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Research Article

Interaction effect of Root Lesion Nematodes and *Fusarium culmorum* Sacc. on the disease complex on some wheat cultivars

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Disease complex,
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Abstract: The study was conducted to assess a possible interaction between fungal pathogen of *Fusarium culmorum* Sacc. and Root lesion nematodes at sequentially and simultaneously inoculation. *Fusarium culmorum* spore suspension effect evaluated on plant disease severity and nematode density on moderate resistant wheat line to *F. culmorum* (2-49) and susceptible wheat variety (İkizce) under controlled condition. The disease severity was higher on İkizce cv. than 2-49. This indicates that there is a considerable important pathogen resistance. Simultaneous and sequential inoculation of *Pratylenchus thornei* and *F. culmorum* reduced the final nematode density and reproduction rate more than inoculation with only nematode on İkizce cv. The simultaneously *P. penetrans* and *F.culmorum* inoculation (N+F) affected the positively of final density on İkizce cv. The N+ F treatment affected the positively of *P. neglectus* density on İkizce cv., whereas negative effect was found in pre or post inoculation of *F. culmorum* treatments. No synergistic interactions were detected on 2-49 when plants were co-infected by the root lesion nematode and fungus. It was determined that *P. thornei* had a positive effect on disease severity when it entered the plant simultaneously and before the *F. culmorum* on İkizce cv.. The disease severity decreased in *F. culmorum* was applied four weeks after the *P. neglectus* treatment on 2-49 wheat line and İkizce cv. In the interaction with *F. culmorum* on İkizce cv., differences were determined between the species of root lesion nematodes.

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Bazı buğday çeşitlerinde Kök Lezyon Nematodları ve *Fusarium culmorum* Sacc.'ın hastalık kompleksi üzerindeki etkileşim etkisi

Makale Bilgileri

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Öz: Çalışma, *Fusarium culmorum* Sacc. ve Kök lezyon nematodları arasındaki olası bir etkileşimi sıralı ve eş zamanlı inokulasyon yapılarak değerlendirmek için yapılmıştır. *Fusarium culmorum* spor süspansiyonunun *F. culmorum*'a orta dayanıklı buğday hattı (2-49) ve duyarlı buğday çeşidinde (İkizce) kontrollü koşullarda bitki hastalık şiddeti ve nematod yoğunluğu üzerine etkisi değerlendirilmiştir. İkizce buğday çeşidinde hastalık şiddeti 2-49 buğday

Anahtar Kelimeler

Hastalık şiddeti,
Fusarium culmorum,
İnteraksiyon,
Kök lezyon nematodu,
Buğday

hattından daha yüksek bulunmuştur. Bu patojene dayanıklılığın oldukça önemli olduğunu göstermektedir. İkizce çeşidinde *Pratylenchus thornei* ve *F. culmorum*'un eş zamanlı ve ardışık inokulasyonu sadece nematod inokulasyonu ile karşılaştırıldığında son nematod yoğunluğunu ve üreme oranını önemli ölçüde azaltmıştır. Eş zamanlı *P. penetrans* ve *F. culmorum* (N+F) inokulasyonu İkizce cv. çeşidinde son nematod yoğunluğunu pozitif etkilemiştir. İkizce çeşidinde N+ F uygulaması *P. neglectus* yoğunluğunu olumlu yönde etkilerken, *F. culmorum*'un önce ya da sonra uygulamalarında olumsuz etki tespit edilmiştir. Bitkiler, kök lezyonu nematodu ve fungus tarafından birlikte enfekte edildiğinde, 2-49 buğday hattında hiçbir sinerjistik etkileşim tespit edilmemiştir. *Pratylenchus thornei*'nin *F. culmorum*'dan önce ve eş zamanlı olarak bitkiye inokulasyonunda hastalık şiddetine olumlu etki yaptığı belirlenmiştir. *Fusarium culmorum* hastalık şiddeti 2-49 buğday hattı ve İkizce buğday çeşidinde *P. neglectus* uygulamasından 4 hafta sonra *F. culmorum* uygulamasında azalmıştır. İkizce buğday çeşidinde *F. culmorum* ile etkileşimde, kök lezyonu nematodlarının türleri arasında farklılıklar tespit edilmiştir.

