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ASSOCIATION OF ROOT LESION NEMATODE, *Pratylenchus thornei* AND SOIL BORNE PATHOGEN, *Fusarium culmorum* ISOLATES IN DISEASE COMPLEX ON DIFFERENT BARLEY VARIETIES

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ABSTRACT

This study was conducted to evaluate the interactive effects of twelve culture filtrates of *Fusarium culmorum* isolates(G14, K8, YLVC16, SK20, CAV6, T21, GOL2, GOL18, YOVA21, YOVA22, YOVA27, K17) and *Pratylencus thornei* (SK24 isolate) on nematode disease severity and nematode reproduction in four barley varities i.e. Tosunpaşa, Tarm92, Burakbey, Aydanhanım in this study. The *F. culmorum* isolates and *P. thornei* were taken from Lake regions in Türkiye and cultured under controlled conditions. Disease severity and nematode reproduction were assessed eight weeks post-inoculation. The experiment included three treatments: (1) only *F. culmorum* culture filtrate isolates (FCUL), (2) only *P. thornei* (N) and (3) co-inoculation of *P. thornei* and *F. culmorum* (N+FCUL). The experiment was laid out following comletely randomized design under factorial arrangement with 5 replications for each barley variety and each isolate treatment. All barley cultivars were found to be susceptible to *F. culmorum*. Culture filtrates of *F. culmorum* K17 and CAV6 isolates resulted in the highest disease severity. Simultaneous inoculation of *P. thornei* and culture filtrates of G14, YLVC16, and SK20 significantly increased disease severity across all barley cultivar. The highest mean nematode reproduction rate occurtsred in Burakbey (7.10), and the lowest mean nematode reproduction was in Tosunpaşa (4.60). The nematode reproduction in the culture filtrates of YOVA27 and K17 isolates increased in all varieties in the co-inoculation (N+FCUL) treatments. However, it was determinated that the nematode reproduction rate was lower in nine culture filtrates of *F.culmorum* (G14, K8, SK20, CAV6, T21, GOL2, GOL18, YOVA22, YOVA21) treatments than only nematode treatment. Lesion nematode reproduction had different in only nematode and the co-inoculation with culture filtrate of *F. culmorum* isolates treatment in barley varieties. However, the culture filtrates of *F. culmorum* negatively affected *P. thornei*; and no direct relationship with fungal aggressiveness was established. These findings enhance our understanding of nematode-fungus interactions and may aid in developing new integrated disease management strategies.

Keywords: Barley, *Fusarium culmorum*, Pathogen interactions, *Pratylenchus thornei*.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops worldwide, ranking fourth in global cultivation. It is used for animal feed, human nutrition, and malting for alcoholic beverages. Moreover, barley straw is widely utilazed as bedding material in developed countries and as livestock feed in developing nations (Miralles *et al.*, 2021). However, barley production is consrained by several biotic factors, including nematodes and fungal pathogens. Among the fungal pathogens, species of the genus *Fusarium* are particularly detrimental. Notably, *F. graminearum*, *F. culmorum* and *F. avenaceum* are responsible for significant yield losses in wheat (*Triticum aestivum* L.) and barley, with global economic impacts exceeding USD 1 billion annually (Ferrigo *et al.*, 2016). The *F. culmorum* (W.G. Smith) is prevalent in cooler temperate regions including Australia, Europe, West Asia, North Africa, andAmerica (Chekali *et al.*, 2013). It gives early symptoms in roots and late symptoms occur in the heads (Arıcı and Koç, 2010; Scherm *et al.*, 2013). The pathogen initially infects plant roots and later colonizes the spikelets, causing symptoms such as brown discoloration, plant lodging, leaf blight, spike rot, and contamination with harmful mycotoxin, ultimately reducing grain yield and quality (Bouarda *et al.*, 2022). The virulence of *Fusarium* species varies by environment and genotype, and their mycotoxins negatively impact grain quality and pose health risks to humans and animals (Obanor *et al.*, 2010; Wang *et al.*, 2015). The *F. culmorum* produces an array of mycotoxins, including deoxynivalenol (DON), fumonisins, zearalenone, T-2 toxin, neosolaniol, diacetoxyscirpenol, moniliformin, fusarin C, wortmannin and fusaric acid. These secondary metobolites are significant contributors to the pathogens virulence (Winter *et al.*, 2013; Pasquali *et al.*, 2016). Root lesion nematodes (*Pratylenchus* spp.) are migratory endoparasites known to cause major yield losses in cereals (Mokrini*et al*., 2019). Among the eight *Pratylenchus* species identified in small grains, *P. thornei* is considered the most destructive, incurring losses exceeding USD 8 million annually in the Australian barley industry (Smiley, 2009). These nematodes cause root necrosis by migrating through epidermal, cortical, and endodermal tissues, compromising cell structure and predisposing the plant to secondary microbial infections (Galal *et al.*, 2014).

