

Status of Root-Knot Nematode (*Meloidogyne* Species) and Fusarium Wilt (*Fusarium oxysporum*) Disease Complex on Tomato (*Solanum lycopersicum* L.) in the Central Rift Valley, Ethiopia

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Abstract

Development of diseases in cultivated crops depends on the complex interrelationship among host, pathogen and prevailing environmental conditions. In nature, plants are rarely, if ever, subject to the influence of only one potential pathogen and this is especially true of soil-borne pathogens like fusarium wilt (*Fusarium oxysporum*). In the present study, the co-occurrence of root-knot nematode (*Meloidogyne* species) and *Fusarium oxysporum* f.sp. *lycopersici* was surveyed in the main tomato growing areas of the central rift valley of Ethiopia. Mineral and organic samples were collected from 59 farmlands representing five districts and two commercial farms during the main cropping season of 2017. Cultural, morphological and virulence analysis of these pathogens revealed their co-existence. Out of 59 samples, 39% were found infected with the disease complex [*Meloidogyne* species.* *Fusarium oxysporum* f.sp. *lycopersici* (FOL)]. Of which, 16.9% and 6.8% resulted from concomitant infection of *Meloidogyne incognita** FOL and *Meloidogyne javanica** FOL, respectively. The percent prevalence of the disease complex ranged between 27.3% and 60%. The co-occurrence of these pathogens within a single host plant within the same ecological niche will indicate the probability of any type of interaction between each other. Therefore, subsequent research studies on their nature of interaction should be done in the future.

Keywords

Disease Complex, *Meloidogyne* species, *Fusarium oxysporum*, Tomato, Status

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops across the world next to potato, McGovern [1]. The fruits of tomato are popular throughout the world and are used in all kinds of vegetables as raw salad and processed products such as paste and juice. Ripe tomato fruit has high nutritive value and a good source of vitamin A, B, C and minerals, MoARD [2]. Recently, it started to gain more medicinal value because of its high content of antioxidant including carotenoids, ascorbic acid, phenolics and lycopene, Oduor [3]. It also serves for export, making a significant contribution to the national economy of Ethiopia with most of the exports to Djibouti, Somalia, South Sudan, Middle East and European markets, Tabor & Yesuf [4].

Tomato production reached more than 163.4 million tons cultivated on more than 4.6 million hectares of land worldwide, FAO [5]. In Ethiopia, there is 6,298.63 hectares area coverage with an annual production of 28,364.83 tones, CSA [6]. There is also large-scale production of tomato in the central rift valley (CRV) under irrigated and rain-fed conditions having unlimited potentials for expansion if certain production constraints are avoided. Despite its rapid spread across the different localities and agro-ecologies, the production and productivity remain low. Several biotic and abiotic factors are impacting its production. Among the biotic factors, plant diseases caused by plant-parasitic nematodes (PPN) are a costly burden. There are over 4100 species of PPN currently identified, collectively, causing an estimated loss of \$80 - \$118 billion per year in damage to crops. Of these species, 15% of them are the most economical species directly targeting plant roots of major agricultural crops and prevent water and nutrient uptake resulting in reduced agronomic performance, overall quality and yields, Bernard *et al.* [7]. In nature, plants are rarely, if ever, subject to the influence of only one potential pathogen and this is especially true of soil-borne pathogens like fusarium wilt (*Fusarium oxysporum*) whereby further opportunities exist for interactions with other microorganisms occupying the same ecological niche, Back *et al.* [8].

The combined effect of wilt causing fungi and PPN causes serious damage to different economically important crops worldwide, Mai & Abawi [9] and Chen *et al.* [10]. Based on its worldwide distribution, extensive host range and involvement with fungi, bacteria and viruses in disease complex, root-knot nematodes (RKN) rank first among the top 10 damaging genera of PPN affecting the world's food supply, Jones *et al.* [11]. A large number of *Fusarium* wilt fungus affected plants are known to be infected with root-knot nematodes, Goswami and Agarwal [12]. *Fusarium oxysporum*, among the *Fusarium* spp., is the most frequently isolated plant pathogenic fungus on many host plants. Thus, obtaining optimum crop quality and economic production of tomato depends on the development and exploitation of an eco-friendly, sustainable, economical and alternative method of nematode-wilt disease complex management. Being aware

of the array of organisms influencing the crop is, therefore, essential, Webster [13]. Therefore, the main objectives of this research work was to assess the co-existence of *Meloidogyne* species and *Fusarium oxysporum* f.sp. *lycopersici* (*FOL*) on tomato in the central rift valley of Ethiopia.

