of tomato. A study was initiated with the objectives of determining the interaction effect of *M. javanica* (MJ) and *F. oxysporum* f. sp. *lycopersici* (FOL) disease complex and evaluating the response of selected tomato genotypes against this disease complex under glasshouse condition. A glasshouse experiment was laid out in a factorial randomized design with 18 treatment combinations of three tomato genotypes (Assila, Cochoro, Marmande) and two pathogens (MJ and FOL) with four replications. At four true leaf stage, tomato seedlings were inoculated with the suspension of MJ at a rate of 3000 second-stage infective juveniles (J_2) and 10 ml FOL suspension ($1x10^6$ spores per milliliter) per pot around the root rhizosphere one week after transplanting except the control which was not inoculated. The result revealed that concomitant inoculation of MJ and FOL (NF) followed by MJ first and FOL ten days after inoculation (N1F2) was found to be highly significant in reducing the tomato growth, biomass and pathogen related parameters compared to the un-inoculated control or single pathogen inoculated treatments. Among the three tomato genotypes evaluated, Assila was found to be moderately resistant as measured by the lower number of root galls and egg-masses per plant compared to the susceptible Marmande genotype. Hence, further study is required to evaluate the performance of Assila genotype in hot spot areas of *Meloidogyne* species and *Fusarium* species infested farmer's field conditions.

Keywords: Co-occurrence, management, resistance, synergistic effects, tomato genotypes

Tomato (*Solanum lycopersicum*) is the second most important Solanaceous vegetable crop after potato. The world tomato production reached to more than 163.4 million metric tons cultivated on more than 4.6 million hectares of land (FAOSTAT, 2016). In Ethiopia, since 1994 up to 2014/15, tomato acreage increased to 5338 ha with a total production of 55,635 MT (Ambecha *et al*., 2015). Recently tomato production is being carried out in the Upper Awash and Central Rift Valley under irrigated and rain-fed conditions having high potential with possible further expansion into different agro-ecology of Ethiopia if production constraints are prevented (Seid *et al*., 2017).

Despite, the importance of tomato in Ethiopia, the average yield was very low (8 MT ha^{-1}) compared to average yields of 51, 41, 36, 34 and 21 MT ha^{-1} in America, Europe, Asia, the entire world and Kenya, respectively (FAOSTAT, 2014). Among the most important pathogens

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that attack tomato are the root-knot nematodes (RKN) and Fusarium wilt. Root-knot nematodes are serious and economically most important pathogen of many cultivated crops around the world (Trifonova *et al*., 2009). They represented an important constraint on the delivery of global food security (Jones *et al*., 2013). According to Nicol *et al*., (2011) damage caused by plant parasitic nematodes (PPN) had been estimated at US $ one billion per year. This was likely to be a significant reduction of the actual figure as many growers in developing nations are unaware of even the existence of PPN (Jones *et al*., 2013). The four major species of RKN i.e., *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* were documented in Ethiopia (Seid *et al*., 2017) which were known to cause the vast majority of crop damage (Moens *et al*., 2009).

Meloidogyne javanica predominately occur in the major tomato growing areas of Ethiopia such as Rift Valley, Upper Awash and East Hararghe (Seid *et al*., 2017). Fusarium wilt of tomato was considered as one of the most important diseases of tomato both in field and glasshouse grown tomatoes worldwide (Sheu *et al*., 2006; Amini *et al*., 2010; Abdel-Monaim, 2012). *Fusarium oxysporum* f. sp. *lycopersici* is the most common pathogen that causes wilt of tomatoes leading to huge economic losses. Most infections originate from the population associated with infected tomato debris (Kumar *et al*., 2017). Many reports on the interactions of soil borne plant pathogens with RKN maintain the observation that the damage caused by nematodes increases damage due to secondary infecting pathogens (Wardle *et al*., 2004). Root diffusates from root-knot and Fusarium infected plants stimulate the fungus in the rhizosphere; at the same time diffusates also suppress actinomycete, antagonists of Fusarium.

A report indicated that co-infection by *Meloidogyne* species and *F. oxysporum* f.sp. *lycopersici* has synergistic effects on tomato. It reduced the number of fruits per plant, plant height, and fruit weight (Muthamia, 2011). Infection of tomato plant by *Meloidogyne* species predisposes the crop to *F. oxysporum* f.sp. *lycopersici* infection, leading to much reduction in fruit weight. Khpalwak (2012) also reported the highest tomato wilt disease incidence due to infection by the nematode before the fungus experimentally. In addition, Lobna *et al*., (2016) reported significant reduction of shoot, root and total plant length on tomato plant inoculated with *F. oxysporum* f.sp. *lycopersici* and *Meloidogyne* spp. simultaneously, and with *Meloidogyne* 10 days before *Fusarium*.

Several studies proved the occurrence of RKN and the Fusarium in major tomato growing areas of Ethiopia but there is a lack of data about the interaction effect of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* disease complex and response of tomato genotypes to the interaction effect of of the co-infestation. Hence, the objectives of this study were 1) to determine the interaction effects of *M. javanica* and *F. oxysporum* f. sp. *lycopersici* disease complex on selected tomato genotypes and 2) to evaluate the response of selected tomato genotypes against the disease complex involving *M. javanica* and *F. oxysporum* f. sp. *lycopersici* under glasshouse conditions.

Materials and Methods

Source, maintenance and inoculation of *M. javanica* population: The *M. javanica* used in this experiment was a pure culture maintained on a known susceptible tomato cultivar Marmande after DNA based identification at Ghent University, Belgium by Seid *et al*., (2017). The pure culture of the *M. javanica* population was maintained for ten weeks on Marmande. The culture was multiplied on several pots using the same genotype to get enough inoculum to help initiate the actual experiment. Infected tomato plants were uprooted and roots were washed gently with tap water to remove the adhered soil particles. Then the washed roots were submerged on Phloxine B (0.15 g/L) for 15 minutes (Holbrook *et al*., 1983) to clearly observe the egg-masses and facilitate

counting. The second stage juveniles (J_2) were extracted from the infected tomato roots using a modification of Baermann funnel technique (Hooper *et al*., 2005). At four leaf stage tomato seedlings were inoculated with the suspension of *M. javanica* at a rate of 3000 second-stage juveniles (J_2) per pot around the root rhizosphere one week after transplanting except the controls which were not inoculated. After inoculation the exposed soil was returned to the roots and lightly watered immediately post inoculation.

Extraction of *Meloidogyne javanica* population: From the pot test a subsample of 100 grams of soil after thoroughly mixing, was taken and processed using a modified Baermann funnel technique (Hooper *et al*., 2005).

Source and maintenance of *Fusarium oxysporum* f.sp. *lycopersici*: Monoconidial isolate of *F. oxysporum* f.sp. *lycopersici* (FOL-white) was used in this study. This isolate was collected from infected tomato plant from the Central Rift valley (CRV), Ethiopia and was characterized according to Leslie & Summerell (2006). Diseased plant specimens (stem bases and roots) were subjected to running tap water and then rinsed with distilled water. Diseased specimens were cut into pieces (2 cm) and surface sterilized with 2% NaOCl for 2 minutes followed by three changes of sterile distilled water and dried in between two sterilized blotting paper. Sterilized and dried specimens were plated out on potato dextrose agar (PDA) media in sterile Petri-dishes and incubated at 25 ± 2 ºC for 7-9 days. Isolated colonies were sub-cultured onto fresh plates until pure cultures were obtained. Pure cultures obtained were identified by visual examination and viewing under compound light microscopes. Identification of *F. oxysporum* isolate was based on the description of pathological, morphological and culture characteristics (Booth, 1971). A loop full of fungal culture grown on PDA plates were taken on a glass slide and observed with image analyzer under 40 X magnifications for the presence of conidia (macro and micro) and conidiophores. After confirming the spores, the cultures were purified by single spore isolation technique.