Interactions between root-lesion nematodes and fungal pathogens are complex and influenced by factors such as plant genotype, pathogen aggressiveness, and fungal metabolites (Back *et al.*, 2002). Although several studies have investigated the nematicidal effects of fungal species, research on the interactions between *Fusarium* spp. and *Pratylenchus* spp. in cereals remains limited (Goze Ozdemir*et al.,* 2022). Some *Fusarium* species produce seconder metabolites capable of suppress in nematode activity (Nitao *et al.*, 2001). For instance, culture filtrate of *F. solani* has shown strong nematicidal activity (Farhat *et al.*, 2022), and *F. oxysporum* has been reported to reduce nematode infections in tomato plants (Martinuz *et al.*, 2013). Given the prevalence of *F. culmorum* in cereal fields and its diverse secondary metabolite profile, it is hypothesized that *F. culmorum* and *P. thornei* may exhibit antagonistic or synergistic interactions (Goze Ozdemir*et al.*, 2023). Therefore, the objective of this research was to evaluate the effects of twelve culture filtrates of *F. culmorum* isolates on *P. thornei* reproduction in four barley varieties, and to explore potential interactive effects on disease severity and nematode population dynamics.

MATERIALS AND METHODS

Four barley varieties (Tosunpaşa, Tarm92, Burakbey, Aydanhanım), 12 *F. culmorum* isolated (G14, K8, YLVC16, SK20, CAV6, T21, GOL2, GOL18, YOVA22, YOVA27, YOVA21, K17) isolated by Goze Ozdemir *et.al.*, (2023), and *P. thornei* SK24 population (Goze Ozdemir, 2021) were used in this study. *F. culmorum* isolates was maintained at +4ºC until use in the experiment. Detailed information on the fungal isolate origin, host plant, and geographic coordinates of each isolate is provided in Table 1.The *P. thornei* population was propagated on carrot discs following the method described by Zuckerman *et al*. (1985).

Nematode inoculum preparation: Carrot discs containing the *P. thornei* SK24 isolate were cut into small pieces and placed in 120 mm petri dishes (Zuckerman *et al*. 1985). Sterile distilled water was added to cover the discs, and nematodes were extracted after six hours using a modified Baermann funnel method. Each replicate of nematode suspensions obtained in measuring cups was reduced to 15 ml and transferred to centrifuge tubes (Mudiope *et al.*, 2004). After waiting for 4 hours for the nematodes to settle to the bottom in the centrifuge tubes, the top liquid was discarded and reduced to 1 ml and the adults + larvae were counted under a light microscope at 40X magnification. Final nematode suspensionswere adjusted to a standard density and stored at +4°C until inoculation.

Table 1. Origins of *Fusarium culmorum* isolates.

Isolate	Place of isolation District/Province	Plant of isolation	Coordinate	Altitude (m)
G14	Gelendost/Isparta	Wheat	N: 38˚12’02.7’’/E: 030˚59’30.7’’	1064
K8	Keçiborlu/Isparta	Wheat	N: 37˚58’48.6’’/E: 030˚17’45.0’’	1163
YLVC16	Yalvaç/Isparta	Barley	N: 38˚19’54.8’’/E: 031˚09’50.7’’	1143
SK20	Şarkikaraağaç/Isparta	Barley	N: 38˚04’59.9’’/E: 031˚24’14.5’’	1211
CAV6	Çavdır/Burdur	Wheat	N: 37˚06’55.8’’/E: 029˚42’41.5’’	1066
T21	Tefenni/Burdur	Wheat	N: 37˚15’14.0"/E: 029˚45’13.3"	1178
GOL2	Gölhisar/Burdur	Barley	N: 37˚05’00.8’’/ E: 029˚31’39.1’’	985
GOL18	Gölhisar/Burdur	Wheat	N: 37˚09’04.5’’/ E: 029˚36’59.7’’	970
YOVA21	Yeşilova/Burdur	Barley	N: 37˚31’25.2"/E: 029˚39’7.3"	1169
YOVA22	Yeşilova/Burdur	Barley	N: 37˚31’19.8"/E: 029˚38’55.3"	1158
YOVA27	Yeşilova/Burdur	Wheat	N: 37˚32’10.6"/E: 029˚37’11.6"	1183
K17	Karamanlı/Burdur	Barley	N: 37˚22’58.4"/E: 029˚53’36.8"	1118