2. Materials and Methods

2.1. Description of the Study Area

The study sites, their geographic locations and characteristic features of surveyed tomato fields are summarized in **Figure 1** and **Table 1**. The survey covered different localities belonging to the five districts (Ada-Chukala, Adama, Dugda, Lomie, and Merti) and two commercial farms [African Juice Tibila Share Company (AJTSC) and Nuraera] of Central Rift Valley (located 8°07' - 8°43' N, 38°46' - 39°59' E) of Ethiopia.

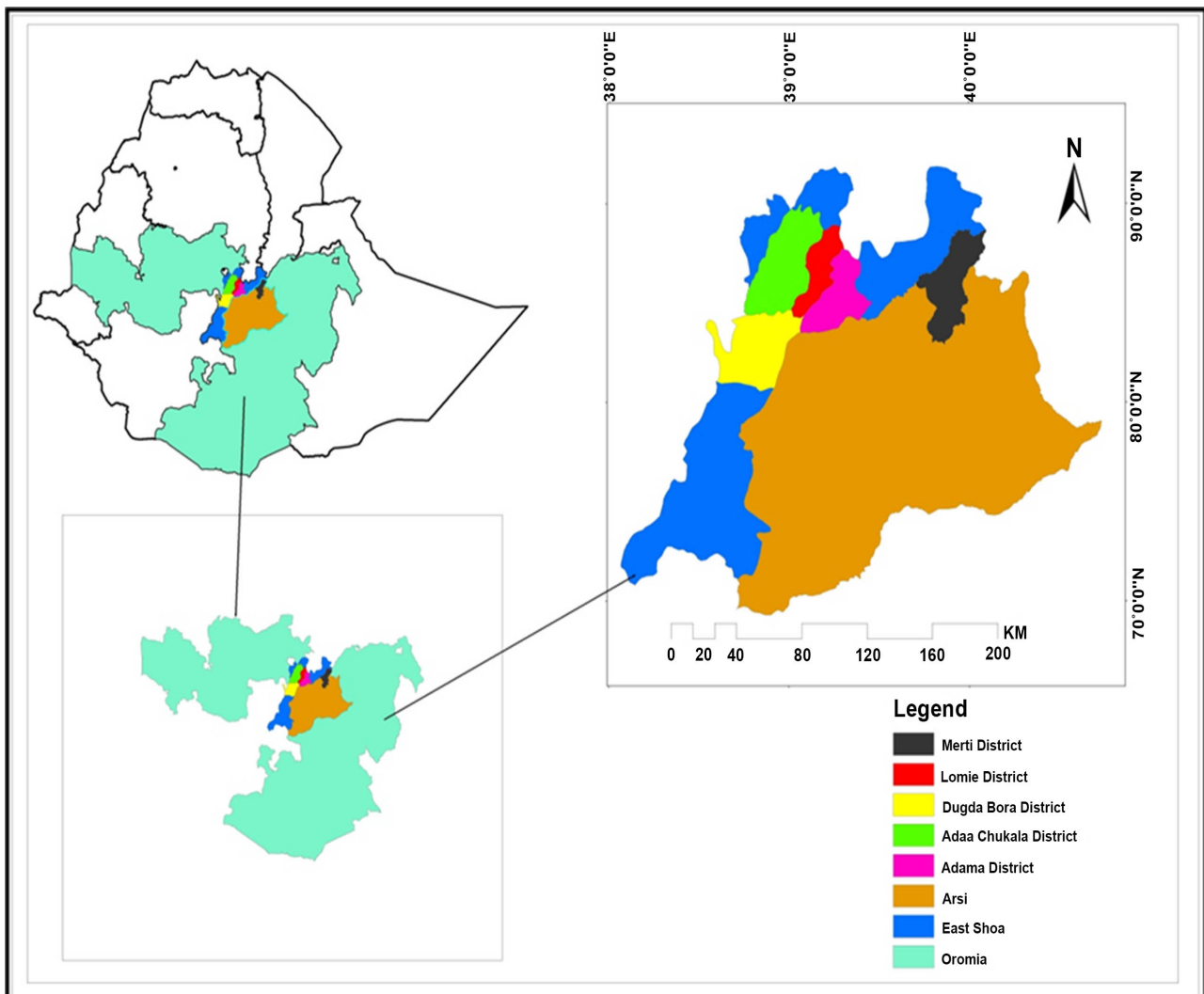


Figure 1. Map showing the sampling districts of tomato-growing areas in the Central Rift Valley, Ethiopia, during 2017/18 cropping season.