Pathogenicity test: Pathogenicity test was used to confirm the identified *F. oxysporum* formae specials. Three week old susceptible (Marmande) tomato genotype seedlings were inoculated by standard root dip method (Srivastava *et al*., 2009). Four treatments: (i) Marmande + White FOL isolate (FOL-W), (ii) Marmande + Pink FOL isolate (FOL-P), (iii) Marmande + Violate FOL isolate (FOL-V) and (iv) un-inoculated check with five replications were set under controlled glasshouse conditions. Pure culture of the aggressive fungal isolate (FOL-W) was used as a starting culture for the disease complex or interaction study. The FOL-W was multiplied with PDA medium on 9 cm diameter petri-dishes to get enough inoculum to help initiate the actual experiment. The three tomato genotypes: Assila, Cochoro and Marmande were inoculated with FOL-W conidia suspension based on the treatment requirement. Inoculum density (conidia concentration) of the pathogen was adjusted to $3x10^6$ conidia/ml/plant (Lobna *et al*., 2016) using a Haemocytometer and 10 ml of this solution was delivered into holes in the soil surface of pots.

Source of the tomato genotypes: The tomato genotype Cochoro was obtained from Melkassa Agriculture Research Center, Ethiopia. Assila was obtained from Seminis (only for research purpose) and Marmande from ILVO, Belgium. Marmande is a known *M. javanica* susceptible genotype. Assila and Cochoro were found resistant and moderately resistant for both *M. incognita* and *M. javanica* (Seid *et al*., 2017).

Soil sterilization and transplanting of tomato seedlings: Soil sterilization was done according to (Walter, 1927) procedure. Soil free of lumps and stones was collected from Haramaya University, Raree Research Station and mixed thoroughly with sand and compost in 1:2:1 proportion. The composite soil has been further sterilized under oven at 100 ºC for 2 h. Two kilograms of autoclaved soil was filled into each

pot of 20 cm diameter size. Seedlings of Assila, Cochoro and Marmande tomato genotypes were raised in glasshouse on a large open plastic pan containing a sterilized soil. The seedlings were irrigated until they were ready for transplanting. When the seedlings had reached four true leaf stages, they were carefully lifted from the plastic pan and transplanted into pots.

Experimental design and treatment combinations: A glasshouse experiment was conducted at Haramaya University, Raree Research Station located in eastern Hararghe, Ethiopia. The experiment was laid in a factorial (tomato genotype and different level of the disease complex) randomized design with four replications using a total of 18 treatment combinations (Table 1). Plants were maintained in a glasshouse at the temperature of 27 ± 3 ºC. The experiment lasted a total of eight weeks after inoculation.

Data Collected: The data on *M. javanica* and *F. oxyspourm* f.sp. *lycopersici* and plant related parameters were collected eight weeks after inoculation.

Number of galls per plant: the plant roots were gently washed with tap water to remove adhering soil particles. Then the number of galls per root system was counted manually aided with hand lens.

Root gall index (RGI) and egg-mass index (EMI): These indices were determined per plant from each pot and based on (Taylor & Sasser, 1978) were scored from 0 to 5 scale; where, 0 = no galls or egg-masses; 1 = 1-2 galls or egg-masses; 2 = 3-10 galls or egg-masses; 3 = 11-30 galls or egg-masses; 4 = 31-100 galls or egg-masses and 5 = >100 galls or egg-masses.

Number of egg-masses per root system: Roots containing egg-masses were soaked in a solution of Phloxine B (15 mg 100 ml-1 in tap water) for 15 min and then the roots were rinsed in tap water to remove residual stain. The egg-masses were stained pink to red and observed and counted (Coyne & Ross, 2014).

Final population density per pot (*Pf*): Pf was estimated from organic (root) and mineral fraction (soil) per pot. The mean number of J_2 in the roots were estimated from the whole root system after extracting nematodes from a subsample of 5 g roots per plant based on (Hussey & Barker, 1973). Nematodes from soil samples were extracted using a modified Baermann funnel technique. It was expressed as J_2 per 100 gram of soil. Reproduction factor (RF) was obtained from the ratio of *Pf/Pi*

Days to 50% flowering (50%FL): Number of days from the date of inoculation to the appearance of 50% flowering was recorded and the mean value was computed.

Root length (RL): The root length per plant was measured once after harvesting of above ground part from the soil level to the tip of 75 % of roots end and expressed in centimeters.

Plant height (PH): PH was measured from the soil level to the main apex of the plant eight weeks after transplanting and mean values calculated per treatment and expressed in centimeters.

Number of flowers per plant (NFPP): The total number of flowers was counted per plant per pot starting from three weeks after transplanting until eight weeks after transplanting once per week and mean values computed.

Fresh shoot weight (FSW): the tomato plant was cut at the crown level in each pot and the fresh shoot weight was measured (in gram) using electronic balance soon after cutting.

Dry shoot weight (DSW): the shoots were put in paper bag and brought to laboratory just after taking the fresh weight and kept in an oven at 105ºC for 24 hours, and the dry shoot weight was measured (in gram) using electronic balance.

Fresh root weight (FRW): After cutting the top parts of the tomato plants, the plant roots were gently washed with tap water to remove adhering soil particles. Then, the fresh root weight was measured (in gram) using electronic balance.

Disease assessment: disease severity (%) in the glasshouse was assessed weekly (visual observation) starting one week after inoculation up to eight weeks and calculated per tomato plant according to Song *et al*., (2004); where, 0 = healthy plant without any wilt symptom; 1= plants showed yellowing of leaves and wilting ranging from (1-20%); 2 = plants showed yellowing of leaves and wilting ranging from (21-40%); 3 = plants showed yellowing of leaves and wilting ranging from (41-60%) and 4 = when all leaves become yellow and complete wilted plant.

Area under the disease progress curve (AUDPC) was calculated according to method of (Shaner & Finney, 1977) using the formula:

$$AUDPC = \sum_{i=1}^{n-1} [0.5(xi + 1 + \text{xi})(\text{ti} + 1 - \text{ti})]$$

Where n is total number of recording made, ti is time of the i^{th} recording in days from the first recording date; xi is disease severity rating at i^{th} recording.

Data analysis: The glasshouse data was subjected to analysis of variance (ANOVA) procedures using Genstat 15^{th} edition statistical software. The differences among treatment means were separated using Fisher's unprotected LSD test at 5% and 1% level of significance.

Results

Identification of *F. oxysporum*. The length*breadth of macro-conidia usually varied between (15.9-46.98 x 1.83-4.88µm) and that of micro-conidia was (6.75-13.56 x 1.93-3.4µm) under 40x magnification. The number of septation of macro-conidia ranges from 1.5-4.3. The shape of macro-conidia varies from straight, slightly curved to sickle shaped. The shape of micro-conidia was oval, elliptical or kidney and usually 0-septated with infrequent occurrence of a single septation (Fig. 1). False head structures with short monophialides were there in the aerial mycelium while it was observed under compound microscope without disturbance of the existing mycelium (Fig. 2).