Fungal culture filtrate preparation: Culture filtrates were prepared from 12 *F. culmorum* isolates. Potato dextrose broth (PDB, Neogen®) medium was used in the culture filtrate and sterilized in 250 ml flasks each containing 50 ml PDB in autoclave at 121 °C for 20 min at 1.2 atm pressure. Culture filtrates were prepared by inoculating 5–7 pieces (1 cm) of 7–10 day-old *F. culmorum* colonies grown on potato dextrose agar (PDA) into 250 ml Erlenmeyer flasks containing 50 ml sterilized potato dextrose broth (PDB). Cultures were incubated at 25±1°C in the dark for 21 days with daily manual shaking. Culture filtrates were filtered through Whatman No. 1 paper to remove fungal mycelia, spores and then aspirated into sterile flasks. The pure culture filtrates were diluted 50% with distilled water and stored at +4°C until application (Bhagawati *et al.,* 2000; Goze Ozdemir*et al.,* 2023).

Experimental design and assessment: The study was conducted between April and July in 2023. Soil (68% sand, 21% silt, 11% clay) was sterilized in an autoclave twice for 1 hour at 121 ºC. The pots were folled with 500 g sterilized soil and were placed in a climate chamber under controlled conditions with a temperature of 25±2°C and a proportional humidity of 60±5%. The experiment included three treatments:(1) only *F. culmorum* culture filtrate isolates (FCUL), (2) only *P. thornei* (N) and (3) co-inoculation of *P. thornei* and *F. culmorum* (N+FCUL) (Hoseini *et al*., 2010). The experiment was laid out following comletely randomized design under factorial arrangement with 5 replications for each barley variety and each isolate treatment.Three seeds of each barley variety were planted per pot and three plants were considered as 1 replicate. The experiment was established after the first tiller stage were formed in barley. Nematode inoculation was performed by pipetting 1000 juveniles + adults around the root zone (2–3 cm depth), while 5 ml of fungal culture filtrate was poured on the soil surface in each pot (Goze Ozdemir *et al.*, 2023). Plants were uprooted and evaluated after approximately 8 weeks. Disease severity was rated using a 0-4 scale (Hestbjerg *et al.*,2002). Disease severity: 0 - no disease symptoms; 1 - brown pin point lesions on the coleoptile and/or roots; 2 - extended browning of the coleoptile and/or browning/reddening of the roots; 3- dead coleoptile and/or extended browning/reddening of the roots; and 4 - browning extending beyond the coleoptile on the above-ground plant parts or dead plant or ungerminated kernel.) Nematode reproduction rate was calculated (RF=PF (final)/PI (initial)). The PF value used to calculate the reproduction rate was determined by measuring the density of nematodes in soil (100 g dry) and roots (1 g wet root) using the Baerman funnel method (Goze Ozdemir, 2021).

Statistical analysis: As the disease severity characteristics in FCUL and N+FCUL applications did not meet the pre requisites of parametric tests (normality and homogeneity of variances); therefore, Kruskal-Wallis, one of the nonparametric tests, was used. When comparing isolates, each variety was analyzed separately, and when comparing varieties, each isolate was analyzed separately using the Kruskal-Wallis test. To determine the differences between subgroup rank averages, the Bonferroni-Dunn test, a nonparametric multiple comparison method, was used. The values obtained in terms of reproduction rate date in N+FCUL application were subjected to angle transformation and then analyzed following two-way ANOVA taking 4 barley varieties and 13 levels of *F. culmorum* isolate factor (12 isolates and 1 only *P. thornei* application) as factors. After the variance analysis, the Tukey's HSD, a multiple comparison method, was used to determine the differences between the means of subgroups.