Table 1. Characteristic features of surveyed tomato fields in the Central Rift Valley, Ethiopia.

District	Altitude (m.a.s.l)	Temperature range (°C)	Relative Humidity range (%)	Cropping system ^a
Dugda	1636.5 - 1656.3	20.55 - 31.75	37.55 - 73.25	sole
A.Chu.	1560.6 - 1605.0	28.90 - 38.90	33.10 - 60.25	sole
Lomie	1563.0 - 1606.0	26.70 - 34.40	28.95 - 46.30	sole
Adama	1530.0 - 1548.0	24.10 - 29.85	31.50 - 58.30	sole
AJTSC	1190.0 - 1248.0	30.10 - 31.35	33.20 - 33.50	sole
Merti	1174.0 - 1207.0	29.85 - 32.85	34.75 - 39.00	sole
Nuraera	1101.0 - 1124.0	26.70 - 31.65	43.00 - 50.70	sole

^aOnly dominant cropping systems are indicated. A.Chu.—Ada Chukallaa; AJTSC—African Juice Tibila Share Company.

2.2. Sampling and Sampling Units of the Disease Complex

The study areas were purposely selected in consultation with the nearby agricultural offices and development agents (DAs), while the sampling fields were selected randomly. Ten to fifteen mineral (soil) and organic (root & stem base) samples per farm were taken from symptomatic plants to be used as composite sample. Mineral samples were collected from 2 cm diameter next to the plant and top 20 - 25 cm depth to obtain a representative sample making sure not to take the top five centimeters layer, Sasser and Carter [14]. Areas infested with weeds or other plant species were avoided to prevent sampling nematodes from non-target hosts, Knight [15]. Mineral fractions were bulked to 2 kg soil per sample. A sub-sample of 1 kg was taken from the composite and packed in a 2 kg size labeled plastic bag. Sub-samples of sufficient amount organic fractions were also packed in separate labeled plastic bags. The hand shovel and other sampling equipment were cleaned and dried after sampling to avoid contamination of soil samples and to prevent the spread of nematodes or pathogens between farms. A total of 118 composite field samples (59 from each fraction) were collected and transported to the plant pathology laboratory of Melkassa agricultural research center. The samples were kept under refrigerator (at 4°C) until further processing.

2.3. Extraction and/or Isolation and Identification of Pathogens

2.3.1. Extraction and Identification of the Root-Knot Nematode(s)

Nematodes were extracted by Modified Baermann's funnel, Hooper *et al.* [16] technique and identified based on the perineal pattern described by Chitwood [17]. Infected roots were washed under running water to make it free from soil (**Figure 2(a)**) and females were dissected out from well-developed galls (**Figure 2(b)**) of the roots under stereo binocular microscope and transferred to Petri dish containing tap water. The posterior portion of the female was cut with a perineal pattern knife, Taylor and sasser [18] and the body contents were cleaned.

