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EFFECT OF *FUSARIUM CULMORUM* SPORE SUSPENSION ON MORTALITY OF ROOT LESION NEMATODES IN VITRO CONDITIONS

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Abstract

Three different *Fusarium culmorum* isolates (B4, ISP, Fc5) were used in the study and the effects of these spore suspension of isolates were investigated on the root lesion nematodes *Pratylenchus thornei*, *P. neglectus* and *P. penetrans* in vitro. As a result of evaluating the percent mortality rate, ISP isolate had the highest mortality effect on root nematodes and more effective on *P. thornei*. The lowest mortality effect of isolate was Fc5. However, Fc5 effect on *P. neglectus* was higher than ISP and B4 isolates. Percentage of mortality rate of B4 isolate were found 16.2 on *P. penetrans*, 13.1 on *P. thornei* and 5.5 on *P. neglectus* and showed the lowest effect on *P. neglectus*. It was found that *F.*culmorum spore suspension could suppress root lesion nematodes at low levels in vitro and changed depending on the isolates.

Keywords: *Fusarium culmorum*, Root lesion nematode, Spore suspension, Mortality

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1. Introduction

Root lesion nematodes (*Pratylenchus* spp.) and soil-borne pathogen *Fusarium* spp. are organisms that cause severe damage to grains. Root lesion nematode is one of the most destructive of the three plants parasitic nematode species and is common worldwide. They migrate through root epidermal and cortical cells and cause lesions in the root as a result of feeding (Agrios, 1997). However, they help to induce many soil-borne diseases (Lasserre et al., 1994; Taheri et al., 1994). One of the most economically important genera of phytopathogenic fungi is *Fusarium* spp. due to wide host range, presence different geographic region and environmental conditions around the world and produce a large number of toxins (van der Lee et al., 2015). *Fusarium culmorum* (W.G. Smith) Saccardo_infects roots and cause dry rot that the bottom of the plant appears to be brown. When infected plants are exposed to water stress, the damage increases and can be seen white spike before ripening (Burgess et al., 2001; Smiley et al., 2004). The main mycotoxins of the *F. culmorum* species are trichothecenes, zearalenone, fusarin C, butenolid (Wagacha and Muthomi, 2007). Trichothecenes have immunosuppressive effect even at

low concentrations and they inhibit eukaryotic protein synthesis by binding ribosomes (Gutleb et al., 2002).

Antagonistic relationships between plant parasitic nematodes and soil-borne pathogens were identified and known that many fungi produce nematicidal compounds (Chen et al., 2000; Meyer et al., 2000; Köpcke et al., 2001). It was reported that the presence of *Fusarium oxysporum* f. sp. *lentis* in the soil were reduced in population density of sedentary endoparasite nematode *Meloidogyne incognita* (Fazal et al., 1994). To developing and egg laying of *Meloidogyne spp.* and *Heterodera spp.* species, nutrients from giant cells or syncytia is necessary. If *Fusarium* spp. were colonized and consumed nutrients, the female nematode would have died without laying the eggs (Nordmeyer and Sikora, 1983). In addition, the presence of nutritional deficiencies reduced the number of females (Triantaphyllou, 1960). Metabolites produced by some pathogenic fungi were suppressed the egg-laying of the nematode larvae (Vaishnav et al., 1985; Ciancio et al., 1988). *Colletotrichum atramentarium* reduces cyst formation of *Globodera rostochienensis* with inhibits larval development, reduces the number of eggs and increased male ratio (Powell, 1971).

While *Fusarium* species are commonly found in soil environments, there must be wide opportunities for these pathogens and their secondary metabolites to interact with plant parasitic nematodes. There are limited studies on the effects of toxins and enzymes of *Fusarium* species on mortality of nematodes (Ciancio, 1995) and no study has been conducted on the effect of *F. culmorum* toxins and enzymes on root lesion nematodes. In order to understand the interactions of *F. culmorum* and root lesion nematodes (*Pratylenchus* spp.), it has been firstly considered necessary to study *in vitro* conditions. In this study, the mortality effect of *F.culmorum* against root lesion nematodes was investigated.

2. Materials and Methods

This study was conducted with *Pratylenchus penetrans* (Cobb, 1917), *P. thornei* Sher et Allen and *P. neglectus* (Rensch) Filipjev Schuurmanns & Stekhoven root lesion nematodes. These nematodes distribution and molecular identification made by Söğüt and Devran, (2011). *Fusarium culmorum* isolated from Adana (B4), Isparta (ISP) and Ankara, Turkey (Fc5/ provided Suleyman Demirel University, Department of Agricultural Biotechnology) that had highest pathogenicity were used in the study (Arıcı, 2006; Arıcı et al., 2013).