Pathogenicity of *F. oxysporum*. Various symptoms on aerial parts and within the stem tissues of tomato plants infected with *F. oxysporum* were noted starting at 33 DAI. Yellowing of the lower leaves at early stage of the plant and leaf necrosis and later, dropping due to the infection were the most prominent symptoms. However, there was no statistically significant difference in virulence among the isolates in mean disease severity and area under disease progress curve (Table 2). As expected, however, there was significant difference ($p<0.05$) between the inoculated treatments and the un-inoculated check regardless of the isolates (Table 2).

Number of root galls per plant (RGPP) and root gall index (RGI): The maximum number of RGPP (539.2) was recorded in simultaneously inoculated *M. javanica* and *F. oxysporum* f.sp. *lycopersici* followed by *M. javanica* inoculated ten days prior to *F. oxysporum* f.sp. *lycopersici* (525.0) on Marmande tomato genotype (Table 3). There was a highly significant difference on the number of RGPP between Cochoro and Marmande when inoculated with *F. oxysporum* f. sp. *lycopersici* ten days prior to *M. javanica*. A highly statistically significant ($p<0.01$) difference was found on the number of RGPP between Assila and Cochoro in all treatment combinations containing *M. javanica*. Less number of RGPP (75.0) was recorded on Assila when inoculated with *M. javanica* alone followed by *M. javanica* ten days prior *F. oxysporum* f.sp. *lycopersici* (79.8). The interaction effect of the three tomato genotypes and *M. javanica* and *F. oxysporum* f. sp. *lycopersici* on the number of RGPP was found non-significant ($p \leq 0.01$).

There was a non-significant ($P \leq 0.05$) interaction effect between the tomato genotypes and *M. javanica* and *F. oxysporum* f. sp. *lycopersici* on RGI. The highest RGI (5.0) was recorded with simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* in all the three tomato genotypes while the lowest RGI was recorded with *M. javanica* inoculated alone and *F. oxysporum* f.sp. *lycopersici* inoculated ten days prior to *M. javanica* (3.0) on Assila (Table 3; Fig.3a-c).

Nematode multiplication, number of galls per plant and number of egg-masses per plant were adversely affected on simultaneous and sequential inoculation of both pathogens in all the treatment combinations.

Number of egg-mass per plant (EMPP) and egg-mass index (EMI): The mean maximum number of EMPP (437.3) was obtained in *M. javanica* inoculated ten days prior to *F. oxysporum* f.sp. *lycopersici* on Marmande genotype followed by simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* on Cochoro tomato genotype (Table 4).

There was a highly significant ($P \leq 0.01$) difference on the mean number of egg-masses produced between Assila and that of Cochoro and Marmande genotypes when inoculated with all treatment combinations containing *M. javanica*. The minimum number of EMPP (26.0) was recorded with *F. oxysporum* f.sp. *lycopersici* inoculated ten days prior to *M. javanica* on Assila tomato genotype.

The highest mean number of EMI (5.0) was observed in *M. javanica* inoculated ten days prior to *F. oxysporum* f.sp. *lycopersici* on Marmande and Cochoro genotypes while the lowest mean number of EMI (3.25) was obtained in *F. oxysporum* f. sp. *lycopersici* inoculated ten days prior to *M. javanica* on the tomato genotype Assila. There was a highly significant difference ($P \leq 0.01$) on the mean number of EMI between Assila and Marmande with *M. javanica* inoculated ten days prior to *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *lycopersici* inoculated first and *M. javanica* ten days later.

However, there was no significant difference obtained on the mean number of EMI between Cochoro and Marmande at all treatment combinations (Table 4).

Final population density (Pf) and reproduction factor (RF): The highest mean *Pf* of *M. javanica* J_2 (76073) from 100 gram soil and the entire root system was recorded from *M. javanica* inoculated ten days prior to the *F. oxysporum* f.sp. *lycopersici* on Marmande with *Pi* of 3000 J_2 per pot while the lowest mean *Pf* of J_2 (13589) was obtained from the treatment combination *F. oxysporum* f.sp. *lycopersici* inoculated ten days prior to *M. javanica* on Assila genotype.

There was a highly significant ($P \leq 0.01$) difference among the three tomato genotypes on the mean number of *Pf* when inoculated with *F. oxysporum* f.sp. *lycopersici* ten days prior to *M. javanica*. There was highly significant difference ($P \leq 0.01$) on the mean number of *Pf* of J_2 recovered from Cochoro and Marmande when inoculated with *M. javanica* alone and *F. oxysporum* f.sp. *lycopersici* ten days prior to *M. javanica* (Table 5).

The highest RF (507.2) was obtained from the treatment combination of *M. javanica* inoculated ten days prior to *F. oxysporum* f. sp. *lycopersici* on Marmande genotype while the lowest RF (90.6) was obtained from *F. oxysporum* f.sp. *lycopersici* inoculated ten days prior to *M. javanica* on Assila genotype.

All the three tomato genotypes showed a highly significant difference ($P \leq 0.01$) on RF when inoculated with *M. javanica* alone and *F. oxysporum* f.sp. *lycopersici* inoculated ten days prior to *M. javanica* (Table 5).

Table 1. Treatment combinations of three tomato genotypes and two pathogens (*Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *lycopersici*) under glasshouse conditions in 2017/18.

S.N0.	Treatment combinations	Abbreviations
1	Assila + MJ alone	Nematode alone (N)
2	Assila + (FOL+MJ) simultaneously	Nematode + Fungus (NF)
3	Assila + MJ 10 days prior to FOL	Nematode first then fungus second (N1F2)
4	Assila + FOL 10 days prior to MJ	Fungus first then nematode second (F1N2)
5	Assila + FOL alone	Fungus alone (F)
6	Assila un-inoculated	Control
7	Cochoro + MJ alone	Nematode alone (N)
8	Cochoro + (FOL+MJ) simultaneously	Nematode + Fungus (NF)
9	Cochoro + MJ 10 days prior to FOL	Nematode first-Fungus second (N1F2)
10	Cochoro + FOL 10 days prior to MJ	Fungus first then nematode second (F1N2)
11	Cochoro + FOL alone	Fungus alone (F)
12	Cochoro un-inoculated	Control
13	Marmande + MJ alone	Nematode alone (N)
14	Marmande +(FOL+MJ) simultaneously	Nematode + Fungus (NF)
15	Marmande + MJ 10 days prior to FOL	Nematode first-Fungus second (N1F2)
16	Marmande + FOL 10 days prior to MJ	Fungus first then nematode second (F1N2)
17	Marmande + FOL alone	Fungus alone (F)
18	Marmande un-inoculated	Control

Table 2. Virulence analysis of the *F. oxysporum* isolates measured by disease severity and AUDPC.

Fusarium oxysporum isolates	Disease severity	AUDPC
FOL-W	3.188 b	71.75 b
FOL-P	3.062 b	71.75 b
FOL-V	2.938 b	64.75 b
Control	0.000 a	0.00 a
LSD (5%)	0.3194	7.80
CV (%)	9.0	9.7

Where, means followed by the same letter (s) within the column in each parameter are not significantly different at 5% level of significance; LSD = list significant difference; CV =coefficient of variation and AUDPC=area under disease progress curve.