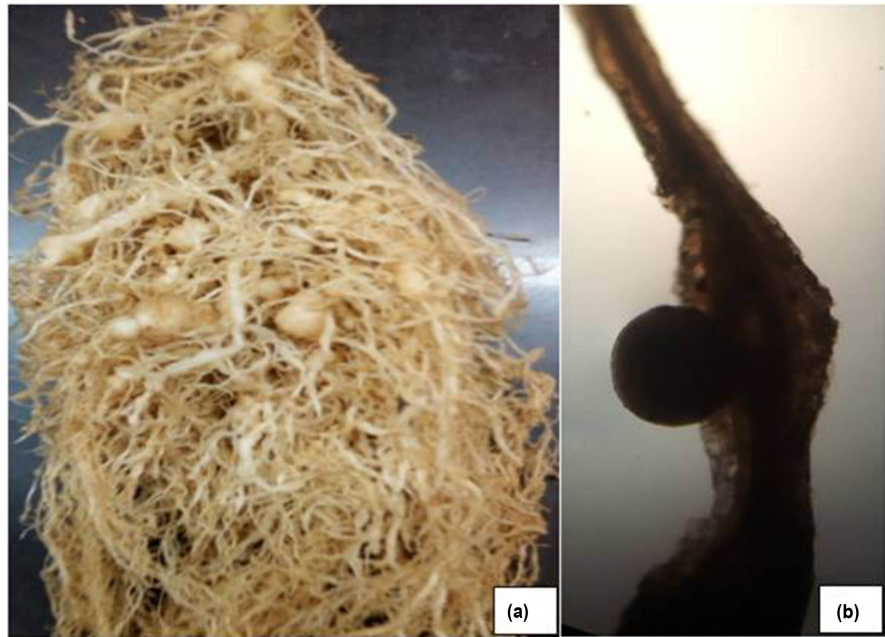


Figure 2. Infected tomato roots after washing (a) and matured female body from well-developed gall (b) during dissection.

Cleaned posterior portion of female was further trimmed and transferred to a drop of glycerin on a clean glass microscope slide. A cover slip was placed and sealed with nail polish and observed under Leica compound microscope. Ten perineal pattern per sample was processed to determine the frequency of each nematode species. Important morphological characters such as body shape of larvae, males and females were also used during identification. Presence and absence of stylet were used to distinguish pathogenic nematodes from free-living ones.

2.3.2. Isolation and Identification of *Fusarium oxysporum*

Fusarium wilt disease-inciting pathogen (*Fusarium oxysporum*) was isolated from organic fractions collected from the survey. Specimens of organic fractions from diseased plant 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 papers. Sterilized and dried specimens were plated out on potato dextrose agar (PDA) medium in sterile Petri dishes. The inoculated plates were incubated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 7 - 9 days. Identification of *F. oxysporum* cultures was made on the basis of cultural and morphological characteristics, Leslie and Summerell [19] followed by pathogenicity test.

2.3.3. Pathogenicity of *Fusarium oxysporum*

To determine the formae speciales, virulence analysis of the isolates was carried out on a susceptible tomato (cv. Marmande), which has no resistance gene for

both Race 0, formerly race 1 and Race 1, formerly race 2, Ibrahim [20]. Inoculum density (conidia concentration) of the pathogen from 10 days old cultures were adjusted to 3×10^6 Conidia/ml/plant, Lobna *et al.* [21] using a hemocytometer. Twenty one days old seedlings were inoculated by standard root dip method, Srivastava *et al.* [22]. Seedlings were removed from the pot tray, shaken to remove the adhering particles and washed carefully under tap water. The roots were trimmed with a sterile scissor and were submerged in the conidial suspension for 30 minutes. The inoculated seedlings were transplanted to 20 cm diameter surface sterilized (5% NaOCl) pots, filled with soil, sand and compost in 1:2:1 ratio, and incubated in greenhouse. Four treatments: (1) Marmande + White FOL Isolate, (2) Marmande + Pink FOL Isolate, (3) Marmande + Violate FOL Isolate; and (4) un-inoculated check (inoculated with sterilized distilled water) with five replications was set in the greenhouse. The prevalence and frequency of occurrence of species and/or disease complex were determined using the following formulae based on species identification of the respective pathogens after extraction and/or isolation of the respective pathogens.

$$\text{Prevalence (\%)} = \frac{\text{Total No. of fields with disease complex}}{\text{Total No. of field surveyed}} \times 100 \quad \text{Hussain [23]}$$

$$\text{Absolute frequency (\%)} = \frac{\text{No. samples containing the species}}{\text{No. of samples collected}} \times 100 \quad \text{Norton [24]}$$

$$\text{Relative frequency (\%)} = \frac{\text{Frequency of occurrence of the species}}{\text{Sum of frequency of all samples}} \times 100 \quad \text{Norton [24]}$$

2.4. Data Analysis

Data obtained from the survey and greenhouse were subjected to analysis of variance (ANOVA) by GenStat software (16th edition) and statistical package for social sciences (SPSS), respectively and means were separated using LSD at $P = 0.05$.

3. Results and Discussion

The results of the survey and subsequent laboratory (pathogen identification) and greenhouse (pathogenicity test) works are discussed hereunder.