1. Introduction

Wheat is one of the most important food sources with cultivated approximately 20% land area of the world (Braun et al., 2010). Among the world's wheat producers, Turkey has been important level with 16-21 million tonnes of wheat production. However, there are several biotic and abiotic stress factors that seriously affect wheat production. *Pratylenchus neglectus* (Rench, 1924) Filipjev Schuurmanns & Stekhoven and *P. thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) cause significant economic yield losses in wheat by reducing quality and quantity in the world (Smiley and Nicol, 2009). It has been determined that *P. thornei* causes yield loss of 38-85% in Australia, 12-37% in Mexico, 70% in Israel, 20-32% in Turkey, and 50% in the USA (Armstrong et al., 1993; Gözel, 2001; Nicol and Ortiz-Monasterio, 2004; Smiley et al., 2005; Toktay, 2008). In addition, 36-85% yield losses in the spring wheat associated with *P. neglectus* populations were reported in Oregon, USA (Smiley et al., 2005; Yan et al., 2010). *Pratylenchus penetrans* have been recorded in wheat-growing fields of Iran (Ghaderi et al., 2010), Morocco (Mokrini et al., 2016), and Turkey (Yüksel, 1974; Göze Özdemir et al., 2021). *Pratylenchus penetrans* affects wheat crops yield by 10–19% in Canada (Nicol and Rivoal, 2008). It has been reported that *P. thornei* and *P. neglectus* were found in different densities and mixed populations in wheat fields in different regions of Turkey (Yavuzaslanoğlu et al., 2012; Yavuzaslanoğlu et al., 2020; Göze Özdemir et al., 2021). *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum* Sacc. are widespread soil-borne fungi in wheat as root and crown rot diseases and decrease the yield in the world and in Turkey (Miedaner et al., 2008; Poole et al., 2012; Köycü and Sukut, 2018; Erginbaş Orakçı et al., 2018). It was determined that yield losses from *F. culmorum* in Turkey reached 43% in winter wheat and 54% in durum wheat in the Central Anatolian plateau (Bağcı et al., 2001; Hekimhan et al., 2004). *Fusarium culmorum* is known to produce toxins and enzymes to cause infection, and these toxins play an important role in the pathogenicity (Hestbjerg et al., 2002; Llorens et al., 2006) and important in the nematode-fungus interaction with synergism or antagonism (Back et al., 2002). *Fusarium culmorum* spore suspension and culture filtrate had negative effects on root-lesion nematodes in different levels of *in-vitro* studies (Göze Özdemir et al., 2018 and 2021). Similarly, several studies were reported antagonistic relationships between nematodes and fungi (Sankaralingam and McGawley, 1994; El-Borai et al., 2002a and 2002b; Poornima et al., 2007).

Organisms occupying the same niche can interact with each other and are not exceptional for plant pathogenic fungi and nematodes. When plant pathogenic fungi and nematodes occur together on the same plant, they can act independently and have an additive effect on damage and yield, or interact with each other in a synergistic or antagonistic causing more and less damage, respectively (Edin et al., 2019; Viketoft et al., 2020). Root lesion nematodes act intracellularly in the root cortex and create a pathway by breaking the cell wall with their stylet (Castillo and Vovlas, 2007). These breaking cells and wounds help many soilborne diseases and pests to enter the plant (Hoseini et al., 2010; Mallaiah et al., 2014). In several earlier studies, it has been reported that nematode penetration increased enzymes secreted by a soil-borne fungal pathogen in roots (Edmund and Mai, 1967; Nord-Meyer and Sikora, 1983). Soil-borne pathogen infection of roots can result in reduced host resistance, which can lead to larger nematode populations (Taheri et al., 1994; Back et al., 2002; Viketoft et al., 2020).

The aim of this study was to investigate the interaction in disease complex between *P. penetrans*, *P. thornei*, *P. neglectus* species, and *F. culmorum* on moderate resistance to *F. culmorum* wheat line and susceptible wheat cultivar in controlled conditions.

2. Material and Methods

2.1. Materials

Three root-lesion nematode species, *Pratylenchus penetrans*, *P. thornei*, *P. neglectus,* collected from Isparta province, were purified. Morphological-molecular identifications of these root-lesion nematode species were made by Söğüt and Devran (2011). The cultures of these species are preserved under laboratory conditions, and the mass production of carrot cultures was continued at regular intervals. *Fusarium culmorum* B4 isolate originated from Adana province (Arıcı, 2006) was used in the study. *Fusarium culmorum* isolate was reisolated periodically to prevent loss of pathogenicity. Wheat variety 2-49 Moderate resistant to *F. culmorum* wheat line used breeding material of *F. culmorum*, spring and bread wheat line (Erginbas-Orakci et al., 2016 and 2018) were provided from CIMMYT (International Maize and Wheat Improvement Center). Susceptible İkizce wheat cultivar was obtained from the Department of Crop Sciences in ISUBU (Isparta University of Applied Sciences).

