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Effect of culture filtrate concentration of *Rhizoctonia solani* Kühn against *Meloidogyne incognita* and *Meloidogyne hapla in vitro*

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Abstract

The effect of culture filtrates of concentration of *Rhizoctonia solani* Kühn against *Meloidogyne incognita* and *Meloidogyne hapla* on juvenile mortality, hatching of egg masses and individual eggs *in vitro* has been investigated. The *Rhizoctonia solani* culture filtrate was diluted from 100% to make 75%, 50% and 25% concentrations. While hatched juvenile of *M. incognita* and *M. hapla* were counted after 7 days and juvenile mortality of *M. incognita* and *M. hapla* counted after 24 h. Culture filtrates of *R.solani* showed negative effects on *M. incognita* and *M. hapla* eggs and juveniles and directly proportional to the concentration of culture filtrates. The highest negative effect was found on juveniles in both nematode species. The negative effects on egg hatching and juvenile mortality of *R. solani* on *M. hapla* was found to be lower than *M. incognita*. This results showed that *R. solani* culture filtrates showed toxic effects on *M. incognita* and *M. hapla* eggs and juveniles *in vitro* and nematode species was important in this effect.

Keywords: *Rhizoctonia solani*, root knot nematode, egg- hatching, juvenile mortality, antagonism

INTRODUCTION

Rhizoctonia solani (Teleomorph: *Thanatephorus cucumeris*) Kühn is a soil-borne pathogen and attacks the hosts roots and lower stems when seeds and seedlings, causing serious yield losses (Parmeter, 1970). Root-knot nematodes are the most economically important plant parasitic nematode group. These obligate endoparasite nematodes cause damage to more than 3,000 plant species (Trudgill and Block, 2001). Infective second-stage juveniles (J2s) penetrate plant roots and settle near the vascular tissues, where they induce the formation of elaborate giant cells (Niu et al., 2016). Southern Root-Knot nematode *Meloidogyne incognita* is the most common species, it can infect almost all plants and causes significant economic damages (Sasser and Freckman, 1987; Johnson & Fassuliotis, 1984). Northern Root-Knot nematode *Meloidogyne hapla* is distributed particularly in cooler and temperate regions, higher altitude areas of the tropics (Whitehead, 1969; Taylor and Buhrer, 1958). In Turkey, nematological studies revealed that *M. incognita* and *M. javanica* were dominant species and cause severe damage to economic crops (Uysal et al., 2017; Özarslandan ve Elekçioğlu, 2010; Devran ve Söğüt, 2009). *Meloidogyne hapla* were determined from pepino, kiwifruit, tomatoes, pepper, patotoes, strawberry and eggplant in Turkey (Özarslandan et al., 2021; Uysal et al., 2017; Akyazı et al., 2017;2012; Özarslandan et al., 2005). *Rhizoctania solani* and *Meloidogyne spp.* are common inhabitants of crop rhizosphere and frequently interact among themselves showing synergistic, antagonistic or antibiotic relationship (Kumar and Haseeb, 2009; Sagar et al., 2012; Misiha et al., 2013; Al-Hazmi and Al-Nadary, 2015). Haseeb (2003), *M. incognita* and *R. solani* damaged tomato fields of western districts of Uttar Pradesh.

