MELOIDOGYNE JAVANICA TREUB, 1885 (CHITWOOD, 1949) AND SOME WEED HOSTS IN TOMATO (*SOLANUM ESCULENTUM* L.) FIELDS IN TEKİRDAĞ, TURKEY

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ABSTRACT: Nematode species from the genus *Meloidogyne* Göldi, 1989 are threatening agricultural production especially in greenhouses in Turkey by causing significant yield loss due to root damage and galling. Within a 2 year period of nematological surveys in Northwestern Turkey several *Meloidogyne* species were identified in vegetable fields and gardens. The majority were isolated from tomato fields and *M. javanica* was one of the most prevalent species. A further research was conducted in infested tomato fields to detemine weed hosts of *M. javanica*. As weeds were examined nematode damage and root galls were observed in 13 species. Galling was significant in *Portulaca oleraceae* L. with up to 30 galls per plant and least in *Lamium amplexicaule* L. and *Trifolium repens* L.

KEY WORDS: *Meloidogyne javanica* L., weed hosts, tomato fields, Turkey

Tomato (*Solanum lycopersicum* L.), is herbaceous annual plant from Solanaceae family. Ranked 4th in the world with 12.6 million tons annual production tomato is one of the most cultivated vegetable in Turkey. Due to favourable climate conditions and soil types tomato can be grown almost everwhere in the country. About 71.3% of produced tomato is used for table consumption and 29.7% is processed in the industry (Tatar & Pirinç, 2017).

Meloidogyne genus was first found on cucumber plants and described by Berkeley in 1855 (Hunt & Handoo, 2009). They are obligate parasites feeding on 5500 different plant species including monocotyledons and dicotyledons (Trudgill & Blok, 2001). Tomato is one of the main hosts of this genus. Until to day 101 species of *Meloidogyne* have been identified and the most destructive species were reported as *M. incognita* Kofoid & White, 1919 (Chitwood, 1949); *M. javanica* Treub, 1885 (Chitwood, 1949); *M. arenaria* Neal, 1889 (Chitwood, 1949); *M. hapla* Chitwood, 1949; *M. chitwoodi* Golden, O'Bannon, Santo and Finley, 1980 and *M. graminicola* Golden and Birchfield, 1965 (Mitkowski & Abawi, 2011; Seid et al., 2015). The damage potential of these nematodes were reported as 25-100% yield reduction (Wesemael et al., 2011) and global economic loss was estimated as $157 billion (Abad et al., 2008). In Western Anatolia Region of Turkey 80% crop losses were indicated in tomato growing areas (Kaşkavalcı, 2007). After feed of juveniles inside plant roots, giant cells is formed as a result of repeated cell division and root galls emerge after hypertrophy in cortical cells.

Due to damaged roots water uptake is restricted and plants start to wilt (Moens et al., 2009).

Many weed species were reported as food source for many root knot nematodes in the absence of crop hosts. Seven weed species, *Ageratum conyzoides* L., *Eleusine indica* (L.) Gaertn., *Portulaca oleracea* L., *Amaranthus* spp., *Cyperus rotundus* L., *Chenopodium album* L. and *Digitaria* spp. were reported as frequently encountered species parasitized by nematodes (Myers et al., 2004; Rich et al., 2008). Weeds produce hundreds of seeds which can survive in soil for several years and germinate under favourable conditions. Weeds present in cultivation areas during crop plant growth and fallow periods are always alternate host for nematodes to maintain populations. In addition variations in weed species and nematode densities depending on several factors such as climate conditions, cultural practices and cropping system may affect populations. The nematode number will be increase parallel to rise of density of weed hosts (Thomas et al., 2005). The control of root knot nematodes is always difficult because of wide host range and virulent races and presence of weeds will make these attempts harder.

The eradication of root-knot nematodes from coinfested areas involves several methods like crop rotation, chemical control and fallow. The main purpose in these management options are to decrease the population densities of the nematodes below damage thresholds prior to next cultivation season. Despite utilization of all these control strategies nematodes may survive and multiply in the presence of host weeds (Kutywayo & Been, 2006).

Although chemical control is most effective to control weed species it is not preferred due to cause significant increase of crop production expenditure. Furthermore herbicides are selective and each herbicide affect certain weeds. Chemical control may not be effective on each weed species under mixed cultivations and unkilled single weed plant in herbicide applied areas may be a host for nematode population. In addition, under low density conditions weed control is not a priority, although weeds may be a good host of plant parasitic nematodes (Rich et al., 2008).