2.2. Nematode inoculum

Mass cultures of *Pratylenchus penetrans*, *P. thornei*, and *P. neglectus* were reproduced and maintained on carrot disks (Zuckerman et al., 1985). Root lesion nematode with carrots transferred to 12 cm diam petri dishes by cutting into small pieces and added sterilized water, then waited for approximately 6 hours for nematode extraction. Nematodes were extracted by using 38 and 20 µm sieve into a centrifuge tube. Nematode inoculum composed of larvae+adults was counted under the light microscope, and a thousand larvae + adult nematodes were prepared to use in the experiments.

2.3. *Fusarium culmorum* spore suspension

In the first step, 120 g rye grains were weighed in the jar and added pure water, and then waited for 24 hours. In the second stage, these jars were autoclaved at 1.2 atm for sterilization for 20 minutes and taken to room temperature to cool. In the third step, jars were kept for two days by a stretched-film cover. Then *F. culmorum* was detected previously pure cultured into potato dextrose agar and was inoculated with 10 discs on rye grains per jar. The inoculated jars were incubated for 10 days. Then, these grains cultured with *F. culmorum* were taken out of the jar by mixing; 1 g sample was placed into 5 ml sterile water and filtered with a micro cloth into sterile tubes. Spore concentration was counted under the light microscope by using a hemocytometer adjusted to 2500 spores g^{-1} soil density (Hassan et al., 2012).

2.4. Interaction between Root lesion nematodes and *Fusarium culmorum* on 2-49 moderate resistant and susceptible İkizce wheat cultivars

The study was conducted between 2018-2019. Root lesion nematodes *Pratylenchus thornei*, *P. neglectus,* and *P. penetrans* and wheat lines susceptible İkizce and moderate resistant 2-49 were used in all interaction experiments. All experiments were carried out under controlled conditions with 25±2 °C temperature and 60±5% relative humidity and were conducted randomized block design with 5 replicates. Wheat seeds were sown into each pot, including autoclaved ca. 200 g sandy soil. Ten days after wheat germination, wheat germination, root-lesion nematodes, or *F. culmorum* were applied to ca. 3 cm soil surface depth around the root zone with plastic pipettes. Six treatments were made up simultaneously and sequentially to determine interactions. Treatments were; untreated control (C), only *F. culmorum* inoculation (F), only root-lesion nematode inoculation (N), simultaneously inoculation of root-lesion nematode and *F. culmorum* (N+F), First *F. culmorum* inoculation- sequentially four weeks after root-lesion nematode inoculation (F+4N) and first root-lesion nematode inoculation – sequentially four weeks later *F. culmorum* inoculation (N+4F). Initial root-lesion nematode density (Pi) was 1000 eggs+larvae+adult each pot^{-1} in all experiments. *Fusarium culmorum*, 2500 spores g^{-1} soil were used in the experiment, then watered with tab-water according to Hasan et al. (2012). Plants were removed from soil approximately 10 weeks later and washed with tab-water. Disease severity of *F. culmorum* on the root and root coller was evaluated 0-4 disease index reported by Wildermuth and McNamara, (1994) where 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 50-75% necrosis and 4 = greater than 75% necrosis. Final root-lesion nematode density (Pf) in soil and root was determined by extraction technique of Baermann funnel and counted under the light microscope. Additionally, at the end of the study, nematode reproduction rates [PF (final) / PI (first)] were recorded.

2.5. Statistical analysis

Disease severity of *F. culmorum*, final root-lesion nematode density, the reproduction rate of each root-lesion nematode species, and plant growth parameters were determined by using SPSS 20.0 (Illionis, US.) analysis of variance (ANOVA). Treatments were compared with TUKEY to determine the differences between means at 0.05 significance levels. Also, a t-test was conducted to determine whether the susceptibility of the wheat was important in the evaluation parameters of each application ($p \leq 0.05$).