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The wide host range provides it easier to survive in the soil for longer periods and crop rotation cannot be used to control root-knot nematodes (Brodie et al., 1993). Chemical control is the most widely used in the world for root-knot nematodes (Wang et al., 2004). However, most of fumigant and nematicides are prohibited due to their harmful effects on humans, animals and the environment (Bhattacharjee & Dey, 2014; El-Nagdi et al., 2017). Therefore, it is very necessary to develop alternative new environmental methods such as biological control in order to improve current management systems (Meyer, 2003). Biocontrol agents of plant parasitic nematodes have been reported with many organisms, including fungi, bacteria, soil invertebrates and predatory nematodes (Stirling, 1991). Relatively little is known about the effects of toxic fungal metabolites on plant parasitic nematode populations in soil. There are studies that report antagonistic relationships between nematodes and fungi (Sankaralingam and McGawley, 1994; El-Borai et al., 2002a, b; Poornima et al., 2007). The nematicidal effect of some *Fusarium* spp. and/or *Rhizoctonia* spp. has been determined (Mani and Sethi, 1984; Ali, 1989; Zareen et al., 2001; Misiha et al., 2013). *Rhizoctonia solani* produces and secretes a non-enzymatic, low molecular weight phytotoxin in liquid culture, as well as cell wall degrading enzymes such as polygalacturonase, cellulase, pectin methylgalacturonase, polygalacturonic acid trans-elimase and pectin methyl trans-elimination (Frank and Francis, 1976; Chen et al., 2006). Fungal pathogens can produce toxic substances that affect nematode activity in their growth medium (Ali, 1989). Culture filtrates from fungal cultures and their active compounds have a potential to be applied as new nematicides in the control against plant parasitic nematodes. A commercial nematicide, DiTera ® (Valent BioSciences Corporation, Libertyville, IL, USA) consist of fungus *Myrothecium* culture filtrates which was originally isolated from *Heterodera glycines* Ichinohe (Soybean Cyst Nematode, SCN) (Meyer et al., 2004).

The objective of the study is to evaluate effect of *Rhizoctonia solani* culture filtrate concentrations onto hatching of egg masses and individual eggs, juvenile mortality of *Meloidogyne incognita* and *M. hapla in vitro* conditions.

MATERIALS AND METHODS

Material

Rhizoctonia solani races were isolated from infected eggplant roots collected from Antalya province in Turkey and identified according to Barnett and Hunter (1998). The root knot nematode material used are DR17 (*Meloidogyne incognita*) and DR15 (*M. hapla*) populations whose mass production continues under climatic chamber conditions (24 ± 1 °C, 60 ± 5% humidity). The DR17 and DR15 populations were taken from in Deregümü eggplant and tomatoes greenhouse of Isparta province, respectively and defined morphologically and molecularly in previous study (Uysal et al., 2017). Since root-knot nematodes are obliged, mass production is continued on living plants and renewed every 2-3 months. Mass production was carried out with the Tueza F1 tomato variety.

Methods

Preparation of *Meloidogyne incognita* and *M. hapla* Egg masses, Eggs and Juvenile larvae (J2)

Egg masses were handpicked from galls of tomato roots. Then, roots surface sterilized in 0.5% sodium hypochlorite for 3 min and washed with sterile water 3 times. Egg-masses were incubated in distilled water for 5 days at 28°C (Misiha et al., 2013). Hatched juveniles were collected daily using a micropipette and stored at 4°C. Eggs were extracted from 0.5–1 cm chopped infested tomato roots suspended in 1% sodium hypochlorite for 5 min at 1800 rpm by centrifugation (Coolen and D'Herde, 1972). Eggs were poured on a 75 µm sieve and collected on 5 µm sieve then the 5µm mesh was washed with tap water to remove sodium hypochlorite (Nico et al., 2004; Liu et al., 2008).

Culture fitrates of *Rhizoctania solani*

Isolates of *R. solani* were grown in potato dextrose broth (PDB). Fifty mL of PDB media was placed in a 250 mL flask and sterilized for 20 minutes at 121 °C. Seven agar discs (8 mm in diameter) were placed in PDB medium and incubated for 8 days at ± 28 °C in the laboratory (Misiha et al., 2013) and shaken by hand every day. The fungal suspension was then vacuum filtered with a sterilized paper filter (Whatmann 3MM) to remove fungal micelles and fungal spores, the pH of the culture filtrates was adjusted to 5.8. Then the culture filtrates were passed through 0.22 µm milipore filters for cold sterilization (Arıcı, 2006).

The obtained fungal filtrates were considered to be 100% concentration as a stock solution. The stock solution was diluted by 75, 50 and 25% by sterilized distilled water (Misiha et al., 2013). After these solutions were prepared, the experiment was set up immediately.

Effect of *Rhizoctania solani* culture filtrates on *Meloidogyne incognita* and *M. hapla* individual egg and egg mass hatch and juvenile larvae *in vitro*

The experiments were conducted in 6 cm diameter autoclaved petri dishes. Sterilized distilled water was used as a positive control, Velum (Fluopyram) (Bayer®) was used as a negative controls, respectively. All experiments were conducted in a completely randomized design with 5 replications. Petri dishes were kept at 25 °C. The experiments were repeated 2 times. The experiment was conducted separately for each *Meloidogyne* species.