RESULTS

In the comparison of the rank averages of isolates in barley varieties in terms of disease severity, the Kruskal Wallis test results indicted that the isolates were statistically significant ($p \leq 0.05$). The Bonferroni-Dunn test results are shown in Latin letters on the rank averages (Table 2). Barley varieties were susceptible to *F. culmorum* culture filtrates, and these susceptibility varies across *F. culmorum* isolates. There was a significant difference in the pathogenicity of culture filtrates obtained from *F. culmorum* isolates. Culture filtrates of *F. culmorum* isolates G14, GOL2, and YOVA21 consistently exhibited the lowest disease severity, while isolates K17, YOVA27, and CAV6 induced the highest levels in all barley varieties. In co-inoculation (N+FCUL) treatments, disease severity generally increased, particularly with culture filtrates of YLVC16, SK20, YOVA27, K17, CAV6, and T21 isolates, which produced severity scores greater than 3.0 in all varieties. Culture filtrates of G14, YOVA 21, GOL 2 isolates were found to have a disease severity score of less than 2 on a 0-4 scale in the FCUL treatment. In addition, G14 isolate caused a severity score increase from 1.4–2.0 (FCUL) to 2.4–2.6 (N+FCUL). Only the YOVA21 isolate maintained a severity score below 2 in both FCUL and N+FCUL treatments (Table 2).

The two-way ANOVA indicated that barley varieties, culture filtrate of *F.culmorum* isolate and interaction among them had a significant effect on nematode reproduction rate (Table 3).

In the co-inoculation (N+FCUL) treatment the highest reproduction rate was determined with the culture filtrate of YOVA27 isolate, while the lowest reproduction rate was recorded in the culture filtrate of K8 isolate in all barley varieties. In addition, There was a statistically significant difference in terms of reproduction rate between these 2 isolates. It was determined that the reproduction rate in YLVC16, K17, YOVA27 and GOL18 isolates were in the same statistical group in Tarm92 and Burakbey barley varieties. In N+FCUL, the reproduction rate varied from 4.28–5.04 in Tosunpaşa, 4.76–6.60 in Tarm92, 6.64–7.52 in Burakbey, and 4.84–5.36 in Aydanhanım (Table 4).Compared to the only nematode treatment, the reproduction rate of *P. thornei* increased in variety Burakbey in the inoculation with culture filtrates of CAV6 and T21 isolates, while it decreased in the inoculation with GOL2 and GOL18 isolates. In the Tarm92 barley variety, the reproduction rate of *P. thornei* increased in co-inoculation with culture filtrates of YLVC16, YOVA27 and K17 isolates but decreased with K8 and T21 isolates. In simultaneous inoculation, a decrease was determined in the culture filtrates of G14, K8, YLVC16, T21, GOL2 and GOL18 isolates in variety Aydan Hanım and Tosunpaşa. The reproduction rate of *P. thornei* decreased with most culture filtrates including G14, SK20, T21, GOL2 and GOL18 isolates (Fig. 2; Table 4).

Table 2. Disease severity of culture filtrate applications of *Fusarium culmorum* isolates in FCUL and N+FCUL treatments on barley.

