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Interaction between culture filtrates of *Fusarium culmorum* isolates and some Root lesion nematodes

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Abstract

This study was conducted with root lesion nematodes *Pratylenchus penetrans*, *P. thornei* and *P. neglectus* and three isolates of *Fusarium culmorum* culture filtrates (ISP, B4, FC5) *in vitro* conditions. The culture filtrates were diluted 1/0,1/10, 1/20, 1/30, 1/40, 1/50, 1/60, 1/70, 1/80, 1/90, 1/100 in 1.5 ml microtubes, 250 µl of root lesion nematodes adult+larvae were placed in each microtubes that containing different concentrations of culture filtrate with 50 µl of purified water together with micropipette and incubated at 25±1°C. Nematode mortality were determined after 24 h, 48 h and 72 h. As a result, The mortality effect of culture filtrate of FC5 isolates was determined to be high on three lesion nematode species. *Fusarium culmorum* culture filtrates of B4 and ISP isolates caused more deaths on *P. penetrans* than *P.thornei* and *P. neglectus.* It has been found to mortality rate increase over time *in vitro*. The lowest mortality rates were generally found at concentrations of culture filtrates of 1/100 and 1/90, while the highest mortality rates were found at concentrations of 1/0 and 1/10. No difference on *P. thornei* mortality was detected between the three isolates of *F. culmorum* culture filtrate at each diluted concentration. In the study, the antagonistic relationship between culture filtrates from *F. culmorum* isolates and root lesion nematodes were determined *in vitro* conditions.

Keywords: Antagonism, Culture filtrate, *Fusarium culmorum*, Root lesion nematodes, Interaction

Introduction

Root lesion nematodes (*Pratylenchus* spp.) and *Fusarium culmorum* (W.G. Smith) Sacc. cause significant yield losses in cereal roots. *Pratylenchus neglectus* and *P. thornei* caused significantly wheat yield losses economically in the world (Smiley and Nicol, 2009). *Pratylenchus* spp. have a migratory endoparasitic feeding behavior (Yeates et al., 1993) and cause brown lesions on the plant roots and loss of root function, and consequently, reduce in plant vigor and yield (Jones and Fosu-Nyarko, 2014). Also, root lesion nematodes assist the invasion of soilborne pathogens into plant root tissue and, this interaction increases the importance for such infections (Smiley & Nicol, 2009). In addition, *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum* are widespread soilborne fungi in cereals root and crown rot diseases, and decrease the yield (Miedaner et al., 2008; Poole et al., 2012). *Fusarium culmorum* has a highly competitive saprophytic capability and as a facultative parasite, it is able to cause foot and root rot (FRR) and Fusarium head blight (FHB) in wheat and barley (Scherm et al., 2013). Root lesion nematode and *F. culmorum* were reported by researchers in wheat in regions of Turkey (Yavuzaslanoğlu et al., 2012, 2020; Yumurtacı et al., 2017; Dolar et al., 2019).

Many experiments have shown that a biological interaction between nematodes and soil-borne fungi are great importance in agriculture (Bhagawathi et al., 2000; Mallaiah et al., 2014).

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While some researchers state nematodes are necessary in interactions for the formation and development of fungal pathogens, Most researchers report that nematodes generally support the pathogenicity due to the changes they make in the host plant as nutrition and migration (Mauza and Webster, 1992; Bowers et al., 1996; Back et al., 2002; LaMondia, 2003; Hoseini et al., 2010; Mallaiah et al., 2014). However, 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). While the plant is damaged in the synergistic interaction between nematode and fungus, the antagonistic interaction reflects positively on the plant (Back et al., 2002). Taheri et al. (1994) reported that in the presence of *P. neglectus* in wheat, the lesion scale values of *F. oxysporum*, *F. equiseti* and *F. acuminatum* in the roots increased, but the nematode density was lower than the initial inoculation density.

It is not known whether the compounds produced by nematode or fungi are involved in natural interactions between fungi and plant parasitic nematodes. However, many compounds secreted by *Fusarium* species have antagonistic properties in most alive (Eriksen, 1998; Bankole and Adebanjo, 2003; Turkington et al., 2014). *Fusarium* species, which secrete 4,15-diacetylnivalenol and 4,15 diacetoxyscirpenol (DON) compounds that inhibit protein synthesis and activation of defense genes, have toxic effects against plant parasitic nematodes (Rotter et al., 1996; Nitao et al., 2001). Some *Fusarium* species, when grown in laboratory conditions, release toxic compounds against plant parasitic nematodes, and these compounds effect egg laying, viability and mobilization larvae (Nitao et al., 1999;2001). Production of toxic metabolites of endophyte *Fusarium* in the plant not only cause nematode paralysis, but can also inhibit host search and the infection processes (Sikora et al., 2003; Athman et al.,2006). Non-pathogenic *Fusarium* Fo162 reduces invasion of *Meloidogyne incognita* and induces systemic resistance against *M. incognita* by altering the chemical composition of tomato root exudates (Dababat and Sikora 2007a; b). Some *Fusarium* isolates induced systemic resistance in banana against burrowing nematodes *Radopholus similis* (Vu et al., 2006).