2.1. Preparation of nematode inoculum

The carrot disc method was used in the mass production of root lesion nematodes (Zuckerman et al., 1985). After 2 months, carrot cultures were transferred to 12 cm diameter petri dishes and cut into pieces. Sterile purified water was placed on it and steep for 4-6 hours for nematodes passing nematodes into water. Then, the nematodes were passed through a 38 and 20 µm sieve and transfered into a centrifuge tube. The larvae + adult densities were counted under light microscope and taken into tubes and made ready for inoculation.

2.2. Preparation of *Fusarium culmorum* inoculum

Fusarium culmorum isolates (B4, ISP, Fc5) were cultured at 24 ± 1^0C on PDA (Potato Dextrose Agar) for 10 days. Then, mycelia were scraped with 5 ml of sterile water and filtering through miracloth into sterile tubes. Spore concentration was determined using a haemocytometer and adjusted to $2.5x10^3$ spores/ml (Arıcı, 2006).

2.3. Effect of *Fusarium culmorum* Spore Suspension on mortality of Root Lesion Nematodes In Vitro Conditions

Fusarium culmorum isolates (B4, ISP, Fc5) and three root lesion nematodes (*P. penetrans, P. thornei, P. neglectus*) were used in the experiment. 200 root lesions nematode larvae + adult and $2.5x10^3$ spores / mL fungus spores were added simultaneously in the 35x10 mm petri dish (Hassan et al., 2012). The dishes were covered with parafilm and they were cultured at 25°C±1 for 3 days. Only nematode-treated petri dishes were taken as controls (C). The treatments were arranged in a randomized complete block design with 3 replicates. After 3 days, live-dead individuals were counted, and percentage deaths were calculated (Liu et al., 2011).

2.4. Statistical analysis

SPSS (version 16.0) program was used for all statistical analysis. One-way ANOVA was conducted to demonstrate variability among treatment means and were separated using Duncan's studentised range test.

3. Results and Discussions

The highest number of living *P. penetrans* individuals was found in the Fc5 *F. culmorum* isolate. The lowest number of living *P. penetrans* individuals determined ISP and B4 isolates, but the differences among them were statistically insignificant ($p \geq 0.05$). On the controry, the number of dead individuals were found to be highest in ISP and B4 isolates and no statistically significant difference was found between them ($p \geq 0.05$). The ISP and B4 isolates had a mortality percentage of 18.6 and 16.2, respectively. The ISP isolate was thought to have a higher mortality than B4. The nematicidal effect of ISP isolate on *P. penetrans* was determinated at low mortality (Table 1).

Table 1. Effect of *Fusarium culmorum* Spore Suspension on *Pratylenchus penetrans*

F. culmorum isolate	Live individuals	Dead individuals	Percentage of mortality
K	192.6±1.45^{c*}	7.3±1.45^{a}	3.6±0.72^{a}
B4	167.6±1.45^{a}	32.3±1.45^{c}	16.2±0.72^{c}
ISP	162.6±1.45^{a}	37.3±1.45^{c}	18.6±0.72^{c}
Fc5	183.6±1.85^{b}	16.3±1.85^{b}	8.2±0.92^{b}

$^{a, b, c}$ Different letters in the same column indicate significant difference ($p < 0.05$).

Pratylenchus neglectus live individuals were found that the highest in the B4 isolate (189.0), the lowest in the ISP (160.0) and the differences ($p \leq 0.05$) among them were statistically significant (Table 2). The number of dead *P. neglectus* individuals were 5.0 in C. The highest mortality between *P. neglectus* (20.0) and *P. penetrans* (18.6) was determined in the application of ISP isolate. The mortality rate of *P. neglectus* was higher than *P. penetrans*, and was found to be at least 5.5 in B4 isolate the mortality effect of Fc5 isolate was higher in *P.neglectus* (12.8) than *P. penetrans* (8.2), whereas B4 isolate was lower in *P.neglectus* (5.5) than *P. penetrans* (16.2). All mortalities rates were found higher than the control group (Table 1 and 2).