3. Results

3.1. Interaction effect of *Pratylenchus neglectus* and *Fusarium culmorum* B4 isolate

In this study, it was found that N+4F treatment had the lowest disease severity with 1.8, and statistically, the difference was determined between F, N+F, and F+4N treatments on 2-49 wheat line ($p<0.05$). Final nematode density and reproduction rate were close to each other in N, N+F, N+4F, and F+4N treatments on 2-49 wheat lines ($p<0.05$). The lowest disease severity with 2.6 indexes was observed on N+4F ($P<0.05$) treatment on susceptible İkizce cv., while F, N+F, and F+4N had the highest disease severity with 4.0 index. The final population density and reproduction rate of *P. neglectus* in N and N+F treatment had higher than F+4N and N+4F treatments on susceptible İkizce cv. Interestingly, the final nematode density and reproduction rate of N+4F treatment was found extremely low level compared to N+F treatment. In the pre-contamination application of *F. culmorum*, the population density of *P. neglectus* decreased compared to the post-contamination application statistically in the Table 1.

Table 1. Interaction effect of *Pratylenchus neglectus* and *Fusarium culmorum* B4 isolate disease complex on moderate resistant 2-49 line and susceptible İkizce

Treatments [1]	2-49, Moderate resistant wheat line to *F. culmorum*			İkizce, Susceptible wheat variety		
	Disease severity*[2]	Final nematode density*[3] (root+200 g soil)	Reproduction rate (PF/PI)*[4]	Disease severity*[2]	Final nematode density*[3] (root+200 g soil)	Reproduction rate (PF/PI)*[4]
N	--	3344.8±313.6 [a]	3.3±0.3[a]	-	3755.2±303.3 [ab]	3.7±0.3[ab]
F	2.6±0.2 [a]	-	-	4.0±0.0[a]	-	-
N+F	2.6±0.2 [a]	3200.0±301.2 [a]	3.1±0.2[a]	4.0±0.0[a]	4037.6±310.7 [a]	4.0±0.3[a]
F+4N	2.6±0.2 [a]	3141.6±247.6 [a]	3.1±0.2[a]	4.0±0.0[a]	2858.4±86.1 [bc]	2.8±0.0[bc]
N+4F	1.8±0.3[b]	3286.4±281.3 [a]	3.2±0.2[a]	2.6±0.2[b]	2246.4±82.8 [c]	2.2±0.0[c]
Control	-	-		-	-	-

[1]N: Only nematode inoculation, F: Only fungi inoculation, N+F: Simultaneously nematode and fungi inoculation, N+4F: Fungus inoculation plant 4 weeks after nematode application, F+4N: Nematode inoculation plant 4 weeks after fungi application.
[2]0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 50-75% necrosis and 4 = greater than 75% necrosis.
[3]Final nematode density composed of larvae + adult in the 200 g soil and all root systems.
[4]Reproduction rate = final nematode density / initial nematode density.
[5]Letters showed statistical difference among means at 0.05 significance level.

It was determined that whether the wheat is resistant or susceptible to *F. culmorum* is important in the disease severity parameter in F, N+F, N+4F, and F+4N treatments. However, it was found that wheat's resistance and susceptibility to *F. culmorum* were important only in N+4F treatment in final nematode density and reproduction rate parameters (Table 2).

Table 2. Effect of *Fusarium culmorum* resistance on disease complex of *Pratylenchus neglectus* and *Fusarium culmorum* B4 isolate suspension

Treatment[1]	Parameter	t	df	p≤0.05
Only nematode inoculation (N)	Disease severity	-	-	-
	Final nematode density	-0.9	8	0.32
	Reproduction rate	-0.9	8	0.37
Only fungi inoculation (F)	Disease severity	-9.7	4	0.001*
	Final nematode density	-	-	-
	Reproduction rate	-	-	-
Simultaneously nematode and fungi inoculation (N+F)	Disease severity	-9.7	4	0.001*
	Final nematode density	-1.9	8	0.08
	Reproduction rate	-1.9	8	0.08
Nematode inoculation plant 4 weeks after fungi application (F+4N)	Disease severity	-9.7	4	0.001*
	Final nematode density	1.0	8	0.31
	Reproduction rate	1.0	8	0.30
Fungus inoculation plant 4 weeks after nematode application (N+4F)	Disease severity	-4.0	8	0.004*
	Final nematode density	3.5	8	0.008*
	Reproduction rate	3.4	4.4	0.02*