Table 3. Response of three tomato genotypes to *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* based on mean number of root galls per plant (RGPP) and root gall index (RGI) at Haramaya University glasshouse in 2017/18.

Treatments	RGPP			RGI		
	Tomato Genotypes					
	Assila	Cochoro	Marmande	Assila	Cochoro	Marmande
'N	75.0 b	446.0 e	421.2 e	3.0 b	5.0 d	5.0 d
NF	145.8 c	463.5 e	539.2 f	5.0 d	5.0 d	5.0 d
N1F2	79.8 b	453.5 e	525.0 f	3.3 c	5.0 d	5.0 d
F1N2	68.8 b	358.2 d	438.0 e	3.0 b	5.0 d	5.0 d
F	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Control	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
LSD at 5%		59.48			0.17	
LSD at 1%		79.21			0.22	
CV%		18.8			3.70	

Where, means followed by the same letter (s) within a row and column in each parameter are not significantly different at 5% level of significance. LSD (5%) = Least significant difference at 5% level of significance; LSD (1%)=Least significant difference at 1% level of significance; CV (%) = Coefficient of variation.

Table 4. Response of three tomato genotypes to *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* based on the mean number of egg-mass per plant (EMPP) and egg-mass index (EMI) at Haramaya University glasshouse in 2017/18.

Treatments	EMPP			EMI		
	Tomato genotypes					
	Assila	Cochoro	Marmande	Assila	Cochoro	Marmande
N	(1.77 cd) 65.2	(2.43 efg) 275.0	(2.36 ef) 244.3	4.00 bcd	5.00 d	4.75 cd
NF	(1.92 d) 87.8	(2.52 fg)344.8	(2.38 efg) 258.8	3.75 c	4.50 bc	4.75 cd
N1F2	(1.54 bc) 36.0	(2.39 efg)250.0	(2.62 g) 437.3	3.75 bc	5.00 d	5.0 d
F1N2	(1.42 b) 26.0	(1.96 d) 121.3	(2.25 e) 225.0	3.25 b	4.30 bcd	4.75 cd
F	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.000 a
Control	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.000 a
LSD at 5%	0.2503			1.0254		
LSD at 1%	0.3334			1.3655		
CV%	12.4			24.7		

Number in the brackets was logarithmic transformations (log y+1), where y, original value. Where, means followed by the same letter (s) within a row and column in each parameter are not significantly different at 5% level of significance.

Table 5. Response of three tomato genotypes to *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* based on the mean number of final population density (*Pf*) and reproduction factor (RF) at Haramaya University glasshouse in 2017/18.

Treatments	*Pf*			RF		
	Tomato Genotypes					
	Assila	Cochoro	Marmande	Assila	Cochoro	Marmande
N	(4.17bc) 15479	(4.653 e) 46425	(4.803 fg) 64440	(1.999 bc) 103.2	(2.478 e) 309.5	(2.628 fg) 429.6
NF	(4.28c) 20539	(4.772 efg) 59447	(4.776efg) 60027	(2.110 c) 136.9	(2.597efg) 396.3	(2.601^{efg}) 400.2
N1F2	(4.212bc) 17075	(4.821fg) 66397	(4.850 g) 76073	(2.040 bc) 113.8	(2.646 fg) 442.6	(2.675) 507.2
F1N2	(4.107 b) 13589	(4.492 d) 31697	(4.688 ef) 49082	(1.936 b) 90.6	(2.318d) 211.3	(2.513 ef) 327.2
F	0.000a	0.000a	0.000a	0.000a	0.000a	0.000a
Control	0.000a	0.000a	0.000a	0.000a	0.000a	0.000a
LSD at 5%		0.148			0.147	
LSD at 1%		0.197			0.196	
CV%		3.4			6.5	

Where, means followed by the same letter (s) within a row and column are not significantly different at 5% level of significance.

Days to 50% flowering (50% FL) and root length (RL): The means of the treatments revealed that the longest days to 50% FL or late flowering (34.75 days) was observed with concomitant inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* followed by *M. javanica* inoculated ten days prior to the *F. oxysporum* f.sp. *lycopersici* (32.5 days). The shortest days to 50% FL (27.75) was obtained on the control treatment on Marmande genotype. There was statistically highly significant ($P \leq 0.01$) difference on the number of days to 50% FL on Assila genotype with simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* (Table 6). A significantly reduced RL (24.5 cm) was recorded from concomitant inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* followed by (26.0 cm) with *M. javanica* inoculation alone. The highest mean (33.75 cm) RL was recorded on the control treatment on Assila genotype. The result from this study indicated a synergy between *M. javanica* and *F. oxysporum* f. sp. *lycopersici* as proved from the increased plant root damage (Fig. 3a-c).

Plant height (PH): The highest mean PH (67.25 cm) was recorded from control treatments while the lowest (53.00 cm) was recorded from concomitant inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici*. Among the three tomato genotypes studied, Assila was found moderately resistant and the highest mean PH (65.67cm) was achieved on it. In all treatment combinations the PH and other growth and biomass parameters were found reduced significantly compared to the control. There was highly significant ($P \leq 0.01$) difference on the mean PH between Assila (moderately resistant and the susceptible Marmande (Table 7).

Number of flowers per plant (NFPP): The highest mean NFPP (19.50) was recorded in the control treatment while the lowest mean NFPP (14.83) was recorded in case of simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* treatment (Table 7). There was

a highly significant difference ($P \leq 0.01$) on the mean NFPP on Assila and Marmande genotypes with simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* and the control treatment. The lowest mean NFPP (14.67) was recorded from Marmande genotype.

Fresh shoot weight (FSW): FSW was significantly reduced in all treatments compared to the control (70.60 g) followed by (63.52 g) with *F. oxysporum* f.sp. *lycopersici* inoculated treatment while the lowest mean FSW was recorded from treatments with simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* (52.04 g) which had highly significant ($P \leq 0.01$) difference (Table 7). The FSW was highly reduced on Marmande (47.78 g) compared to the moderately resistant Assila (79.74 g) genotype.

Dry shoot weight (DSW): DSW was found to be reduced with highly statistical significant ($P \leq 0.01$) difference in all treatments among the studied tomato genotypes compared to the control treatment. The lowest mean DSW (5.52) of the studied tomato genotypes was obtained from concomitant inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* treatment. There was a highly significant ($P \leq 0.01$) difference among tomato genotypes on the mean DSW. The highest mean DSW (7.86) was recorded from Assila while the lowest DSW (4.89) was obtained from Marmande Genotype (Table 7).

Fresh root weight (FRW): The lowest mean FRW was obtained from simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* (16.69 g) followed by *M. javanica* (17.72 g) alone treatment compared to the control (23.22 g). In general, it was observed that the effect of *M. javanica* was more pronounced on RL than on shoot parameters. However, there was no statistically significant ($P \leq 0.05$) difference on FRW among the studied tomato genotypes (Table 7).