Individual egg hatch suppression: One ml of egg suspension (approximately 100 eggs) of *M. incognita* or *M. hapla* and 2 ml of filtrate of different dilutions was put in one after another in each petri dish. Hatched J2 were counted after 7 days. Percentages of suppression hatch were calculated (Liu et al., 2008).

Egg mass hatch suppression: Nearly uniform size two egg masses of *M. incognita* or *M. hapla* were transferred to petri dishes containing 3 ml filtrate of different dilutions. Hatched J2 were counted after 7 days. Percentages of suppression hatch were calculated (Liu et al., 2008).

Juvenile larvae mortality: One ml of J2 suspension (approximately 100 J2) of *M. incognita* or *M. hapla* and 2 ml of filtrate of different dilutions were added in each petri dishes. The dead J2 which they did not move on probing with a fine needle (Cayrol et al., 1989) were counted after 24 h. The percentages of mortality was calculated (Liu et al., 2008).

Statistical analyses

SPSS (version 20.0) program was used for the statistical analysis of the data obtained in the experiments, and analysis of variance (ANOVA) was performed to test the differences between the means. Means were compared by Tukey HSD test at P≤ 0.05.

RESULTS AND DISCUSSION

Culture filtrates of *Rhizoctania solani* was significantly reduced numbers of *Meloidogyne incognita* hatched larvae from individual egg and egg masses compared to the water control as shown in Table 1. The nematicide Velum was suppressed higher hatch of individual egg and egg mass than *Rhizoctania solani* culture filtrates. The highest percentages of J2 mortality was determined as 93.8±0.8 at 100% concentration. The difference between the culture filtrate concentration of *R. solani* and Velum (Fluopyram) was statistically significant (p≤0.05). The lowest percentages of J2 mortality (21.7), individual egg of *M. incognita* (29.6) and egg mass suppression hatch (13.0) were found at 25% *R. solani* culture filtrate concentration. The suppression hatch and J2 mortality decreased as the culture filtrate concentration was diluted. There was a statistically significant difference between the *R. solani* culture filtrate concentrations for percentages of J2 mortality, individual egg and egg mass suppression hatch. The *R. solani* culture filtrate was found to hatch individual egg suppression more effectivetely than the egg mass. In addition, the percentages of J2 mortality was higher than percentages of the individual egg and egg mass suppression hatches. The present results showed that *R. solani* has a negative effect against *M. incognita in vitro* conditions (Table 1).

Table 1. Effect of culture filtrate concentration of *Rhizoctonia solani* Kühn against *Meloidogyne incognita*

Concentration	Percentages of individual egg suppression hatch ±S.E*			Percentages of egg mass suppression hatch ±S.E			Percentages of J2 mortality±S.E		
25%	29.6±0.7	e	A	13.0±0.8	e	C	21.7±1.0	e	B
50%	49.9±0.8	d	A	26.7±0.6	D	B	48.2±0.7	D	A
75%	67.3±1.1	c	B	38.1±0.6	c	C	79.7±0.8	c	A
100%	86.3±0.8	b	B	67.0±0.7	B	C	93.8±0.8	B	A
Control	0.0±0.0	f	B	0.5±0.2	f	AB	0.7±0.2	f	A
Velum	99.2±0.4	a	A	99.4±0.3	a	A	100.0±0.0	a	A

*Different uppercase letters in the same line and different lowercase letters in the same column indicate that the means are significantly different (p≤0.05).

Culture filtrates of *Rhizoctania solani* was significantly supressed *Meloidogyne hapla* individual egg and egg masses hatch compared to the water control as shown in Table 2. However, the effect of *R. solani* culture filtrates on *M. hapla* larvae mortality, hatching individual egg and egg masses were lower than Velum. While the highest percentages of J2 mortality, individual egg and egg mass suppression hatch were found at 100% *R. solani* culture filtrate concentration, the lowest was found at 25% *R. solani* culture filtrate concentration. The number of *M. hapla* was hatched larvae from individual egg and egg masses increased when the culture filtrate concentration was diluted. A statistically significant difference was found between the *R. solani* culture filtrate concentrations in percentages of J2 mortality, individual egg and egg mass suppression hatch. *Rhizoctania solani* culture filtrate more effective *M. hapla* J2 mortality. In the study, *R. solani* was a negative effect against *M. hapla in vitro* conditions. The lowest negative effect was found egg mass supression of *M. hapla* (Table 2).