3.2. Interaction effect of *Pratylenchus penetrans* and *Fusarium culmorum* B4 isolate

No statistically significant difference was found among treatments on 2-49 lines in disease severity, final nematode density, and nematode reproduction rate. The disease severity was found 4.0 index in F, N+F, N+4F, and F+4N applications on İkizce cv. No statistically significant difference was found between N, F+4N, and N+4F applications in total nematode density and reproduction rate on İkizce cv (p≥0.05). Total nematode density and reproduction rate were found 3402.4 nematodes root^{-1}+200 g soil and 3.3, respectively in N+F applications that the highest value among the applications on İkizce cv. It appears that simultaneous nematode and fungus inoculation on İkizce cv affects the *P. penetrans* density positively (Table 3).

Table 3. Interaction effect of *Pratylenchus penetrans* and spore suspension of *Fusarium culmorum* B4 disease complex on moderate resistant 2-49 line and susceptible İkizce

Treatments[1]	2-49, Moderate resistant wheat line to *F. culmorum*			İkizce, Susceptible wheat variety		
	Disease severity*[2]	Final nematode density*[3] (root+200 g soil)	Reproduction rate (PF/PI)*[4]	Disease severity*[2]	Final nematode density*[3] (root+200 g soil)	Reproduction rate (PF/PI)*[4]
N	-	2200.0±203.0[a]	2.1±0.1 [a]	-	2422.4±63.8 [b]	2.3±0.0 [b]
F	1.8±0.3[a]	-	-	4.0±0.0 [a]	-	-
N+F	2.2±0.3[a]	2067.6±98.5 [a]	2.0±0.1 [a]	4.0±0.0 [a]	3402.4±212.6 [a]	3.3±0.2 [a]
F+4N	1.8±0.3[a]	2114.4±105.4 [a]	2.0±0.1 [a]	4.0±0.0 [a]	2296.0±98.4 [b]	2.2±0.0 [b]
N+4F	1.6±0.2[a]	2109.6±104.9 [a]	2.0±0.1 [a]	4.0±0.0 [a]	2543.2±54.2 [b]	2.4±0.0 [b]
Control	-	-	-	-	-	-

[1]N: Only nematode inoculation, F: Only fungi inoculation, N+F: Simultaneously nematode and fungi inoculation, N+4F: Fungus inoculation plant 4 weeks after nematode application, F+4N: Nematode inoculation plant 4 weeks after fungi application.
[2]0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 50-75% necrosis and 4 = greater than 75% necrosis.
[3]Final nematode density composed of larvae + adult in the 200 g soil and all root systems.
[4]Reproduction rate = final nematode density / initial nematode density.
[5]Letters showed statistical difference among means at 0.05 significance level.

It was determined that whether the wheat is resistant or susceptible to *F. culmorum* was important in the disease severity parameter in F, N+F, N+4F and F+4N treatments. However, it was found that wheat's resistance and susceptibility to *F. culmorum* was important in N+F and N+4F treatments in final nematode density and reproduction rate parameters (Table 4).

Table 4. Effect of *Fusarium culmorum* resistance on disease complex of *Pratylenchus penetrans* and *Fusarium culmorum* B4 isolate suspension

Treatment[1]	Parameter	t	df	p≤0.05
Only nematode inoculation (N)	Disease severity	-	-	-
	Final nematode density	-1.0	8	0.32
	Reproduction rate	-1.0	8	0.32
Only fungi inoculation (F)	Disease severity	-3.8	8	0.005*
	Final nematode density	-	-	-
	Reproduction rate	-	-	-
Simultaneously nematode and fungi inoculation (N+F)	Disease severity	-4.9	8	0.001*
	Final nematode density	-5.6	8	0.00*
	Reproduction rate	-5.4	8	0.001*
Nematode inoculation plant 4 weeks after fungi application (F+4N)	Disease severity	-5.8	4	0.004*
	Final nematode density	-1.2	8	0.24
	Reproduction rate	-1.3	8	0.20
Fungus inoculation plant 4 weeks after nematode application (N+4F)	Disease severity	-9.7	4	0.001*
	Final nematode density	-3.6	8	0.006*
	Reproduction rate	-3.4	8	0.009*