3.1. Identification of the Test Pathogens

3.1.1. Root-Knot Nematodes (*Meloidogyne* Species)

Important diagnostic character used for species identification was the perineal pattern found in the female *Meloidogyne* species. The perineal pattern cut showed high squarish, round to flat dorsal arch. The lateral ridges were absent in many of the samples and few in others. The striae were coarse, smooth to wavy marked with breaks and forks (Figure 3). These characters were compared with the descriptions given by Eisenback *et al.* [25] and were inline. The female body was pear-shaped with no visible protruding posterior. The posterior was globose and

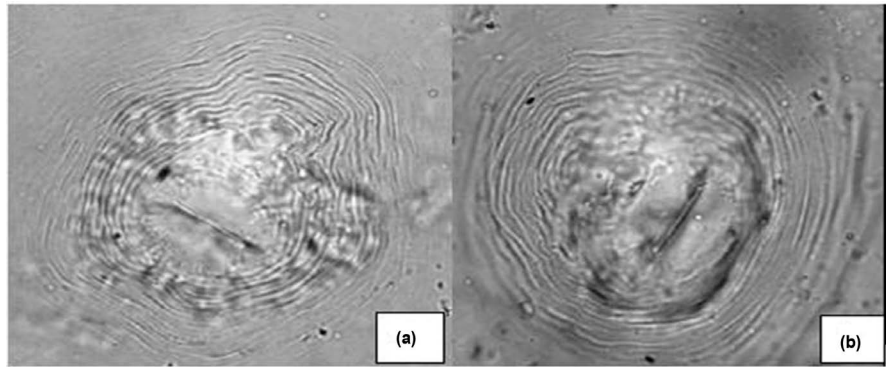


Figure 3. Female perineal pattern of RKN during identification; (a) *M. incognita* and (b) *M. javanica*.

the neck projects anteriorly (**Figure 4(d)**). Characteristic offset head shape and narrow and conical, tapering to a finely rounded hyaline tail tip (**Figure 4(a)** & **Figure 4(b)**) was also noted during the identification. The stylet was evident (**Figure 4(c)**) and it affirms, as the nematode was not free-living. *M. incognita* and *M. javanica* were the predominant species found in the survey areas (on the basis of perineal pattern characteristics). This result is also in line with previous reports of Seid *et al.* [26] and Seid *et al.* [27].

3.1.2. Fusarium Wilt (*Fusarium oxysporum* f.sp. *lycopersici*)

Diseased plant organic specimens from survey were used to isolate (identify) wilt-causing pathogen, it was found mainly *Fusarium oxysporum* and affirm with pathogenicity test for its formae speciales. Pure cultures of 7-9-days-old were morphologically characterized. Morphological characteristics described by Leslie and Summerell [19] and Rafai *et al.* [28], such as colony color, colony growth pattern, size of macro- and micro-conidia and absence and presence and number of septation of both macro-and micro-conidia were mainly used to narrow down the identification under laboratory condition. The mycelium was found to be floccose (abundant) with three morphotypes or colors (white, pink and violet) as indicated in **Figure 5**, which was used to separate isolates in the pathogenicity test.

The length*breadth of macroconidia usually varied between (15.9 - 46.98 × 1.83 - 4.88) μm and that of microconidia was (6.75 - 13.56 × 1.93 - 3.4) μm under 40× magnification. The number of septation of microconidia ranges from 1.5 - 4.3 μm. The shape of macroconidia 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. 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 (**Figure 6**). This result is in line with the description of *Fusarium* species of Rani and Ramya [29] & Leslie & Summerell [19].