Treatments	*Fusarium culmorum* isolate	Barley varieties							
		Tosunpaşa	Average ranks	Tarm92	Average ranks	Burak Bey	Average ranks	Aydan Hanım	Average ranks
FCUL	G14	1.4±0.5	9.8 ^{e}	2.0±0.71	17.2 ^{ef}	2.0±0.01	14.0 ^{ef}	1.6±0.55	11.2 ^{e}
	K8	2.4±0.5	27.7 $^{b-d}$	2.6±0.55	29.4 $^{c-e}$	2.6±0.55	28.4 ^{cd}	2.6±0.55	31.7 $^{b-d}$
	YLVC16	2.6±0.55	32.3 ^{bc}	2.8±0.45	34.2 $^{b-d}$	3.0±0.01	38.0 ^{c}	2.6±0.55	31.7 $^{b-d}$
	SK20	2.6±0.55	32.3 ^{bc}	2.6±0.55	29.4 $^{c-e}$	2.4±0.55	23.6 ^{de}	2.6±0.55	31.7 $^{b-d}$
	ÇAV6	3.4±0.55	47.7 ^{a}	3.6±0.55	49.2 ^{ab}	4.0±0.01	55.5 ^{a}	3.4±0.55	47.7 ^{ab}
	T21	3.0±0.0	41.5 ^{ab}	3.0±0.01	39.0 $^{a-c}$	3.0±0.01	38.0 ^{c}	2.8±0.45	36.6 $^{a-c}$
	GOL2	1.8±0.45	15.6 ^{de}	2.0±0.01	15.0 ^{ef}	1.6±0.55	9.0 ^{f}	2.0±0.01	17.0 ^{de}
	GOL18	2.2±0.45	23.1 $^{c-e}$	2.4±0.55	24.6 $^{c-f}$	2.4±0.55	23.6 ^{de}	2.2±0.45	21.9 $^{c-e}$
	YOVA 21	1.4±0.55	9.8 ^{e}	1.6±0.55	9.8 ^{f}	2.0±0.01	14.0 ^{ef}	1.6±0.55	11.2 ^{e}
	YOVA 22	2.4±0.55	27.7 $^{b-d}$	2.2±0.45	19.8 $^{d-f}$	2.6±0.55	28.4 ^{cd}	2.4±0.55	26.8 $^{c-e}$
	YOVA 27	3.4±0.55	47.7 ^{a}	3.4±0.55	45.8 ^{ab}	3.2±0.45	41.5 ^{bc}	3.4±0.55	47.7 ^{ab}
	K17	3.6±0.55	50.8 ^{a}	3.8±0.45	52.6 ^{a}	3.8±0.45	52.0 ^{ab}	3.6±0.55	50.8 ^{a}
N+FCUL	G14	2.4±0.55	19.6 ^{bc}	2.6±0.55	20.1 ^{cd}	2.4±0.55	18.0 $^{d-f}$	2.4±0.55	18.4 ^{ef}
	K8	2.4±0.55	19.6 ^{bc}	3.0±0.71	28.2 ^{bc}	2.8±0.45	27.0 $^{c-e}$	2.6±0.55	22.6 $^{d-f}$
	YLVC 16	3.6±0.55	43.0 ^{a}	3.0±0.71	45.1 ^{a}	3.0±0.01	31.5 $^{b-d}$	3.4±0.55	39.4 $^{a-c}$
	SK20	3.6±0.55	43.0 ^{a}	3.6±0.55	40.7 ^{ab}	3.0±0.01	31.5 $^{b-d}$	3.4±0.55	39.4 $^{a-c}$
	ÇAV 6	3.6±0.55	43.0 ^{a}	3.8±0.45	45.1 ^{a}	4.0±0.0	53.5 ^{a}	3.8±0.45	47.8 ^{ab}
	T 21	3.4±0.55	39.0 ^{a}	3.4±0.55	36.3 ^{ab}	3.6±0.5	44.7 ^{ab}	3.2±0.45	35.2 $^{b-d}$
	GOL 2	2.0±0.7	13.8 ^{bc}	2.2±0.45	12.7 ^{d}	1.8±0.45	7.4 ^{f}	2.2±0.45	14.2 ^{ef}
	GOL 18	2.4±0.55	19.6 ^{bc}	2.6±0.55	20.1 ^{cd}	2.6±0.55	22.5 ^{de}	2.4±0.55	18.4 ^{ef}
	YOVA 21	1.6±0.55	8.0 ^{c}	1.8±0.45	7.4 ^{d}	2.2±0.45	13.5 ^{ef}	1.8±0.45	8.2 ^{f}
	YOVA 22	2.6±0.55	23.4 ^{b}	2.6±0.55	20.1 ^{cd}	2.8±0.45	27.0 $^{c-e}$	2.8±0.45	26.8 $^{c-e}$
	YOVA 27	3.6±0.55	43.0 ^{a}	3.6±0.55	40.7 ^{ab}	3.4±0.55	40.3 $^{a-c}$	3.6±045	43.6 ^{ab}
	K 17	4.0±0.0	51.0 ^{a}	4.0±0.01	49.5 ^{a}	3.8±0.45	49.1 ^{a}	4.0±0.01	52.0 ^{a}

*Lower case letters in the same column indicate statistical differences in disease severity between isolates in treatments. (FCUL:Only culture filtrate of *F.culmorum* , N+FCUL:*Pratylenchus thornei*+ culture filtrate of *F.culmorum*)

Table 3. Analysies of variance table highlight the significance of individual and interactive effects of barley varieties and isolates on nematode reproduction rate.