Disease severity and Area under Disease Progressive Curve (AUDPC): Mean disease severity was higher on Marmande (the susceptible check) than on Assila (moderately resistant cultivar) (Table 8). Effects of nematode *Fusarium* complex significantly differed on all dates of disease assessment except on the first two weeks of disease assessment (Fig. 4). The control invariably had the lowest disease incidence values with assessment dates and genotypes. On the other hand, simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* and *M. javanica* inoculated ten days prior to *F. oxysporum* f.sp. *lycopersici* treatments had the highest severity compared to un-inoculated control in all genotypes. The AUDPC calculated for disease severity rating was significantly different ($P = 0.05$) among treatments. The maximum AUDPC value of 138, 114 and 96 was estimated when simultaneously *M. javanica* and *F. oxysporum* f.sp. *lycopersici* was inoculated on the genotype Marmande, Cochoro and Assila, respectively and the lowest AUDPC value was obtained from control, which was also significantly lower than AUDPC values for the rest of the other treatments (Table 8).

Discussion

Nematode multiplication, number of galls and egg-masses were found adversely affected after concomitant inoculation of nematode and fungus. In this study, there was no relationship observed between the number of root galls per plant and egg-masses per plant. This is in agreement with the findings of Trudgill & Blok (2001) in which they reported the absence of direct correlation between root galls and egg-masses number per plant. The reproduction of nematode and galling on roots decreased with pre-inoculation of fungus, while the infection of fungus increased in the presence of nematode (Haseeb *et al*., 2007). Ganaie & Khan (2011) also reported significant reduction of tomato plant growth, biomass and yield on simultaneous inoculation of *M. incognita* and *F. solani*. The minimum number of egg masses recorded with *F. oxysporum* f. sp. *lycopersici* inoculated ten days prior to *M. javanica* suggested the role

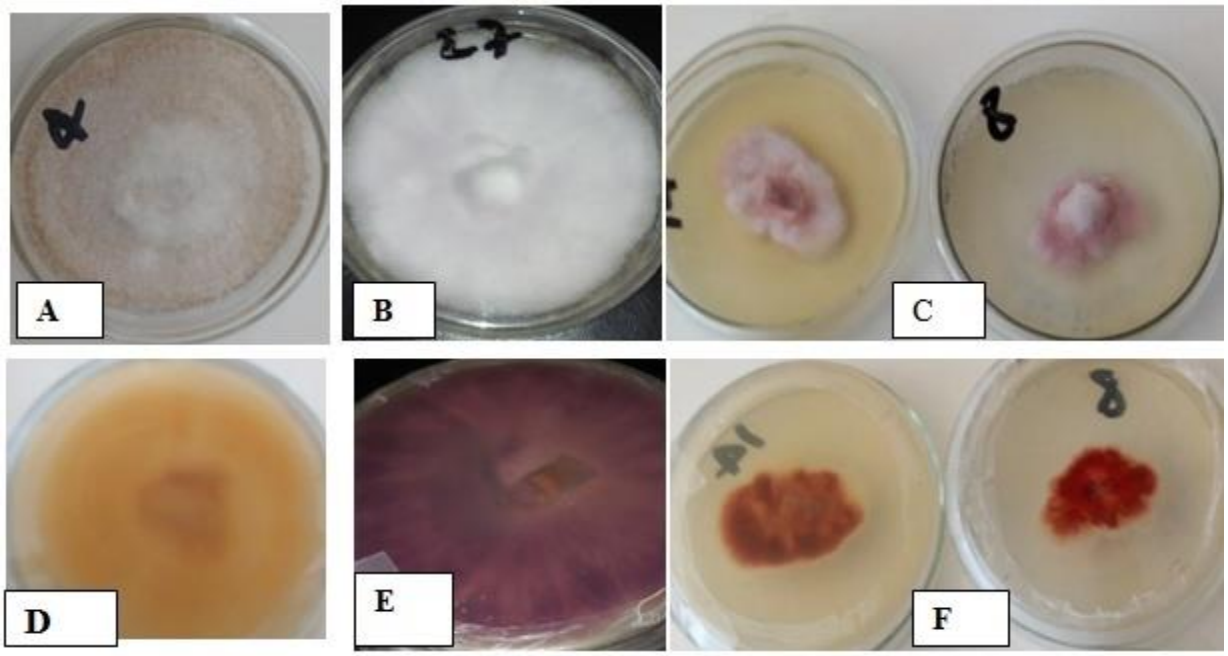

Fig. 1. Morphotypic isolates of *Fusarium oxysporum*; White (A: in face, D: back), Violet (B: in face, E: back) and Pink (C: in face, F: back)

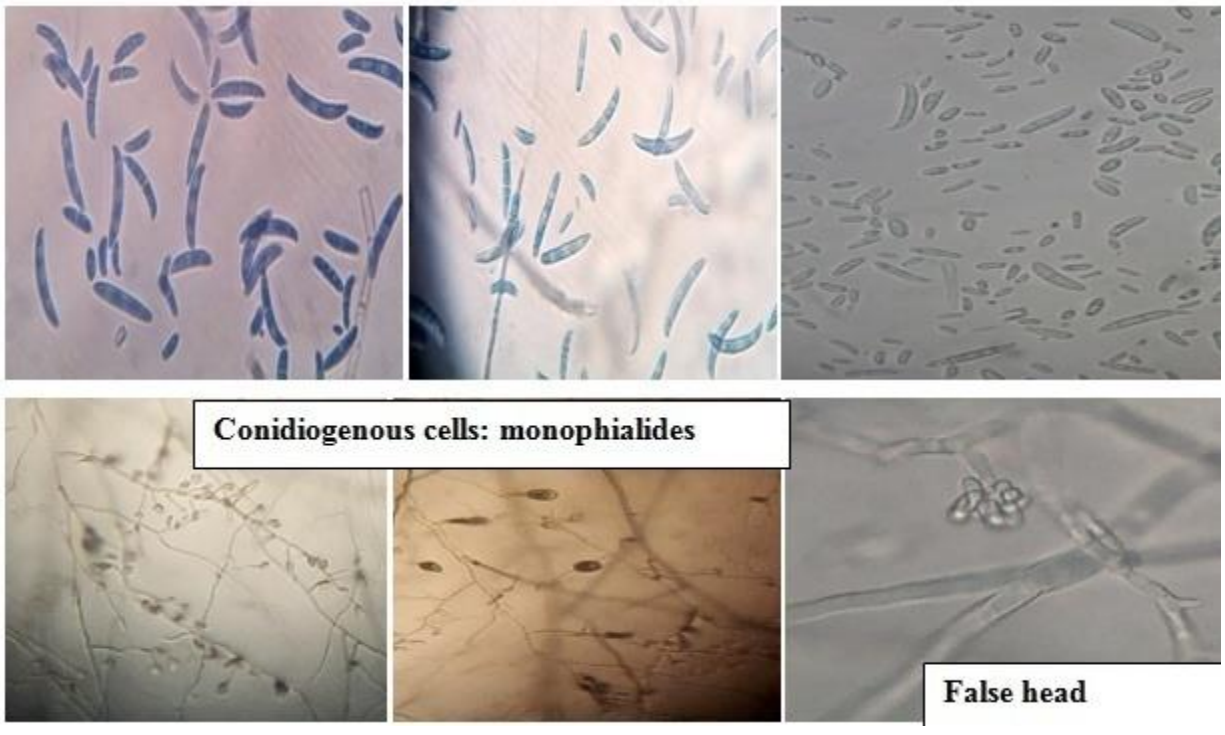

Fig. 2. Macro-and micro-conidia, Conidiogenous cells and false heads of FOL.