Table 2. Effect of culture filtrate concentration of *Rhizoctonia solani* Kühn against *Meloidogyne hapla*

Concentration	Percentages of individual egg suppression hatch ±S.E*			Percentages of egg mass suppression hatch ±S.E			Percentages of J2 mortality±S.E		
25%	25.3±1.0	e	A	11.3±0.7	E	B	15.4±1.0	e	B
50%	37.5±1.5	d	A	25.2±1.3	d	C	30.4±1.2	d	B
75%	57.9±0.8	c	A	40.1±0.9	C	B	61.8±1.4	c	A
100%	76.7±1.3	b	B	54.2±1.3	b	C	83.2±1.7	b	A
Control	1.0±0.2	f	A	0.7±0.2	F	A	1.6±0.2	f	A
Velum	95.8±1.2	a	A	94.2±1.8	A	A	91.3±2.9	a	A

*Different uppercase letters in the same line and different lowercase letters in the same column indicate that the means are significantly different (p < 0.05).

The present study reveals that *Rhizoctania solani* culture filtrates was showed negative effects on *Meloidogyne incognita* and *M. hapla* eggs and juveniles. The negative effect of *R. solani* culture filtrate on *M. incognita* was higher than on *M. hapla*. The percentages of J2 mortality, individual egg and egg mass suppression hatch were found to be 92.8%, 86.3%

and 67.0%, respectively on *M. incognita* at 100% *R. solani* concentration, while in *M. hapla* was determinated in 83.2%, 76.7% and 54.2%, respectively. The detection of high J2 mortality and supression hatch in the culture filtrate suggested that the antagonistic effect could be caused by the enzyme or toxins secreted by *R. solani* in this study. These results showed that root-knot nematode population was affected by presence of *Rhizoctonia solani*. Ali (1989) reported that *R. solani* culture filtrates showed toxic effects on *M. javanica* eggs and juveniles. Misiha et al. (2013) showed that culture filtrates of *F. solani* and *R. solani* significantly reduced number of hatched juveniles and increased juvenile mortality of *M. incognita*. Culture filtrates of *Fusarium solani* and *R. solani* have been reported to have some toxic substances that inhibit the hatching of *M. incognita in vitro* (Sakhuja et al., 1978). Al-Hazmi and Al-Nadary (2015) found that the reproduction of *M. incognita* was supressed in the presence of *R. solani* in okra. It was reported that reduction of galling and population of root knot nematode in presence of *R. solani* (Sagar et al., 2012; Kumar and Haseeb, 2009; Roy and Mukhopadhyay, 2004; Mehta et al., 1995; Choo et al., 1990). Also, the negative effects of several soil-borne fungi on the reproduction of Meloidogyne species on several crops were determined in many previous studies (Mokbel et al., 2007; Moussa and Hague, 1988; Al-Hazmi, 1985). There are not many studies with *M. hapla* and *R. solani*. Irvine (1964) determined that the most of plants died were in the *M. hapla* — *R. solani* treatment and followed by *M. hapla* alone treatment but no plants died in the *R. solani* alone treatment. In many and present study showed that root knot nematode population may decrease in the presence of *R. solani*. The results indicate that require field studies for control of *M. incognita* and *M. hapla* infesting plants.

CONCLUSION

The results of the study showed that the culture filtrate of *Rhizoctania solani* had a negative effect on Meloidogyne species. More detailed studies are needed on this subject. In particular, there is a need to determine the content of the culture filtrate of *R. solani*, which has an nematicide effect. In addition, new nematicides can be developed from active compounds obtained from fungal cultures that have a nematicidal effect on root knot nematodes. Another result of this work can provide us with information about the explanation of the disease complex of *R. solani* and Meloidogyne species. Both cause significant product losses in many crops. It is important to consider both pathogens when designing disease and nematode control methods. Different methods should be developed to suppress the population of both agents in the field.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

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