3.3. Interaction effect of *Pratylenchus thornei* and *Fusarium culmorum* B4 isolate

The statistical difference was found in disease severity in N+F (2.6 indexes) and N+4F (1.6 indexes) treatments on 2-49 lines. It was determined that disease severity decreased in the N+4F treatment on the 2-49 lines. It appears that before inoculation of the *P. thornei* affects the density of *F. culmorum* on the 2-49 lines. No statistical difference was found among treatments on 2-49 lines in final nematode density and nematode reproduction rate. While the highest disease severity was found 4.0 and 3.6 indexes in N+4F and N+F treatments on İkizce cv., the lowest was found 2.2 indexes in F+4N. The final nematode density and reproduction rate on İkizce cv. were higher N and N+4F treatments than N+F and F+4N treatments. The lowest reproduction rate was found in F+4N treatment on İkizce cv. It is seen that the density of *P. thornei* decreases in all applications where fungus and nematodes are together on İkizce cv. (Table 5).

Table 5. Interaction effect of *Pratylenchus thornei* and spore suspension of *Fusarium culmorum* B4 disease complex on moderate resistant 2-49 line and susceptible İkizce

Treatments[1]	2-49, Moderate resistant wheat line to *F. culmorum*			İkizce, Susceptible wheat variety		
	Disease severity*[2]	Final nematode density*[3] (root+100 g soil)	Reproduction rate (PF/PI)*[4]	Disease severity*[2]	Final nematode density*[3] (root+100 g soil)	Reproduction rate (PF/PI)*[4]
N	-	2572.0±213.8 [a]	2.5±0.2 [a]	-	3892.0±252.3 [a]	3.8±0.2 [a]
F	2.2±0.2 [ab]	-	-	3.2±0.2 [b]	-	-
N+F	2.6±0.2 [a]	2608.0±215.2 [a]	2.5±0.2[a]	3.6±0.2 [ab]	2296.0±274.9 [b]	2.2±0.2 [b]
F+4N	2.2±0.2 [ab]	2584.0±94.8 [a]	2.5±0.a	2.2±0.2 [c]	1328.0±137.6 [c]	1.3±0.1 [c]
N+4F	1.6±0.2 [b]	2521.6±194.3 [a]	2.4±0.1 [a]	4.0±0.0 [a]	2376.0±223.2 [ab]	2.3±0.2 [b]
Control	-	-	-	-	-	-

[1]N: Only nematode inoculation, F: Only fungi inoculation, N+F: Simultaneously nematode and fungi inoculation, N+4F: Fungus inoculation plant 4 weeks after nematode application, F+4N: Nematode inoculation plant 4 weeks after fungi application.
[2]0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 50-75% necrosis and 4 = greater than 75% necrosis.
[3]Final nematode density composed of larvae + adult in the 200 g soil and all root systems.
[4]Reproduction rate = final nematode density / initial nematode density.
[5]Letters showed statistical difference among means at 0.05 significance level.

It was determined that whether the wheat is resistant or susceptible to *F. culmorum* is important in the disease severity parameter in F, N+F and N+4F treatments. However, it was found that wheat's resistance and susceptibility to *F. culmorum* was important only in N and F+4N treatment in final nematode density and reproduction rate parameters (Table 6).

Table 6. Effect of *Fusarium culmorum* resistance on disease complex of *Pratylenchus thornei* and *Fusarium culmorum* B4 isolate suspension

Treatment[1]	Parameter	t	df	p≤0.05
Only nematode inoculation (N)	Disease severity	-	-	-
	Final nematode density	-3.9	8	0.004*
	Reproduction rate	-4.0	8	0.004*
Only fungi inoculation (F)	Disease severity	-3.5	8	0.008*
	Final nematode density	-	-	-
	Reproduction rate	-	-	-
Simultaneously nematode and fungi inoculation (N+F)	Disease severity	-2.8	8	0.02*
	Final nematode density	0.8	8	0.39
	Reproduction rate	0.7	8	0.45
Nematode inoculation plant 4 weeks after fungi application (F+4N)	Disease severity	0.0	8	1.00
	Final nematode density	7.5	8	0.00*
	Reproduction rate	7.3	8	0.00*
Fungus inoculation plant 4 weeks after nematode application (N+4F)	Disease severity	-9.7	4	0.001*
	Final nematode density	0.4	8	0.63
	Reproduction rate	0.4	8	0.69