Table 2. Effect of *Fusarium culmorum* Spore Suspension on *Pratylenchus neglectus*

F. culmorum isolate	Live individuals	Dead individuals	Percentage of mortality
K	195.0±1,15^{c*}	5.0±1.15^{a}	2.3±0.72^{a}
B4	189.0±2,08^{b}	11.0±2.08^{b}	5.5±1.04^{b}
ISP	160.0±1,15^{a}	40.0±1.15^{d}	20.0±0.6^{d}
Fc5	174.3±2,3^{b}	25.6±2.3^{c}	12.8±1.16^{c}

$^{a, b, c, d}$ Different letters in the same column indicate significant difference ($p < 0.05$).

Pratylenchus thornei live individuals were found the highest in the Fc5 (190.3) and the lowest in ISP (147.3) and the differences among them were statistically significant ($p < 0.05$). The lowest mortality percentage of *P. thornei* was found in the Fc5 isolate and was lower than the control. Death effect on B4 isolate was lower than ISP, higher than Fc5. Percentage mortalities rate of *P.thornei* were 9.6, on control, 13,1 on B4 and 26.3 on ISP and 4.8 on Fc5 (Table 3).

Table 3. Effect of *Fusarium culmorum* Spore Suspension on *Pratylenchus thornei*

F. culmorum isolate	Live individuals	Dead individuals	Percentage of mortality
K	180.6±1.76^{bc*}	19.3±1.76^{ab}	9.6±0.8^{ab}
B4	173.6±6.38^{b}	26.3±6.38^{b}	13.1±3.19^{b}
ISP	147.3±5.04^{a}	52.6±5.04^{c}	26.3±2.5^{c}
Fc5	190.3±2.60^{c}	9.6±2.6^{a}	4.8±1.3^{a}

$^{a, b, c}$ Different letters in the same column indicate significant difference ($p < 0.05$).

ISP was found the most effective isolate on three-root lesion nematode (Table 1, 2 and 3). Percentage mortalities rate of ISP were 18.6 on *P. penetrans*, 20.0 on *P. neglectus* and 26.3 on *P. thornei* (Table 1, 2 and 3). Fc5 isolate had the lowest mortality effect among the other isolates (Table 1, 2 and 3). *F. culmorum* isolates did not have the same effect on root lesion nematodes. It suggested that each species of root lesion nematodes could have the morphological and physiological structure.

In the study, it was showed that *F. culmorum* spore suspension could suppress root lesion nematodes in vitro, even at low levels, and suppressing depending on the lesion nematode species and isolate of *F.culmorum*. Spore suspension of *F.culmorum* should have a nematicidal effects because of secretion of metabolites, enzymes or mycotoxins (Ciancio, 1995). It has been reported that some *Fusarium* species was secreted toxic compounds against plant parasitic nematodes and these compounds were affected egg laying, viability and larval mobility (Nitao et al., 1999). El-Borai et al. (2002a) reported that *Tylenchus semipenetrans* eggs were inhibited by mycelial growth of *Phythopthora nicotianae* and *F. solani in vitro*. Metabolites produced by some pathogenic fungi have been suppressed the laying of nematode larvae (Vaishnav et al., 1985, Ciancio et al., 1988).

The results from our study indicated that there may be an antagonistic relationship between *F. culmorum* and root lesion nematodes (*Pratylenchus* spp.). Similar results have been found by some investigators to have antagonistic relationships between different nematodes and fungi. It was reported that *T. semipenetrans* population density was increased in citrus plants, while *P. nicotinae* infection was decreasing (Graham and Duncan, 1997; El-Borai et al., 2002b). In addition, it was determinated negative interaction between *Helicotylenchus multicinctus* and *F. oxysporum* f.sp. *cubense* (Poornima et al., 2007). Sankaralingam and McGawley (1994) reported that there was antagonistic interaction between *Rhizoctonia solani* and *Rotylenchulus reniformis* in cotton plant and when the

intensity of *R. solani* increases, population density of nematodes decreases significantly. Some researchers were reported that Nematode and fungal species, population density, plant species, variety, line and soil structure was important between nematode and fungal interactions (Riedel et al., 1985; Khan and Husain, 1989; Bowers et al., 1996; Back et al., 2002; Mohiddin and Rahman-Han, 2014). For these reasons, more detailed studies are needed to determinate the association between *F. culmorum* and root lesion nematodes. In addition, the same studies are planned with culture filtrates of *F. culmorum*. The work will be supported by pots and field studies. This planned study will contribute to a better understanding of the antagonistic relationship.

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