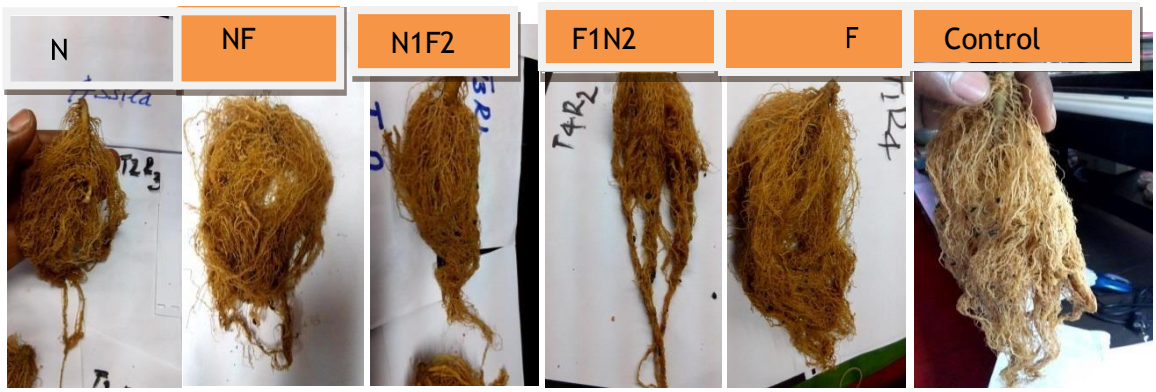

Fig.3a. Root damage of 'Assila' genotype after *Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *lycopersici* sequential inoculation on pot experiments under glasshouse conditions.

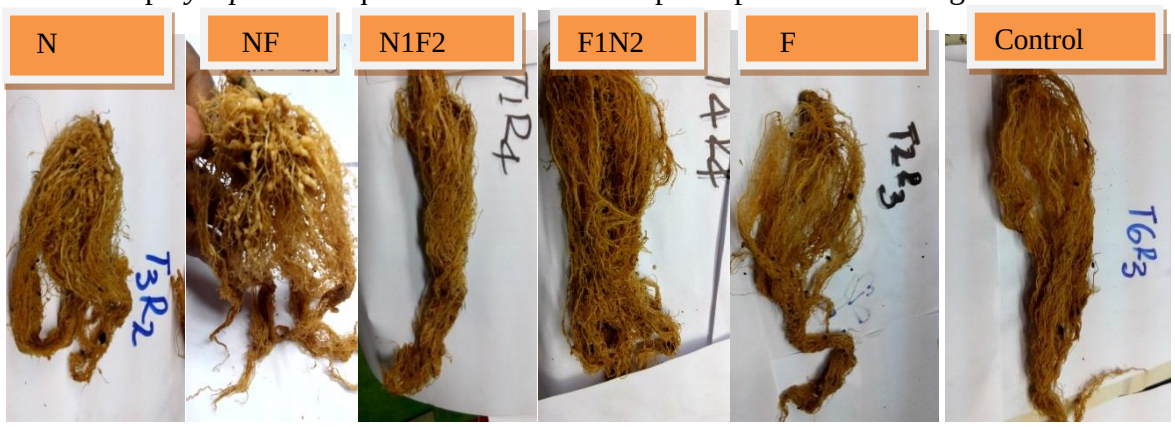

Fig.3b. Root damage of 'Cochoro' genotype after *Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *lycopersici* sequential inoculation on pot experiments under glasshouse conditions.

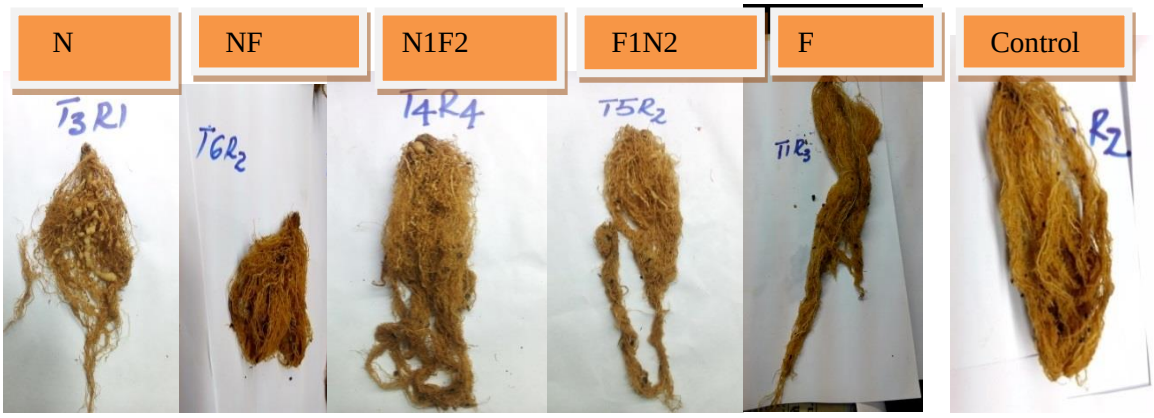

Fig.3c. Root damage of 'Marmande' tomato genotype due to *Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *lycopersici* sequential inoculation on pot experiments under glasshouse conditions.