Sources of variations	Degree of freedom	Adj SS	Adj MS	F-Value	P-Value
Barley variety	3	352.27	117.424	262.51	0.000
Fusarium culmorum isolate	12	15.69	1.308	2.92	0.001
Barley varietyX *F. culmorum* isolate	36	26.14	0.726	1.62	0.020
Error	208	93.04	0.447		
Total	259	487.15			

Interestingly, high-pathogenic isolates like K17 and CAV6 did not consistently suppress nematode reproduction. Conversely, low-pathogenic isolates such as G14 and GOL2 were associated with significant reductions in RF, particularly in Tosunpaşa, and Aydanhanım varieties. The high-pathogenic isolate K17 did not suppressed nematode production in the Tarm92 variety in the co-inoculation. Low-pathogenic isolates G14 and GOL2 showed a negative effect on *P. thornei* in Aydanhanım and Tosunpaşa barley varieties in N+FCUL treatment. In addition, only in Tosunpaşa barley variety, an increase in the nematode reproduction rate (5.04) was observed in N+FCUL application compared to N application (4.60) (Table 3; Table 6). While it was thought that high pathogenic isolates would be suppressive on nematodes, low pathogenic isolates caused a decrease in the reproduction rate. It is surprising that *F. culmorum* isolates gave results contrary to expectations. These results suggest that while fungal pathogenicity contributes to disease development, its relationship with nematode reproduction is not linear and may depend on isolate-specific secondary metabolites or barley genotype interactions.

Table 4. Reproduction rate of *Pratylenchus thornei* in simultaneous application of nematode+ culture filtrates of *Fusarium culmorum* on barley varieties.

Culture filtrate of *F. culmorum* isolate	Barley varieties			
	Tosun Paşa*	**Tarm 92**	**BurakBey**	**Aydanhanım**
G14	4.42±0.65$^{a\ B}$	5.52±0.40^{a}-$^{c\ B}$	7.04±0.27$^{a\ A}$	4.84±0.50$^{a\ B}$
K8	4.68±0.75 $^{a\ B}$	4.76±0.49$^{c\ B}$	7.08±0.28$^{a\ A}$	4.98±0.43$^{a\ B}$
YLVC16	4.70±0.71$^{a\ B}$	6.40±0.51$^{aB\ A}$	7.02±0.43$^{a\ A}$	4.80±0.32$^{a\ B}$
SK20	4.44±0.71$^{a\ B}$	5.36±0.62^{a}-$^{c\ B}$	7.12±0.13$^{a\ A}$	5.12±0.23$^{a\ B}$
CAV6	4.32±0.78$^{a\ C}$	5.46±0.18^{a}-$^{c\ B}$	7.52±0.36$^{a\ A}$	5.04±0.46$^{a\ BC}$
T 21	4.56±0.34$^{a\ B}$	4.98±0.41$^{Bc\ B}$	7.38±0.36$^{a\ A}$	4.86±0.72$^{a\ B}$
GOL2	4.28±0.53$^{a\ C}$	5.48±0.37^{a}-$^{c\ B}$	6.90±0.29$^{a\ A}$	4.88±0.62$^{a\ BC}$
GOL18	4.48±1.00$^{a\ B}$	5.54±0.43^{a}-$^{c\ AB}$	6.64±0.68$^{a\ A}$	4.92±0.62$^{a\ B}$
YOVA21	5.04±0.53$^{a\ B}$	5.62±0.33^{a}-$^{c\ B}$	7.18±0.26$^{a\ A}$	5.04±0.28$^{a\ B}$
YOVA22	4.62±1.09$^{a\ B}$	5.52±0.40^{a}-$^{c\ B}$	7.14±0.23$^{a\ A}$	5.04±0.54$^{a\ B}$
YOVA27	4.92±0.43$^{a\ B}$	6.58±0.44$^{a\ A}$	6.98±0.63$^{a\ A}$	5.36±0.23$^{a\ B}$
K17	4.66±0.91$^{a\ B}$	6.60±0.37$^{a\ A}$	7.28±0.27$^{a\ A}$	5.14±0.28$^{a\ B}$
Only nematode PT	4.90±0.35$^{a\ B}$	5.92±0.42^{a}-$^{c\ B}$	7.12±0.29$^{a\ A}$	5.16±051$^{a\ B}$

*Lower case letters in the same column indicate statistical differences in nematode reproduction factor between isolates in N+FCUL application and upper case letters in the same line indicate the statistical difference between the mean reproduction rate of *P. thornei* in barley varieties at p≤0.5.