Culture filtrates from fungal cultures and their active compounds have the potential to be applied as new nematicides in the control against plant parasitic nematodes. The 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). Various non-endophytic *Fusarium* isolates have been shown to produce filtrates toxic to plant-parasitic nematodes *in vitro* (Athman et al., 2006). *Fusarium solani* culture filtrate is composed of long chain alkanes and shows toxic effect on *M. incognita* (Mani et al., 1986). Several commercial mycotoxins produced by *Fusarium* species have been tested against *M. javanica* and have been found to have nematicidal activity even at low concentrations (Ciancio, 1995). *Fusarium* secondary metabolites are thought to be used control of *R. similis* (Dubois and Coyne, 2011). Göze Özdemir et al. (2018) reported that *F. culmorum* spore suspension was able to suppress root lesion nematodes at low levels *in vitro* conditions and this situation changed depending on the isolates.

The objectives of the current study were to screen *F. culmorum* isolates of antagonistic to Root Lesion Nematode species *(Pratylenchus thornei, P. neglectus, P. penetrans*) and to determine the effects of culture filtrate concentrations on mortality of Root Lesion Nematode species *in vitro* bioassay varied from 1/0-1/100 % dilution ratio.

Materials and Methods

Materials

This study was conducted with *Pratylenchus penetrans*, *P. thornei* and *P. neglectus* root lesion nematodes and three isolates of *Fusarium culmorum* culture filtrates. The root lesion nematodes and *F. culmorum* isolates used in the study are identified in a previous study that conducted in Turkey (Söğüt and Devran, 2011; Arıcı 2006; Arıcı et al., 2013).

Preparation of Root Lesion Nematode

Pure, sterile cultures of *Pratylenchus penetrans*, *P. thornei,* and *P. neglectus* were maintained on carrot disks (Zuckerman et al., 1985). Carrot cultures transferred to 12 cm diameter petri dishes and cut into pieces. Sterile purified water is placed on it and steeps for 4-6 hours for nematodes to pass into the water. Then, the nematodes were passed through a 38 and 20 µm sieve and taken to a centrifuge tube. The larvae+adult density counted under a light microscope and taken into tubes in order to use in the experiments.

Preparation of Culture Filtrates

Fungal culture filtrates were obtained from *Fusarium culmorum* isolates B4 (Adana), ISP (ISP) and FC5 (Ankara). Fifty mL of PDB (Potato Dextrose Broth) media was placed in a 250 mL flask and sterilized for 20 minutes at 121 ° C. Seven agar discs (8 mm in diameter) from each fungal isolate were placed in PDB medium and incubated for 21 days at ± 25°C in the laboratory and shaken by hand every day. The fungal suspension was then vacuum filtered with a sterilized paper filter (Whatmann 3MM) and aspirator, and fungal micelles and spores were removed. The pH of the culture filtrates was adjusted to 5.8. Then the culture filtrates were passed through 0.22 µm milipore filters (Badea et al., 1997; Arıcı, 2006).

Antagonism of Diluted Culture Filtrates

The culture filtrates obtained from *Fusarium culmorum* ISP, B4 and FC5 isolates were diluted 1/0, 1/10, 1/20, 1/30, 1/40, 1/50, 1/60, 1/70, 1/80, 1/90, 1/100 in 1.5 ml microtubes, average 250 µL of root lesion nematode adult+larvae were placed in each microtubes that containing different concentrations of culture filtrate with 50 µl of purified water together with micropipette and incubated at 25±1°C. Total volume in each microtubes was 1100 µL. Three replicate microtubes were included for each concentration. Experiments for each isolate and 3 root lesions nematode were established separately. Pure water was used in the control. All experiments were conducted in a completely randomized design with 3 replications. Nematode mortality was determined after 24 h, 48h and 72 hours. The evaluation was made on mortality rate that the percentage of death. Nematodes were considered

dead if they didn't move when investigated with a fine needle (Cayrol et al, 1989).

Statistical analysis

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. "Tukey" was used in cases where the variances were homogeneous at P≤0.05 significance level to determine the different group averages.