4. Discussion

It was observed that wheat susceptibility of *Fusarium culmorum* directly affects disease severity according to the results of the t-test. The disease severity was found to be lower on 2-49 wheat line than İkizce cv. in all experiments. This suggests that the use of pathogen-resistant plants is important to reduce the damage caused by pathogens. However, there were differences in treatments where plant susceptibility of *F. culmorum* was important in final nematode density and reproduction rate parameters according to the results of the t-test. In the study, the resistance of the plant to the *F. culmorum* was found to be important in terms of nematode development. The final nematode density and reproduction rate changed in treatments where root lesion nematode and *F. culmorum* together on İkizce susceptible variety. Pre or post inoculation of *F. culmorum* can increase or decrease the final nematode density and reproduction rate. The wheat susceptibility of *F. culmorum* in final nematode density and reproduction rate parameters was found to be statistically significant only in N+4F treatment in *P. neglectus* and *F. culmorum* B4 disease complex, whereas it was found significant in N and F+4N treatments in *P. thornei* and *F. culmorum* B4 disease complex. Final nematode density and reproduction rate of N+4F treatment in 2-49 wheat line were higher than İkizce cv. in *P. neglectus* and *F. culmorum* B4 disease complex. In *P. thornei* and *F. culmorum* B4 disease complex, Final nematode density and reproduction rate of N treatment on 2-49 wheat line were lower than İkizce cv. However, The F+4N treatment's final nematode density and reproduction rate on İkizce cv. were lower than 2-49 wheat line. The wheat susceptibility of *F. culmorum* in final nematode density and reproduction rate parameters were found to be statistically significant in N+F, and N+4F treatments in *P. penetrans* and *F. culmorum* B4 disease complex and final nematode density and reproduction rate of N+F treatment on 2-49 wheat line were lower than İkizce cv. However, The N+4F treatment's final nematode density and reproduction rate on İkizce cv were higher than 2-49 wheat lines. As reported by Castillo et al. (2003) the reaction of two different *Meloidogyne artiellia* populations with partially and very resistant chickpea lines and cultivars were investigated under controlled conditions. They found that *M. artiellia* infection generally significantly increased the severity of Fusarium wilt in partially resistant genotypes, but in highly resistant lines, it was determined that the resistance could be broken with increasing fungal inoculum density in the presence of nematodes. In the presence of Root lesion nematodes on 2-49 wheat line, no increases in disease severity was determined, and it was found that the resistance of *F. culmorum* was not broken. Ahmadi et al. (2021) investigated the interactions among *Heterodera filipjevi*, *F. culmorum*, and drought on a set of wheat germplasm with differing levels of resistance/tolerance to cereal cyst nematode, crown rot, and drought. They found that co-occurrence of water stress and *H. filipjevi* increased the cyst density of wheat plants, particularly in accessions susceptible to Heterodera, while co-inoculation of *F. culmorum* and *H. filipjevi* reduced cyst density. Also, 2-49 (resistant to *F. culmorum*) plants were the least affected in all treatments of *H. filipjevi* and *F. culmorum*. The simultaneous nematode and fungus inoculation in the disease complex affected the positively of *P. neglectus* density on İkizce cv., whereas a negative effect was found in pre or post inoculation of *F. culmorum* treatments. *Pratylenchus penetrans* density increased nematode and fungus inoculation on İkizce simultaneously. In *P. thornei* and *F. culmorum* disease complex, the nematode density and reproduction rate decreased in all treatments where fungus and nematodes were together on İkizce. No interaction was detected between *P. thornei, P. penetrans, P. neglectus* species, and *F. culmorum* on 2-49 wheat lines. Root lesion nematodes were found to reproduce on İkizce cv. wheat variety and 2-49 wheat line. However, the development of *F. culmorum* differentiated on İkizce cv. and 2-49, and these differences affected the interaction between nematodes and fungi. This result shows that the density of *F. culmorum* is important in the interaction between nematodes and fungi.