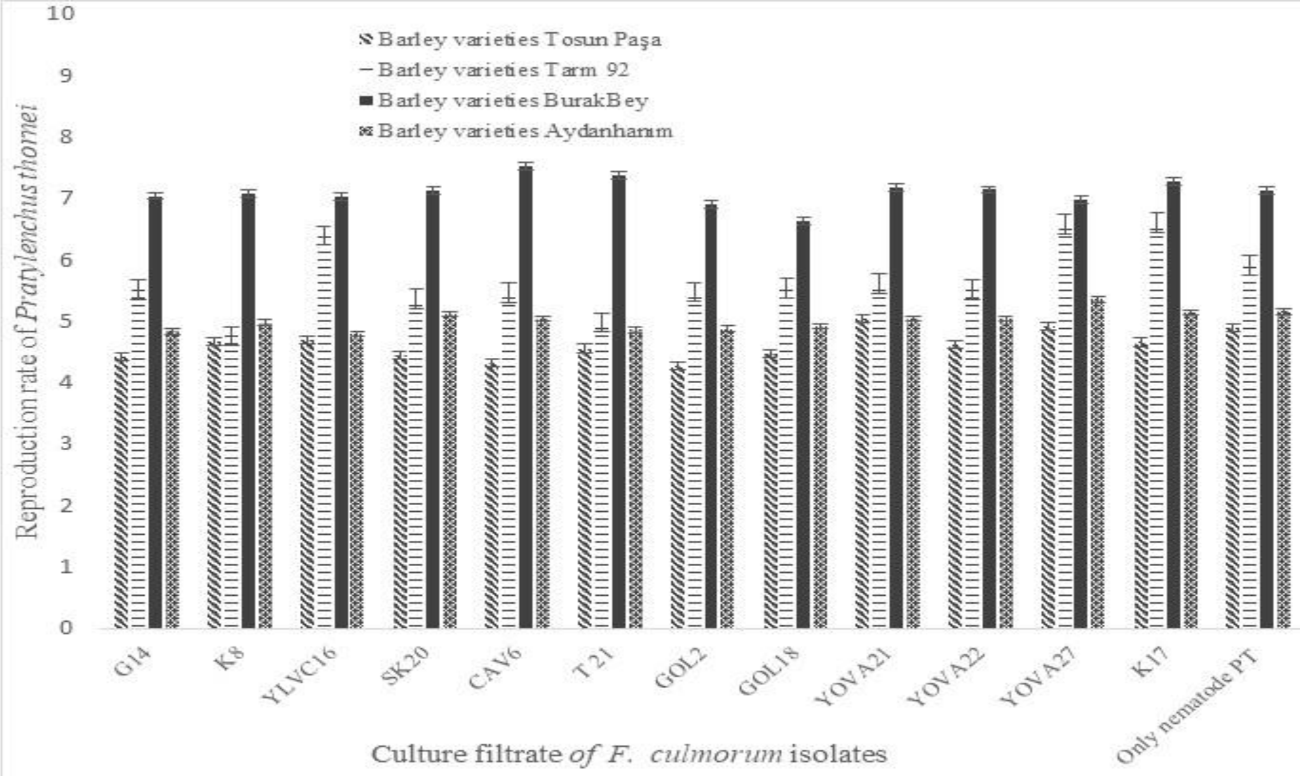

Fig 2. Reproduction rate of *Pratylenchus thornei* on barley varieties in N+FCUL treatment

DISCUSSION

The effects of simultaneous inoculation of different *F. culmorum* culture filtrates and *P. thornei* on disease severity and nematode reproduction rate in barley were compared. The interactions between *F. culmorum* and *P. thornei* appeared to be both antagonistic and mutualistic, depending on the isolate and barley genotype. Interestingly, highly pathogenic isolates did not consistently suppress nematodes, whereas less virulent isolates such as G14 and GOL2 had a strong negative impact on nematode reproduction. However, the negative effect of culture filtrates of *F. culmorum* isolates on *P. thornei* could not be directly linked to aggressiveness or pathogenesis. The nature of interactions between phytophagous nematodes and phytopathogenic fungi varies among different fungal and nematode species. Host plant genotype, availability of organic matter and nutrients, and other biotic and abiotic factors can influence the outcome of infections by nematodes and plant pathogenic fungi. Nematodes can create wounds or physiological changes in plant roots, facilitating fungal infections. For example, some plant pathogenic nematodes can cause physical damage such as small wounds to their host plants. Such wounds can allow fungal pathogens to easily penetrate plant tissues and cause infections. Some nematodes can cause physiological changes to their host plants, leading to changes in fungal pathogen populations around their host plants and increasing the likelihood that their population size and/or pathogenicity will increase. Hovewer, fungal metabolites may inhibit nematode development and reproduction. Abiotic factors such as soil moisture and soil physicochemical properties also play an important role in infections by plant pathogenic fungi and nematode and may further modulate these dynamics (Zhang *et al.*, 2020).