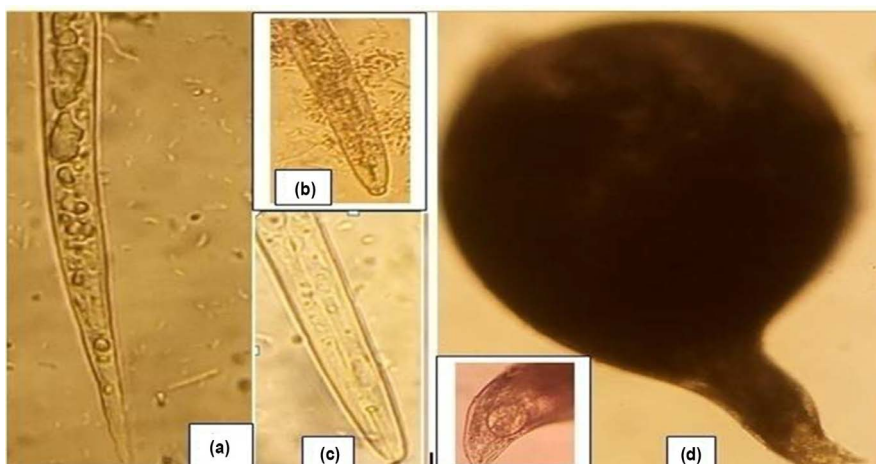


Figure 4. Light micrograph (40×) of the tail (a), head (b), stylet (c) and female *Meloidogyne* species (d).

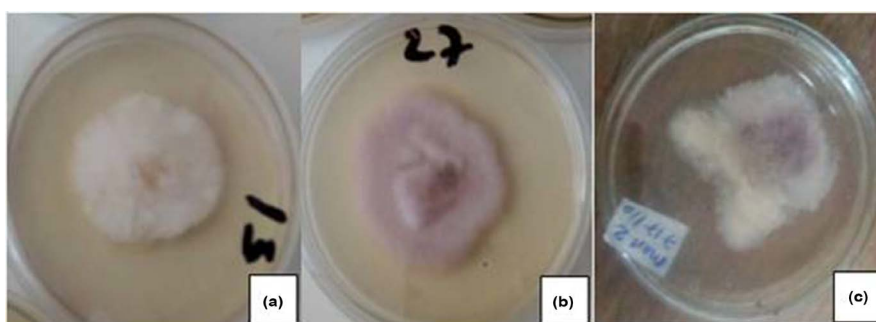


Figure 5. Morphotypic isolates of 5-days-old pure culture *Fusarium oxysporum*; White (a), pink (b) and Violet (c) PDA media.



Figure 6. Macro- and micro-conidia (a), false heads (b) and conidiogenous cells (c) of 10-days-old *Fusarium oxysporum* on PDA media under 40× magnification.

3.1.3. Pathogenicity of *Fusarium oxysporum*

Various symptoms on aerial parts and within the stem tissues of tomato plants infected with *F. oxysporum* were noted starting at 33 days after inoculation (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 (Table 2). As expected, however, there was significant difference ($p < 0.05$) between the

Table 2. Virulence analysis of the FOL isolates measured by mean disease severity and AUDPC.

<i>Fusarium oxysporum</i> isolates	Disease severity	AUDPC
White	3.188 ^b	71.75 ^b
Pink	3.062 ^b	71.75 ^b
Violet	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: Least significant difference; CV: coefficient of variation and AUDPC: area under disease progress curve.

inoculated treatments and the un-inoculated check regardless of the isolates. Moreover, successful re-isolation of the pathogen from artificially inoculated symptomatic organic tissue affirms as the pathogen was pathogenic to tomato. Even though, there is no statistically significant difference ($p < 0.05$) in mean disease severity and AUDPC, numerically higher disease severity and AUDPC were noted from whitish isolate.

3.2. Distribution and Prevalence of *Meloidogyne* Species and *Fusarium oxysporum* f.sp. *lycopersici* Disease Complex

The percent prevalence of the disease complex in this study was determined based on species identification of RKN and fusarium wilt after extraction and/or isolation of the respective pathogens under the laboratory condition. The results of the survey showed the co-occurrence of *Meloidogyne* species and *F. oxysporum* f.sp. *lycopersici* in all assessed areas in the CRV of Ethiopia. This study provides the quantification of occurrence of *Meloidogyne* species and FOL disease co-occurrence on tomato crop.

Root-knot nematode species morphologically identified from infected roots and rhizosphere soil included *M. incognita* and *M. javanica*, which occurred either separately or concurrently. The result showed that 39% of tomato fields were infected with the disease complex (MI*MJ*FOL); off which, 16.9% and 6.8% resulted from concomitant infection of MI*FOL and MJ*FOL, respectively (**Figure 7**). The prevalence of the disease complex (MI*MJ*FOL) varied in surveyed districts with the highest at Dugda (60%) and the lowest at Lomie, 27.3% (**Figure 7**). The prevalence of *Meloidogyne* species and FOL co-occurrence has not been reported before in Ethiopia. However, mixed population of *M. ethiopica* (32.7%) and *M. incognita* with *M. javanica* (3.9%) have been reported, Mandefro and Mekete [30] and Abebe *et al.* [31]. The remarkable co-occurrence of these pathogens in tomato crop grown in the CRV of Ethiopia depicts sever direct or indirect damage to this crop.