In the present study, while there was no change in final nematode density of *P. neglectus* and *P. thornei* in *F. culmorum* was applied 4 weeks after the nematode treatment (N+4F) compared to only nematode inoculation on 2-49 wheat lines, it was observed that disease severity decreased. However, on İkizce cv., the final nematode density of *P. neglectus* decreased in N+4F treatment compared to only nematode inoculation, and accordingly, disease severity was found decreased. In contrast, the disease severity decreased in *P. thornei* inoculation 4 weeks after the application of *F. culmorum* (F+4N), increased in N+4F treatment on İkizce cv. Interestingly, it was determined that *P. thornei* final nematode density was lower than only *P. thornei* treatment in N+4F and F+4N treatments. It was determined that *P. thornei* had a positive effect on disease severity when it entered the plant simultaneously and before the *F. culmorum.* Hajihassani et al. (2013) investigated the interactions of *Heterodera filipjevi* and *F. culmorum* at different inoculation times in the Sardari winter wheat variety in 2009 and 2010 under field conditions, and it was observed that the nematode was suppressed in *H. filipjevi* application 4 weeks after the *F. culmorum* application. Hassan et al. (2012) have investigated the interaction of *H. avenae* and *F. culmorum* in Sham 3 durum wheat under greenhouse conditions, and as a result of the experiment, it was stated that which organism entered the plant first was important. It was found that *P. sudanensis* was adversely affected when *F. oxysporum* inoculated before nematode (Saadabi and Yassin 2007).

Differences have been determined among Root lesion nematode species in nematode-fungus interaction. The different pathogenicity of nematode species, cuticle permeability, and biochemical differences between nematode species may have affected this situation. When only N applications were examined, the reproductive rate of *P. penetrans* was found to be lower than *P. neglectus* and *P.thornei*. While parthenogenesis is observed in most of the *Pratylenchus* species, sexual reproduction is observed in *P. penetrans* (Castillo and Vovlas, 2007). Previous studies revealed that there was biodiversity in terms of pathogenicity even among populations of the same species, including *P. brachyurus* (Payan and Dickson, 1990), *P. goodeyi* and *P. penetrans* (Hafez et al., 1999), and *P. vulnus* (Pinochet et al., 1994) species are reported (Mudiope et al., 2004). Göze Özdemir (2021) reported that two out of five populations of *P. thornei* had a higher reproduction rate. Furthermore, it has been reported that the cuticle of nematode species affects the penetration of compounds and that biochemical differences between nematode species cause detoxification or degradation of these compounds (Tsoo and Yu, 2000). In the present study, While *P. neglectus* and *P. penetrans* were synergistically in relationship with *F. culmorum* in simultaneous nematode and fungus inoculation, *P. thornei* was an antagonistic relationship with *F. culmorum* in all treatments. Riedel et al. (1985) and Bowers et al. (1996) reported that *Verticillium dahliae* developed in the presence of the *P. penetrans* population in potatoes; however, it did not disease formation with *P. crenatus* and *P. scribneri* presences. Hafez et al. (1999) reported that the *P. neglectus* population in Canada had a synergistic relationship with *V. dahliae*, whereas the Parma and Idaho populations did not have a disease complex with *V. dahliae* and had no yield losses. Bhattarai et al. (2009) found that *Rhizoctonia solani* damage increased in the combination of *Globodera pallida* with *R. solani* or *G. rostochiensis* with *R. solani,* and stem canker index increased significantly in co-inoculation with *G. pallida* and *R. solani* compared with *R. solani* alone.

The results of this study clearly show that the interaction between Root lesion nematode species and *F. culmorum* was affected by multiple factors and synergistic or antagonistic interaction changes according to the treatments difference.Unlike previous studies was found disease severity of *F. culmorum* did not increase in the presence of the Root lesion nematode species in all treatments on İkizce wheat in the study. The density of *F. culmorum* used in the study may have also been effective in this result. The interaction effect may change with increasing fungal inoculum densities, which constitutes a separate study subject. Gao et al. (2006) found that reproduction of *Heterodera glycines* was significantly reduced by high levels of *F. solani,* and severe root necrosis was observed when soybean plants were co-inoculated. In addition, it was determined that *F. culmorum* was effective on nematode density on İkizce cv. Here, the plant defense mechanism should not be overlooked. The reason why the treatment priority of the organism (nematode or fungus) was important in interaction on İkizce may be that the secretions of the organism entering the plant first act as an effector and stimulate the plant defense mechanism. Also, the fact that no interaction could be determined between *F. culmorum* and Root lesion nematodes species on 2-49 wheat line once again showed the importance of plant resistance in control.

In this study, it was shown that both organisms cause significant yield loss in cultivated cereal areas. It is important to consider both pathogens when designing control methods. The priority is to prevent the contamination of the field and the spread of the two organisms. It is seen that it is important to suppress both with early applications in disease complexes. Therefore, are recommended different methods should be established to suppress the population of both pathogens in the field.

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