Results and Discussion

In the present study, the culture filtrates of *Fusarium culmorum* isolates have a mortality effect on *Pratylenchus neglectu*s, *P. thornei* and *P. penetrans*, and this effect has been found to increase over time. The effect of culture filtrates of FC5 and ISP isolates on root lesion nematodes were determined higher than B4 isolate and isolate diversity seems to be important factor in terms of their antagonism *in vitro* conditions (Table 1). The mortality rate of *Fusarium culmorum* isolates on root lesion nematodes was evaluated in terms of time. The highest mortality rate was obtained generally after 72 hours, while the lowest mortality rate was determined after 24 h (Table 1). After 24 h, mortality rates were determined to vary between 19.3-58.7% depending on the isolate and nematode species. However, it was observed that mortality rate of nematodes after 48 h were 25.7-73.9%. In the experiment conducted with B4 isolate of *F.culmorum* culture filtrate and *P. penetrans,* mortality rate was found 63.8% while *P. neglectus* and *P. thornei* mortality rates were 32.0% and 45.9%, respectively after 72 h. The culture filtrate of the B4 isolate has a higher lethal effect on *P. penetrans* . After 72 h, mortality rates of *P. neglectus, P. penetrans* and *P. thornei* were found 77.6%, 70.4% and 63.5%, respectively in the culture filtrate of FC5 isolate. There was a statistically significant difference between the mortality rate of the 24, 48 and 72 h only *P. thornei* in culture filtrate of FC5 (P≤0.05) (Table 1). Mortality rate of *P. neglectus* was found 61.4%, while *P. penetrans* and *P. thornei* mortality rates were found 84.8% and 62.4%, respectively in ISP isolate culture filtrate after 72 h. The culture filtrate of the ISP isolate has a higher mortality rate on *P. penetrans* than *P. thornei* and *P. neglectus* (Table 1). There was no statistically significant difference between the mortality rate of 48 h and 72 h in the experiment conducted with the culture filtrate of ISP isolate with *P. neglectus*.and *P. penetrans* (P≥0.05) (Table 1). In addition to difference of isolate, time, and nematode species are also important in the effect of culture filtrate on nematodes *in vitro* conditions.

Differences were found in the mortality effect of different concentrations of culture filtrates on root lesion nematodes *in vitro* conditions. Mortality was found to decrease as the culture filtrate concentrations were diluted. In the study conducted with three root lesion nematodes, the lowest mortality rates were generally detected at 1/100 and 1/90 concentrations of culture filtrates, while the highest mortality rates were found at 1/0 and 1/10 concentrations *in vitro* conditions. However, it seems that even the mortality rates at 1/100 and 1/90 concentrations are higher than control (Table 2). All concentrations of the culture filtrate of the FC5 isolate were showed higher mortality effect on *P. neglectus* than other isolates (Table 2). *Pratylenchus neglectus* mortality rate were found statistically significant between ISP, B4 and FC5 isolate at 1/80, 1/70, 1/60, 1/50, 1/40, 1/30, 1/20, 1/10 and 1/0 culture filtrate concentrations (P≤0.05) (Table 2). ISP isolate is more effective on *P. penetrans* even at low concentrations (Table 2). However, There was no statistically significant difference between *F. culmorum* ISP, B4 and FC5 isolates of mortality rate at the concentrations of 1/50, 1/40, 1/30, 1/20, 1/10 and 1/0 culture filtrate on the *P. penetrans* (P≥0.05) (Table 2). There was no difference between isolates in terms of *P. thornei* mortality rate at all concentrations (Table 2). It was determined that isolate concentrations was significant for mortality on *P. neglectus* but not significant factor on *P. thornei in vitro* conditions (Table 2).

Table 1. Effect of culture filtrates of *Fusarium culmorum* isolates on root lesion nematode

Fusarium culmorum isolate	Hours	Mortality rate (%) ±STD error of mean					
		Root Lesion Nematode					
		Pratylenchus neglectus		*Pratylenchus penetrans*		*Pratylenchus thornei*	
B4 Adana isolate	24	21.2±2.4	b*	35.3±3.6	b	21.3±2.7	b
	48	25.7±2.8	ab	41.2±3.9	b	37.4±4.2	a
	72	32.0±3.1	a	63.8±5.2	a	45.9±3.6	a
FC5 Ankara isolate	24	58.7±5.3	b	38.9±4.8	b	21.2±2.1	c
	48	70.4±5.5	ab	54.1±5.7	ab	47.6±4.7	b
	72	77.6±5.4	a	70.4±5.9	a	63.5±5.4	a
ISP Isparta isolate	24	25.6±2.6	b	34.6±3.1	b	19.3±2.1	c
	48	50.0±5.0	a	73.9±5.3	a	45.3±4.8	b
	72	61.4±5.8	a	84.8±4.7	a	62.4±5.5	a

The effect of each isolate on root lesion nematodes was evaluated within itself.