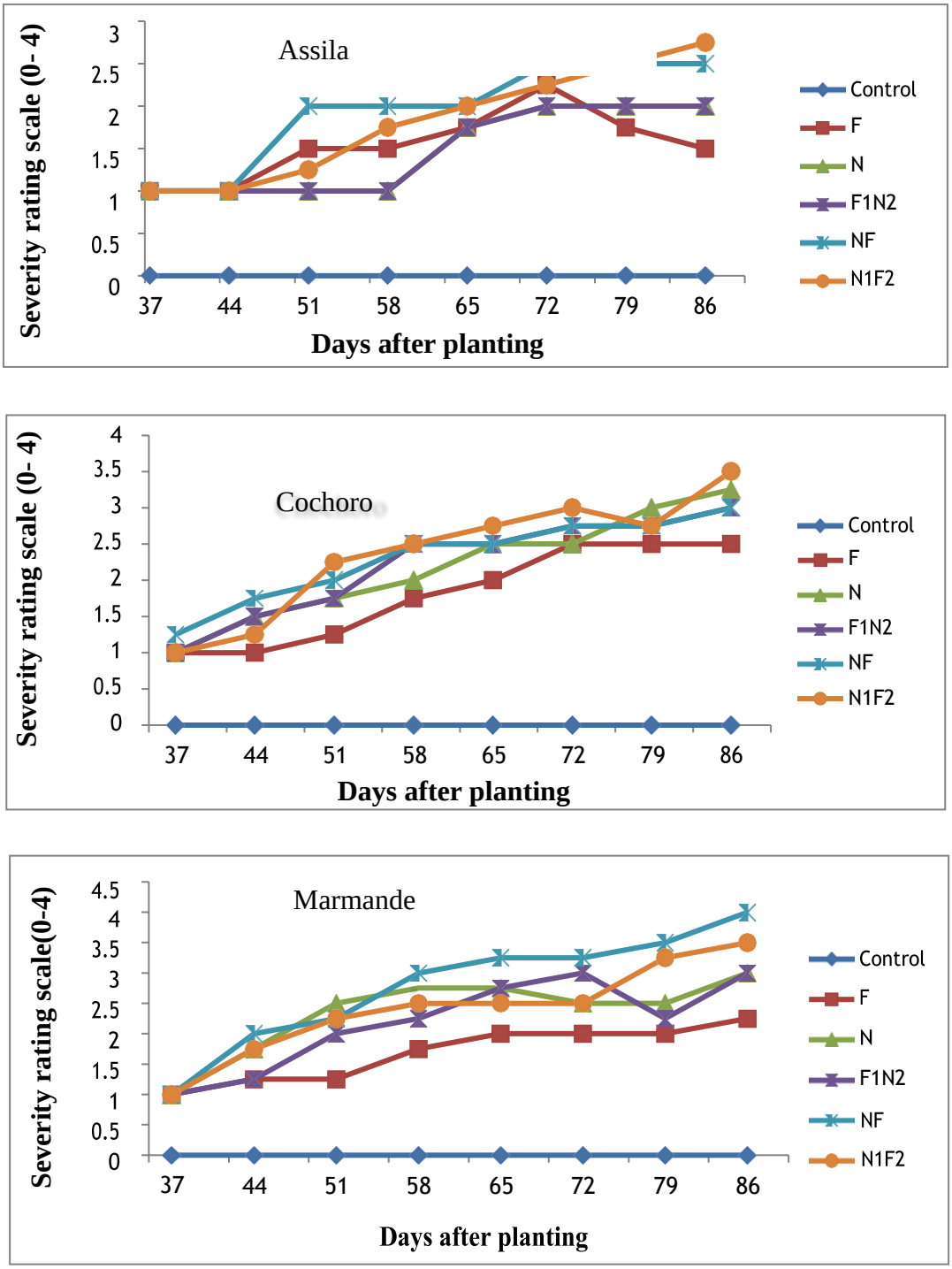

Fig. 4. Effect of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* on the three selected tomato genotypes on disease progress curve at Haramaya University, Raree Research Station in glasshouse during 2017/18.

Table 6. Response of three tomato genotypes to *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* based on the mean number of days to 50% flowering (50%FL) and root length (RL) at Haramaya University glasshouse in 2017/18.

Treatments	50%FL			RL(cm)		
	Tomato Genotypes					
	Assila	Cochoro	Marmande	Assila	Cochoro	Marmande
N	29.50 abc	28.50 ab	30.50 abc	26.00 ab	26.00 ab	27.00 abc
NF	34.75 d	31.00 abc	31.50 bcd	24.50 a	28.50 abcd	27.25 abc
N1F2	30.25 abc	29.25 abc	32.50 cd	26.50 ab	27.25 abc	27.25 abc
F1N2	29.25 abc	29.75 abc	32.25 cd	29.50 bcde	28.00 abcd	26.00 ab
F	30.25 abc	29.25 abc	31.75 bcd	32.50 de	27.50 abc	31.75 cde
Control	28.25 ab	29.50 abc	27.75 a	33.75 e	31.75 cde	29.75 bcde
LSD at 5%	3.621			4.808		
LSD at 1%	4.822			6.404		
CV%	8.4			12.0		

Where, means followed by the same letter (s) within a row and column in each parameter are not significantly different at 5% level of significance.

Table 7. Main effect *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* on different growth and biomass parameters such as plant height, number of flower per plant, fresh shoot weight, dry shoot weight and fresh root weight of the three tomato genotypes at Haramaya University glasshouse in 2017/18.

	Tomato genotypes growth and biomass parameters				
Treatments	PH(cm)	NFPP	FSW(g)	DSW(g)	FRW(g)
N	58.75 b	17.67 ab	62.11 ab	5.608 a	17.72 a
NF	53.00 a	14.83 a	52.04 a	5.524 a	16.69 a
N1F2	59.25 b	17.75 ab	59.23 ab	5.861 a	18.29 ab
F1N2	61.42 b	16.25 ab	62.36 ab	6.306 a	17.99 a
F	57.33 ab	16.67 ab	63.52 ab	6.102 a	20.97 ab
Control	67.25 c	19.50 b	70.60 b	8.147 b	23.22 b
LSD at 5%	5.423	2.967	12.06	1.326	3.846
LSD at 1%	7.222	3.951	16.07	1.766	5.122
CV%	11.1	21.2	23.9	25.9	24.5
	Tomato Genotypes				
Assila	65.67 b	18.62 b	79.74 b	7.865 c	19.24 a
Cochoro	62.04 b	18.04 b	57.41 a	6.014 b	19.54 a
Marmande	50.79 a	14.67 a	47.78 a	4.896 a	18.66 a
LSD at 5%	3.835	2.098	8.53	0.938	2.720
LSD at 1%	5.107	2.794	11.36	1.249	3.622
CV%	11.1	21.2	23.9	25.9	24.5

Table 8. Effect of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* and the three selected tomato genotypes on disease severity and area under disease progressive curve (AUDPC) at Haramaya University glasshouse in 2017/18.

Tomato Genotypes	Pathogen Combination	Final severity rating [a]	AUDPC
Assila	N	2.0 bc	71.75 c
	NF	2.5 ab	96.25 a
	N1F2	2.8 a	88.38 ab
	F	1.5 c	77.00 bc
	F1N2	2.0 bc	71.75 c
	C	0.0 d	0.00 d
	LSD(0.05)	0.64	16.24
	CV (%)	20.24	14.10
Cochoro	N	3.3 a	107.63 a
	NF	3.0 a	114.63 a
	N1F2	3.5 a	117.25 a
	F	2.5 a	89.25 a
	F1N2	3.0 a	110.25 a
	C	0.0 d	0.00 d
	LSD (0.05)	1.03	29.2
	CV (%)	24.8	19.7
Marmande	N	3.0 b	117.25 b
	NF	4.0 a	138.25 a
	N1F2	3.5 ab	119.00 b
	F	2.3 c	83.13 c
	F1N2	3.0 b	108.50 b
	C	0.0 d	0.00 d
	LSD(0.05)	0.55	16.75
	CV (%)	12.57	10.78

Where, means followed by the same letter (s) within a row and column in each parameter are not significantly different at 5% level of significance. LSD (5%) = Least significant difference at 5% level of significance; LSD (1%) = Least significant difference at 1% level of significance; CV (%) = Coefficient of variation. [a] Scale 0 to 4: 0=healthy plant without any wilt and 4=when all leaves become yellow and complete wilted plant (Song *et al*., 2004), [b] AUDPC was calculated using 1–9 scale rating; Means followed by the same letter (s) within a row and column in each parameter are not significantly different at 5% level of significance.

of nematode in providing a ready-made means of entry for the fungus into the host plant. This could be due to the superficial root injury caused by *M. javanica* which enhanced entrance for fusarium. This is in line with the findings of Sharma & McDonald (1990) in that the presence of *Meloidogyne* species aggravated the disease situation by *Fusarium oxysporum* f.sp. *cicero* on chickpea. The same results were obtained by (Morrell & Bloom, 1981; Fattah & Webster, 1983) who studied the predisposition of *Meloidogyne* species in host plant broke wilt resistance.