The nematode population can be reduced by removing host weed species from infested areas. Therefore the determination of weed hosts of local Meloidogyne species is essential. Thus this study was carried out in tomato fields in Northwestern Marmara Region to evaluate weed hosts of major root knot nematode *Meloidogyne javanica*. A research covered observations in tomato fields, identification of root knot nematode and host weed species in laboratory and assesment of root galling severity of weeds.

MATERIAL AND METHOD

Survey and nematode identification

Within a period of 2015-2018 a survey has been carried out in Malkara, Süleymanpaşa and Şarköy districts of Tekirdağ province. Soil and tomato root samples were collected from 47 randomly selected tomato fields. Soil samples of approximately 1 kg were taken from 5 different points in each field from 0 - 30 cm soil depths. In order to isolate *Meloidogyne* juveniles centrifuge flotation method (Jenkins, 1963) was used while females were collected with the help of forceps under microscope.

During surveys *Meloidogyne javanica* was identified in several locations. *M. javanica* species present were identified by perineal pattern morphology of females. In addition molecular diagnosis of identified species were performed

with Fjav (5'-GGTGCGCGATTGAACTGAGC-3') forward and Rjav (5'-CAGGCCCTTCAGTGGAACTATAC-3') reverse primers (Zijlstra et al., 2000).

In order to examine perinneal pattern adult females were collected from galled roots by crushing with a needle in a Petri dish filled with tap water. Extracted females were cut from posterior and the posterior part of the body were placed into glycerine and slides were prepared for further observation under microscope.

For molecular analysis nematode DNA was purified with Sigma Aldrich Extract N Tissue PCR kit. Juveniles of *M. javanica* were hand picked under microscope and placed into microcentrifuge tubes containing 2.5 µl tissue preparation and 10 µl extraction solution. This mixture was incubated at 55 °C for 10 minutes followed by 95°C for 3 minutes. The DNA concentration in extracted samples (A260/280 A260/230) were measured in spectrophmmeter.

All PCR reactions was prepared in a final volume of 20 µl (10 µl 2X PCR Ready Mix (Sigma Aldrich), 1 µl reverse primer, 1 µl forward primer, 2 µl template DNA, 6 µl ddH2O). PCR reaction was programmed as 95°C, 3 min; (95°C, 50 sec.; 59°C, 50 sec.; 72°C, 1 min) × 35; 72°C, 10 min. Final amplification products were seperated on a 1.5 % agarose gel the gel was run 50 minutes at 50V. At final the gel was stained with ethidium bromide and visualised UV transluminator.

Meloidogyne javanica was present in 11 tomato fields and the damage on tomato plants were evident. Stunting, yellowing and in some cases wilting was prevalent symptoms in tomato plants. Distinct root galling and egg masses were observed on the roots of tomato plants depending on susceptibility of tomato cultivar (Fig. 1).

Weed hosts of *Meloidogyne javanica*

A further study was conducted in *Meloidogyne javanica* infected 11 fields in order to determine weed hosts. Weed samples were collected by removing entire plants from soil without damaging roots. During weed sampling each plant roots were examined, presence of galls were recorded, galls on each root were counted. Galling severity per plant was graded based on the 0-5 scale described by Taylor and Sasser (1978). Samples were put seperately in a nylon bag and transferred to our Institute for further examination and species identifications. For species identification the herbarium of each species were prepared by slow drying of plant samples at room temperature. After complete drying each weed sample were sticked on to cardboard, covered by nylon and labeled. Collection site, province, collection date were recorded on each label. Weeds were identified by comparing plant morphology with published literatures.

RESULTS

Based on weed identifictions and field observations 13 of 18 weed species including *Lactuca serriola* L., *Heliotropium europaeum* L., *Portulaca oleracea* L., *Cynodon dactylon* (L.) Pers., *Amaranthus retroflexus* L., *Amaranthus viridis* L., *Chenopodium album* L., *Lamium amplexicaule* L., *Trifolium repens* L., *Anagallis arvensis* L., *Eleusine indica* (L). Gaertn, *Lepidium draba* L. and *Galium aparine* L., were found infected with *Meloidogyne javanica*. Females were present in galled roots of each weed species.

Females of our *M. javanica* perineal pattern had rounded dorsal arch and lateral lines that seperate dorsal and ventral parts. Population had slender, vermiform juveniles. The juveniles had slender body with continuous body contour. The spear was short, knobs were enlarged transversely. The tail was tapering with rounded tail tip. These characteristics were similar with original

description of Chitwood (1949). The morphopetrics of J2 match the values of Özaslandan & Elekcioğlu (2010) and Whithead (1968).