*There is no statistical difference between the mean shown with the same letter in the same column (P ≤0.05). N:36

Table 2. The effect of culture filtrate concentrations of *Fusarium culmorum* isolates on root lesion nematodes

Fusarium culmorum		Mortality rate (%)±STD error of mean		
		Root Lesion Nematode		
Concentration	Isolate	*Pratylenchus neglectus*	*Pratylenchus penetrans*	*Pratylenchus thornei*
1/100	ISP	9.8±1.3 b*	33.0±5.6 a	8.4±2.1 a
	B4	11.2±0.7 b	15.2±2.3 b	13.5±3.0 a
	FC5	25.5±3.6 a	8.2±1.7 b	12.5±2.3 a
1/90	ISP	19.3±2.9 b	44.9±9.4 a	18.8±4.5 a
	B4	13.7±1.3 b	25.3±4.6 b	15.3±3.3 a
	FC5	37.0±2.8 a	22.8±4.7 b	20.5±4.2 a
1/80	ISP	27.3±3.1 b	63.1±10.0 a	23.9±4.3 a
	B4	15.3±1.3 c	31.2±3.5 b	18.3±3.4 a
	FC5	49.7±2.4 a	29.7±4.6 b	26.2±4.4 a
1/70	ISP	34.3±3.8 b	67.4±10.0a	32.7±5.0 a
	B4	16.3±1.5 c	36.9±3.5 b	23.3±4.3 a
	FC5	69.5±6.3 a	42.4±5.2 b	34.2±4.9 a
1/60	ISP	43.2±4.9 b	71.6±9.6 a	39.1±6.3 a
	B4	19.1±2.1 c	41.6±4.7 b	27.7±4.6 a
	FC5	76.4±5.4 a	56.3±8.0 ab	41.1±6.2 a
1/50	ISP	48.6±5.8 b	75.8±9.9 a	48.1±7.7 a
	B4	23.4±1.7 c	48.3±5.1 a	35.3±4.0 a
	FC5	86.4±5.0 a	64.8±8.3 a	48.7±7.6 a
1/40	ISP	63.2±8.9 b	77.2±9.7 a	56.8±8.7 a
	B4	28.5±1.8 c	57.5±6.1 a	45.9±4.8 a
	FC5	89.3±5.3 a	70.3±7.7 a	58.4±8.5 a
1/30	ISP	66.0±9.4 b	80.1±9.4 a	63.1±9.1 a
	B4	34.7±1.7 c	68.4±6.2 a	50.9±4.2 a
	FC5	94.8±2.6 a	81.5±6.0 a	65.4±9.2 a
1/20	ISP	74.0±9.0 b	85.0±7.4 a	67.6±9.5 a
	B4	45.7±2.5 c	75.4±6.3 a	55.5±4.5 a
	FC5	96.8±1.6 a	87.1±5.2 a	69.7±9.3 a
1/10	ISP	77.8±7.8 b	85.6±7.0 a	71.6±9.6 a
	B4	51.0±2.2 c	78.8±5.3 a	60.0±4.6 a
	FC5	99.5±0.4 a	92.6±3.5 a	72.6±9.3 a
1/0	ISP	83.2±7.1 b	88.3±5.8 a	76.7±8.8 a
	B4	56.1±2.5 c	81.6±4.9 a	70.7±4.6 a
	FC5	100.0±0.0a	96.7±1.6 a	78.0±8.6 a
Control	ISP	1.7±0.2 a	0.9±0.2 a	1.4±0.2 a
	B4	1.7±0.1 a	1.2±0.1 a	2.1±0.2 a
	FC5	1.6±0.1 a	1.3±0.1 a	2.8±0.3 a

The mortality rate of each concentration on root lesion nematodes was evaluated in terms of *Fusarium culmorum* isolates.
* There is no statistical difference between the means shown with the same letter in the same column ($p < 0.05$).N:9
ISP: Isparta isolate,B4: Adana isolate, FC5: Ankara isolate