The mere co-existence of the two pathogens within a single host plant does not necessarily depict their synergism and therefore, there is a need to investigate

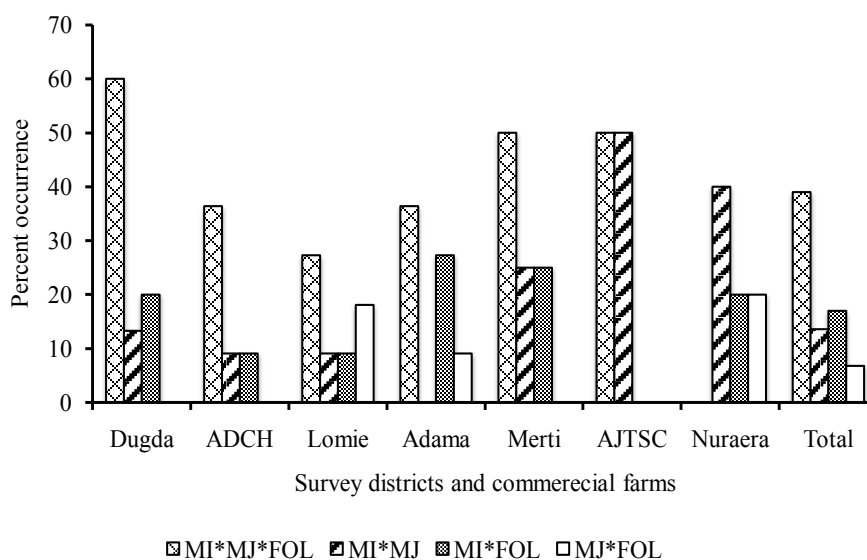


Figure 7. District-wise variation in prevalence (%) of type of species and/or disease complex. Where, ADCH: Adaa chukala and AJTSC: African Juice Tibila Share Company.

their nature of interaction. However, greenhouse experiments elsewhere in the world reported the synergistic effects of different soil-borne pathogens and *Meloidogyne* species on a range of host plants, Al-Hazmi and Al-Nadary [32]; Ansari *et al.* [33]; Golden and van Gundy [34]; Kumar [35]; Kumar [36]; Lobna *et al.* [21] and Onkendi [37]. Fusarium wilt-RKN disease complex is one of the most widely recognized and economically important diseases in the world, Wanjohi *et al.* [38]) as they both affect the function of the root system. The two often infect tomato simultaneously, forming a complex that increases the incidence and severity of Fusarium wilt, Onkendi *et al.* [37]. Presence of *Meloidogyne* species greatly reduced resistance of tomato cultivars to the fusarium wilt. Among the *Meloidogyne* species, *M. incognita* is the most common in tomato production worldwide, Gautam *et al.* [39]. The presence of fungal survival structures (chlamydospores) that enhance its ability to persist in the soil for a long time and its wide host range makes its (*F. oxysporum*) management fairly challenging, Wanjohi *et al.* [38].

3.3. Frequency of Disease Complex

Disease complex involving MI*MJ*FOL was the most frequent (51%) followed by MI*FOL (22.2%) and the least was MJ*FOL, 8.9% (Figure 8). Out of 59 samples, 51 samples were found carrying *Meloidogyne* species. There was the highest absolute (16.9%) and relative (22.2%) prominence of MI*FOL as compared to MJ*FOL (6.8%, 8.9%, respectively). This probably indicated that *M. incognita* appears to be the most important species associated with damage inflicted to tomato production in the area due to the disease complex as it was the frequently found species.