There were significant variations between weed species in the point of gall size index. Within these weed species *Portulaca oleraceae* and other weeds supported moderate to small root galls while *Lamium amplexicaule, Amaranthus retroflexus* and *Lepidium draba* had slightly large galls. Approcimately 21 galls were counted in *Chenopodium album* while this number increased to 28 in *Portulaca oleraceae*. The lowest gall number was counted as 7 and 3 galls in *Trifolium repens* and Lamium amplexicaule respectively. The lowest gall number was counted on roots of *Trifolium repens* and *L. amplexicaule* which has almost 4-5 galls. In contrast *Capsella bursa-pastoris*, *Euphorbia helioscopia, Geranium dissectum, Sinapis arvensis* and *Solanum nigrum* had no galls despite they were present in all nematode infected fields.

DISCUSSION

Several weed species including *Amaranthus retroflexus* L., *Echinochloa crus-galli* (L.) P. Beauv., *Malva sylvestris* L., *Portulaca oleraceae* L., *Rumex chryspus* L, *Avena sterilis* L., *Chenopodium album* L., *Ecballium elaterium* (L.) A. Rich., *Solanum nigrum* L., and *Sorghum halepense* L. were reported in tomato fields all around the world (Brito et al., 2008). In our study 18 weed species were identified in tomato fields located in Tekirdağ.

There are several weed hosts of these nematodes with different susceptibility levels. Highly susceptible weeds have the possibility of maintaining higher nematode populations even when cultivated crops were harvested. The *Meloidogyne javanica* is confirmed as a good host for several weed species all around the world including *Amaranthus hybridus, Bidens pillosa, Sesbania aculeata, Digitaria horizontalis, Euphorbia heterophylla, Chenopodium album, Gutenbergia cordifolia, Melilotus alba, Amaranthus hybridus, Solanum americanum, Portulaca oleracea* (Desaeger & Rao, 2000; Lorenzo et al., 2002; Walker et al., 2002; Khan & Murmu, 2004; Gharabadiyan et al., 2012).

After our extensive plant observations *M. javanica* infection was detected in 13 weed species and severity of infection was highest in three species including *C. album, A. arvensis* and *P. oleracea*.

Being among major pests competing for water, light, and nutrients these weeds are good hosts for many nematode species. They have many impacts on nematode populations by reducing the effect of nematode management strategies, protection of nematodes from bad soil conditions and pesticide applications (Thomas et al., 2005). In addition the survival and feed of nematodes on weed roots may result in development of resistance - breaking strains (Samaliev & Stoyanov, 2007). In our study areas nematode populations were still higher even when tomato plants were removed and new infections was observed in the following growing seasons.

According to our gall index results 9 weed species were found to have moderate to high rates. It is considered that *M. javanica* juveniles prefer these plants to feed and maintain its populaton. Our findings suggest that under heavy nematode infestations especially management of these weeds is essential for the the appropriate nematode population suppression.

This report was first which describes root-knot species and host status of some weeds in Northwestern Marmara Region Turkey.

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Table 1. Root galling index of Taylor and Sasser (1978).

Grade	Presence of gall %
0	No gall
1	1-20%
2	21-40%
3	41-60%
4	61-80%
5	81-100%

Table 2. Comparative measurements of J2 of *Meloidogyne javanica* In addition PCR technique using Fjav and Sjav primer pair of *M. javanica* was yielded DNA fragment of 670 bp (Fig. 3).

	J2 Tekirdağ	**Özaslandan& Elekcioğlu, 2010**	**Whithead, 1968**
n	**10**	**10**	**10**
Body length (L)	430 (423.5-456.2)	408.0-454.4	387-459
Tail lenght	51.7 (49.6-57.3)	46.40-59.20	36-56
Hyaline terminus lenght	12.28 (11.6-15.3)	11.20-15.20	-
Spear lenght	12.5 (12.1-12.7)	11.20-14.40	9.4-11.4
DGO-stylet knob	3.21 (3.1-3.5)	3.2-4.0	4
a	31.2	30.33	27.1-35.9
c	7.95-8.31	8.33	7.3-1.1

Table 3. Name of all weeds identified in tomato fields and *Meloidogyne javanica* gall index rates in infected weed species.