It was observed that *Fusarium culmorum* culture filtrate obtained from the B4, ISP and FC5 isolates have higher mortality rates on *Pratylenchus thornei, P. neglectus, P. penetrans* with respect to previous study conducted with spore suspension of same *F. culmorum* isolates (Göze Özdemir et al., 2018). Additionally, found that spore suspension of ISP isolate of *F. culmorum* had the highest mortality effect on root nematodes and more effective on *P. thornei* while the lowest mortality effect of isolate was FC5 (Göze Özdemir et al., 2018). However, in this study determined that culture filtrate of FC5 isolate of *F. culmorum* highest mortality rate in the three lesion nematode species. FC5 was found to be the most effective isolate on *P. neglectus* in the study conducted with both spore suspension (Göze Özdemir et al., 2018) and culture filtrate. It has been determined that the application of *F. culmorum* isolates as spore suspension or culture filtrate has significant mortality effect of root lesion nematodes. The antagonistic effect of the culture filtrate appears to be higher than the spore suspension. Also, Mani and Sethi (1984), found that culture filtrates of *F. solani* inactivated 100% *Meloidogyne incognita* juveniles only after 48 h. Non-diluted culture filtrate of *F. roseum vat. arthrosporoides* inactivatied 100% of *M. arenaria* juveniles but a 10% dilution caused no alteration in nematode activity compared to control (Cayrol and Djian, 1990). Hallmann and Sikora(1996), non-pathogenic *F. oxysporum* strain 162 metabolites reduced *M. incognita* mobility within 10 minute of exposure and 98% of juveniles were inactivated at 60 minute, 50% of the juveniles were dead after 5 hours and resulted in 100% mortality at 24 hours. Zaraeen et al. (2001) tested the effects of 10 different *F. solani* strains on *M. javanica in vitro* and under controlled conditions and reported that the strains' culture filtrates varied in terms of parasitism and larval death in the eggs and females of *M. javanica*. Athman et al. (2006) reported that *Radopholus similis* mortality rates after 24 h exposure in culture filtrates ranged from 76.4% to 100% and the length of exposure time to culture filtrates increased the percentage of paralysed nematodes. Mwaura et al. (2010) investigated the lethal effect of 5 endophytic *F. oxysporum* isolates on *P. goodeyi* and found that the percent mortality rate (62.3-72.8) after 48 hours was higher than the control (17.3-34.6%). Yan et al., (2011), reported that among the 294 isolates screened which endophytic fungi from cucumber seedlings, 23 significantly (*Fusarium* (5), *Trichoderma* (1), *Chaetomium* (1), *Acremonium* (1), *Paecilomyces* (1), and *Phyllosticta* (1)) reduced galls formed by *M. incognita* in greenhouse test. Van Dessel et al. (2011) found that 3 different *F. oxysporum* strain culture filtrates caused high mortality rates against *Helicotylenchus multicinctus*, *Radapholus similis* and *P. goodeyi* within 24 hours. In the same study, it was found that *H. multicinctus* was less sensitive than *R. similis* to culture filtrate applications, while *R. similis* was more susceptibly than *P. goodeyi*.

Conclusion

In the study, the antagonistic relationship between culture filtrates from *Fusarium culmorum* isolates and root lesion nematodes were determined *in vitro* conditions. This suggests that *F. culmorum* were able to suppress root lesion nematodes in natural conditions. The highest mortality rate in the three lesion nematode species was also determined in FC5 and ISP isolates. It is not clear whether the compounds produced by *Fusarium* play a role in the natural interactions between fungi and the plant parasitic nematodes. However, the detection of high mortality rates in the culture filtrate suggested that the antagonistic effect might be caused by the enzyme or toxins secreted by *F. culmorum* in this study.

The present study shows that interaction between *F. culmorum* and root lesion nematodes is caused by the ability of isolates from some *Fusarium* spp. to produce toxins. The maximum effect of culture filtrates were observed indicating these isolates can be used as a potential of biocontrol management of lesion nematodes. Therefore, more detailed studies are required, and in particular the determination of the culture filtration content is of great importance. The identification of nematode antagonistic compounds may be a step toward examining such interactions and activities related to antinematode effects in the future. In addition, new nematicides could be developed from active compounds obtained from fungal cultures that have a nematicidal effect on plant parasitic nematodes. As a result of the study, when studying interactions between *Fusarium culmorum* and root lesion nematodes, researchers should take into account that *Fusarium* isolates can produce lethal toxins in nematodes.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Fatma Gul GOZE OZDEMIR, Bulent YASAR, Serife Evrim ARICI designed the experiments. Fatma Gul GOZE OZDEMIR performed the laboratory works and analysed the data. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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filtrate of FC5 isolate and root lesion nematode at International Agriculturel Science Congress, 09-12 May 2018, Van, TURKEY and *F. culmorum* culture filtrate of ISP isolate and root lesion nematode at Agriculture for life, Life for Agriculture,7-9 June, 2018, Bucharest, Romania.

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