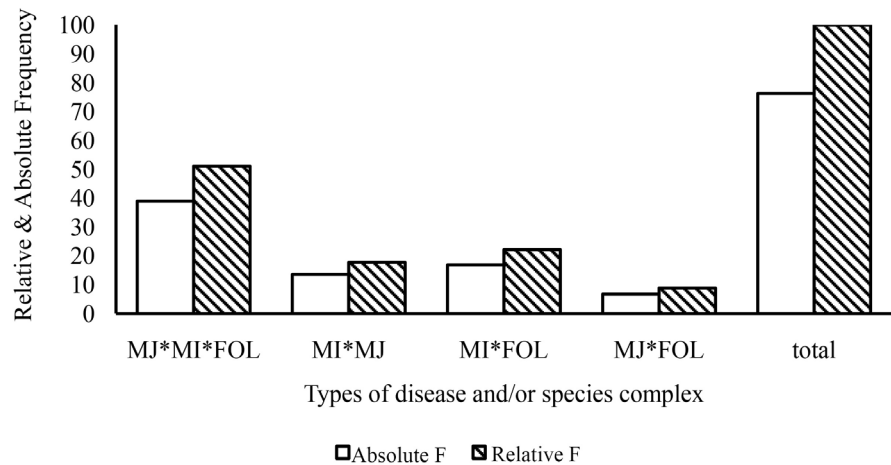


Figure 8. Absolute and relative frequency of occurrence of species and/or disease complex in five districts and two commercial tomato farms of CRV during 2017/18 main cropping season.

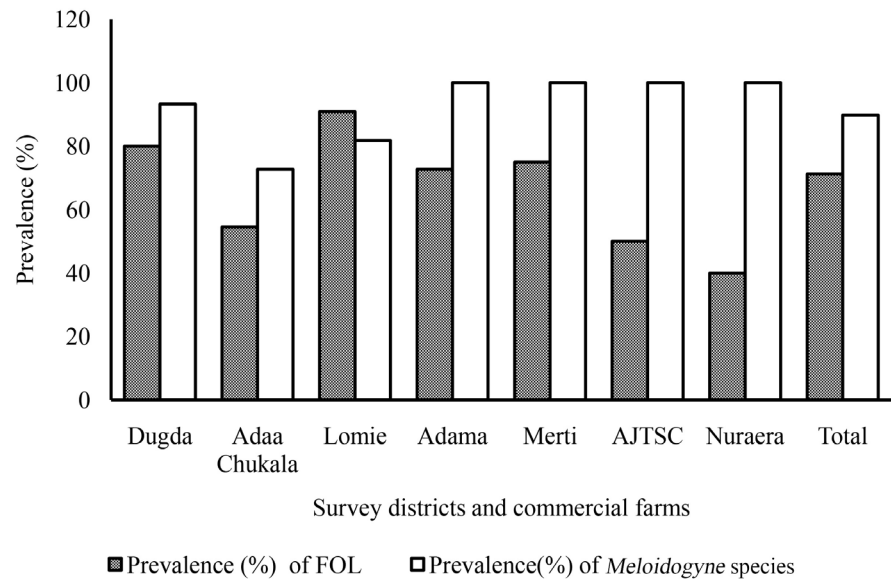


Figure 9. Prevalence of *Meloidogyne* species and *Fusarium oxysporum* f.sp. *lycopersici* in the survey area in 2017/18 main cropping season.

3.4. Status of *Meloidogyne* Species and *Fusarium oxysporum* f.sp. *lycopersici*

The prevalence of *Meloidogyne* species and *Fusarium oxysporum* f.sp. *lycopersici* regardless of their co-occurrence is summarized in **Figure 9**. There was high prevalence of *Meloidogyne* species and *F. oxysporum* f.sp. *lycopersici* (89.8%, 71.2%, respectively). It is evident from the figure that *Meloidogyne* species was prevalent (up to 100%) in most the areas assessed. On the other hand, maximum (90.9%) prevalence of FOL was recorded from Lomie district while the minimum (54.5%) was from Adaa Chukala district. This result probably showed high chance of getting synergistic reaction (if it is their nature) or any kind of disease

interaction provided that other conducive environmental factor for the complex to occur.

4. Conclusion

The present survey revealed the existence of the disease complex (co-existence of *Meloidogyne* species and *Fusarium oxysporum* f.sp. *lycopersici*) in the central rift valley of Ethiopia. The mere co-existence of the disease complex in the same host plant with similar niche will not be a sole evidence for the existence of their synergism. However, the pathogens were pathogenic to tomato and indicated the existence of some type of interaction between them. Therefore, the nature of their interaction need to be investigated in the future.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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