Weed species	Common name	Family	Lifespan	Gall index
Amaranthus retroflexus L.	Pig weed	Amaranthaceae	Annual	2
Amaranthus viridis L.	Slender Amaranth	Amaranthaceae	Annual	1
Anagallis arvensis L.	Scarlet pimpernel	Primulaceae	Annual	3
Capsella bursa-pastoris L.	Shepherd's purse	Brassicaceae	Annual	0
Cynodon dactylon L	Bermuda grass	Poaceae	Annual/ Perannual	2
Chenopodium album L.	Lambs quarters	Chenopodiaceae	Annual	3
Eleusine indica (L.) Gaertn	Goosegrass	Poaceae	Annual	2
Euphorbia helioscopia L.	Sun spurge	Euphorbiaceae	Annual	0
Gallium aparine L.	Stickwilly	Rubiaceae	Annual	2
Geranium dissectum L.	Cutleaf geranium	Geraniaceae	Annual	0
Heliotropium europaeum L.	Heliotrope	Boraginaceae	Annual	1
Lepidium draba L.	Hoary cress	Brassicaceae	Biannual	2
Lamium amplexicaule L.	Henbit	Lamiaceae	Annual	1
Solanum nigrum L.	Black nightshade	Solanaceae	Annual/ perannual	0
Sinapis arvensis L.	Charlock mustard	Brassicaceae	Annual	0
Lactuca serriola L.	Pricky lettuce	Asterceae	Biaannual	2
Portulaca oleraceae L.	Common purslane	Portulacaceae	Annual	3
Trifolium repens L.	White clower	Fabaceae	Perannual	1

Figure 1. Root damage on highly susceptible and moderate susceptible tomato cultivars.

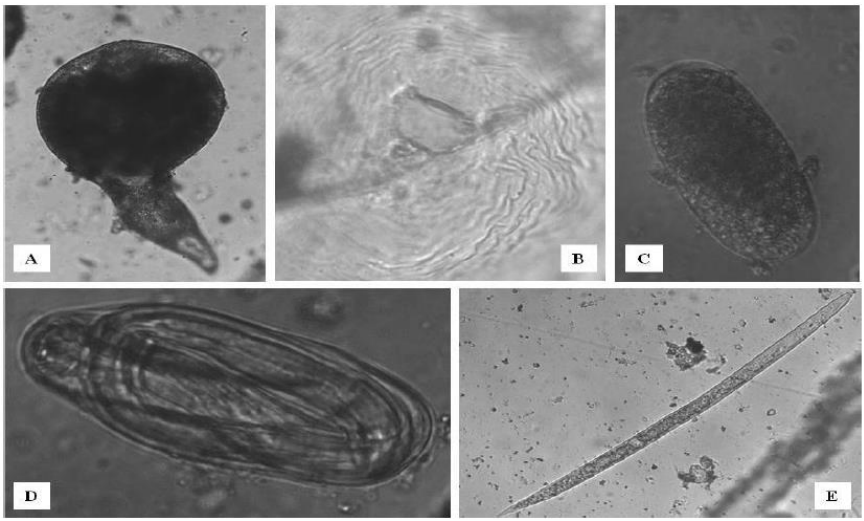

Figure 2. A) *Meloidogyne javanica* female B) *M. javanica* female perineal pattern C) *M. javanica* egg D) J1 inside *M. javanica* egg E) *M. javanica* J2 Morphometric measurements of J2 individuals were given in Table 2.

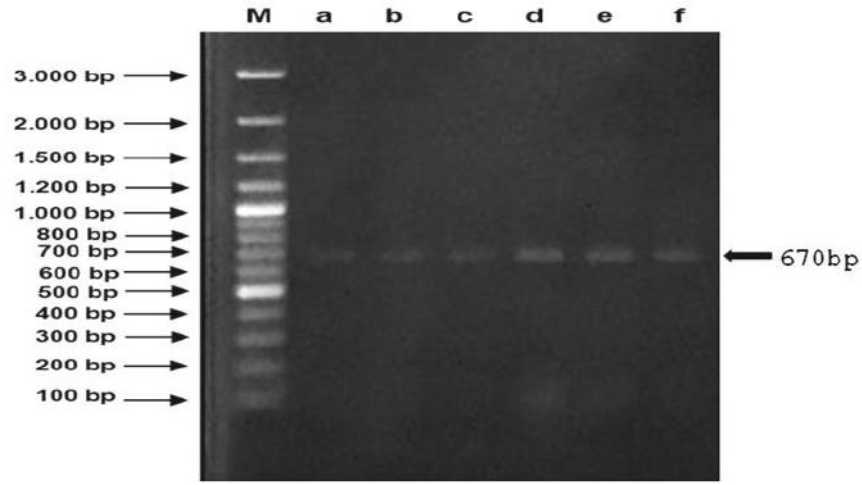

Figure 3. Electrophoresis of the amplified products of *Meloidogyne javanica*.

Figure 4. Weed roots infected with *Meloidogyne javanica.*