There was a highly significant difference on the mean number of final population density of J_2 recovered from Cochoro and Marmande when inoculated with *M. javanica* alone and *F. oxysporum* f.sp. *lycopersici* ten days prior to *M. javanica*. Seid *et al*., (2017) reported that Assila was resistant for four populations of *M. javanica* and *M. incognita* originated from tomato growing districts of Ethiopia. In this study, Assila was also found moderately resistant for the disease complex involving *M. javanica* and *F. oxysporum* f.sp. *lycopersici*. This is proved from the lowest RF obtained on the tomato genotype Assila. Lobna *et al*., (2017) reported that when *F. oxysporum* f.sp. *lycopersici* and *M. javanica* simultaneously inoculated gall index, root galls per plant, egg-masses per plant and reproduction factor were found increasing. In our study, a significantly reduced root length was recorded from concomitant inoculation of *M. javanica* and *F. oxysporum* f. sp. *lycopersici* followed by *M. javanica* inoculated alone. The highest mean root length was recorded from the control treatment on genotype Assila. This is in complete agreement with the works of Kumar *et al*., (2017) when they inoculate both pathogens simultaneously reduced root length compared to the control treatment. The result from this study indicated a synergy between *M. javanica* and *F. oxysporum* f. sp. *lycopersici* as it is proved from the increased plant root damage. A similar report has been attributed to the increased root damage due to nematodes modifying and increasing substrates in the rhizosphere which favored fungal growth (Bhabesh *et al*., 2007).

Among the three tomato genotypes studied, Assila was found moderately resistant and the highest mean plant height was achieved from it. In all other treatment combinations, except the control, plant height and other growth and biomass parameters were found reduced significantly which is in agreement with the findings of Kumar *et al*., (2017). There was a highly significant difference on the mean number of flower per plant on Assila and Marmande genotypes with simultaneous inoculation of *M. javanica* and *F. oxysporum* f.sp. *lycopersici* and the control treatment. The lowest mean flower number per plant was recorded from Marmande genotype due to low level of resistance against the inoculated pathogens. This finding demonstrated that both fresh shoot weight and dry shoot weight of the studied tomato genotypes reduced with concomitant inoculation of *M. javanica* and *F. oxysporum* f. sp. *lycopersici* and separate inoculation of the two pathogens compared to the control treatments.

The concomitant inoculation of *M. incognita* and *F. oxysporum* or inoculation of *M. incognita* ten days prior to *F. oxysporum* significantly reduced plant height and fresh shoot weight with high fusarium wilt incidence (Singh *et al*., 1981). Similarly, Padilla *et al*., (1980) studied the interaction between *M. incognita* and *F. oxysporum* f. sp. *pisi* on pea. They found that the concomitant inoculation of the two pathogens caused the death of plants after 45 days of inoculation. The inoculation of *M. javanica* one week prior to *F. oxysporum* inoculation to seedling of chickpea or simultaneous inoculation of both pathogens and inoculation of fungus one week prior to nematode resulted in reduced plant growth compared to individual inoculations (Goel & Gupta, 1986; Samuthiravalli & Sivakumar, 2008). It is in agreement with our study where maximum and significant reduction of shoot length and fresh root weight were observed when both pathogens inoculated simultaneously compared to un-inoculated checks. Additionally, Haseeb *et al*., (2007) studied individually as well as the combined

effect of *M. incognita* and *F. oxysporum* f. sp. *pisi* causing significant reduction in all the growth parameters of *Pisum sativum* compared to the un-inoculated plants. Index of leaf damage after 30, 45 and 60 days of planting increased when *F. oxysporum* and *M. javanica* was simultaneously inoculated. The plant height and fresh root weight also found to reduce in nematode-fungus simultaneous inoculation. Lobna *et al*., (2017) found that *F. oxysporum* f.sp. *lycopersici* and *M. javanica* reduced plant height, root length, fresh shoot weight and fresh root weight under glasshouse conditions on the tomato cultivar Riogrande. Moreover, the result of this study was in complete agreement with the finding of Haseeb *et al*., (2006) who exhibited the simultaneous inoculation of both pathogens (*M. incognita* and *F. oxysporum f.sp. pisi*) and nematode inoculation 10 days prior to fungus significantly reduced plant growth.

In a sequential inoculation, one pathogen of the disease complex infects the host before the invasion of the other pathogen and it brings about certain changes of tissue due to disease and biochemical alterations within the host, making it more suitable substratum for establishment and growth (Anwar & Khan, 2002). In our study, the same trend was observed when *M. javanica* was simultaneously inoculated or inoculated ten days prior to *F. oxysporum* f.sp. *lycopersici*. The EMI, *Pf* and RF were found to be high in the studied tomato genotypes though there was a significant variation between genotypes in simultaneous inoculations of *M. javanica* and *F. oxysporum* f.sp. *lycopersici*. The presence of *M. javanica* enhances more *F. oxysporum* spores gained entry through the wound tissue created by *M. javanica*. This was in complete agreement with Nagesh *et al*., (2006) who had performed a separate study on tomato that *M. incognita* is an important factor in increasing the severity of Fusarium wilt. A disease complex involving *M. javanica* and *F. oxysporum* f.sp. *lycopersici* resulted in alteration of mineral absorption, physiological damage and biochemical changes within the host plant (Lobna *et al*., 2017). It seems reasonable to expect that infection by one pathogen may alter the host response to subsequent infection by another (Zacheo, 1993).

The disease severity on Assila, Cochoro and Marmande at different days after inoculation was found different. The AUDPC calculated for disease severity rating was significantly different among treatments. The maximum AUDPC value of 138, 114 and 96 was estimated when simultaneously *M. javanica* and *F. oxysporum* f. sp. *lycopersici* was inoculated on the genotype Marmande, Cochoro and Assila, respectively. The result is in agreement with the findings of Dababat *et al*., *(*2011) that nematode interfere with the metabolic balance of the inoculated plant and inhibit hydrostatic water pressure that results in stunted growth and wilting. The same results were obtained by Choo *et al*., (1990) who studied the influence of *M. incognita* on the development of Cucumber (*cucumis sativus*) wilt caused by *F. oxysporum* f. sp. *cucumerinum* indicate that wilt was more severe in plants when inoculated with both nematode and fungus simultaneously than with fungus alone. Yen *et al*., (2003) reported that *M. incognita* was able to increase the incidence of Fusarium wilt of watermelon caused by *F. oxysporum* f. sp. *niveum* and also decrease resistance ability of water melon genotypes to Fusarium wilt.

Generally, the findings of this study indicated that plant growth, biomass and pathogen related parameters were synergistically affected when the tomato genotypes were inoculated with *M. javanica* and *F. oxysporum* f. sp. *lycopersici* simultaneously followed by *M. javanica* inoculated ten days prior to *F. oxysporum* f. sp. *lycopersici* compared to the un-inoculated control treatment. The synergistic effect of two pathogens has been reported by Marley & Hillocks (1996) who checked that the Fusarium wilt resistance character was broken by RKN (*Meloidogyne* species) in pigeon pea wilt resistance cultivar. Among tomato genotypes evaluated in this study Assila was found moderately resistant to the two pathogens because it reduced the reproduction of *M.*

*javanica a*nd *F. oxysporum* f. sp. *lycopersici* compared to Marmande while Cochoro was found tolerant under glasshouse conditions. Hence, further study is required to evaluate the performance of Assila genotype in hot spot areas of *M. javanica* and *F. oxysporum* f. sp. *lycopersici* in field conditions.

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