The significant variations were determined in the impact of *F. culmorum* isolates on nematode reproduction rates. Variation in the effects of fungal isolates may also be attributed to environmental conditions, geographic origin, and mycotoxin production. Due to various geographical and meteorological factors, the dominance of various *Fusarium* species can exhibit considerable variability, ranging from global regions to field scales, between seasons, and over longer time periods (Hofer *et al.,* 2019). Mitter *et al.*,(2006) emphasized the high genetic diversity among field populations of *F. culmorum* isolates, even among isolates obtained from a single field. Furthermore, Gamba *et al.* (2017) reported that the agricultural ecology of different regions in Morocco supported the formation of different pathotypes of *Pyrenophora triticirepentis* while attempting to map the race structure of the pathogen. Bouarda *et al.*(2022) investigated the pathogenicity of 10 isolates of *F. culmorum* on 20 durum wheat genotypes in Morocco. The study results showed significant statistical differences between genotypes, isolates, and their interactions. A total of 53% of the phenotypic variation has been explained by interactions between host and pathogen genes, leading researchers to propose that resistance to root rot diseases can only be achieved by pyramiding a few resistance genes. Both genotype and isolate interactions accounted for a significant proportion of disease severity variation in durum wheat. Plant breeding is one of the most effective tools to enhance resistance against adverse climate conditions, various epidemic pathogens, and pests. Potential genetic resources are available, including barley (Ingvordsen *et al.,* 2015). Understanding various interactions between genotype, pathogen, and environment is crucial for the progress of breeding studies. Interactions with root lesion nematodes in breeding studies for resistance against *Fusarium* species can be evaluated. This way, effective gene combinations against both pathogens can be created, especially in genetic pyramids.

Mycotoxin profiles are crucial in defining fungal pathogenicity and nematicidal effects. Inbaia *et al.* (2023) stated that the mycotoxin profile is important in the pathogenicity of *F. avenaceum* isolates isolates in barley plants. In a study by Manka *et al.* (1985), the pathogenicity of 37 *Fusarium* isolates (including 20 *F. culmorum*) was assessed on wheat, rye, and triticale seedlings, and the same isolates were evaluated for their ability to produce trichothecenes in wheat grains. Isolates with high pathogenicity have been reported to produce DON toxin and/or 3-acetyl DON mycotoxins. However, pathogenic isolates that do not produce DON toxin have also been reported. In a study by Hestbjerg *et al.* (2002), on 70 fungal isolates from different geographical regions, it was found that the ability to produce DON toxin may play a role in the aggressiveness of *F. culmorum* against barley seedlings. Gang *et al.*(1998) identified a correlation between the degree of spike blight and DON toxin content in winter rye grains that were inoculated with 34 DON toxin-producing of *F. culmorum* isolates. These studies underscore the importance of mycotoxin profile, type, and quantity in *Fusarium* pathogenicity, and these variations are likely to influence the entire nematode and fungus relationship. In our study, we found that simultaneous inoculations generally decreased nematode reproduction rates. It is possible that mycotoxins, which are also effective in pathogenicity, had a negative effect on nematodes. *Fusarium* species are a rich source of hundreds of compounds belonging to various chemical classes such as butenoides, alkaloids, terpenoids, cytochalasins, phenalenones, xanthones, sterols, diphenyl ether, and anthraquinone derivatives, as well as hundreds of compounds with unique chemical structures (Ahmed *et al*., 2023). *Fusarium* species secrete toxic binary metabolites against plant parasitic nematodes in laboratory cultures. These compounds affect egg hatching, larval viability, and movement (Nitao *et al*., 2001; Singh and Mathur, 2010). Furthermore, some researchers reported that 4,15-diacetylnivalenol and 4,15 diacetoxyscirpenol toxins have toxic effects on plant parasitic nematodes. The culture filtrate of *F. solani* showed strong nematicidal potential and their secondary metabolites were isolated from n-hexane fraction of culture filtrate (Nitao *et al.*, 2001; Farhat *et al.*, 2022). And also, researchers have found that *F. oxysporum* produces nematicidal compounds in tomato plants and reduces nematode infection (Martinuz *et al.*, 2013). Morover, they inhibit host-seeking and infection processes (Dababat and Sikora, 2007). According to the results we obtained, there are different amounts of toxins and enzymes produced by *Fusarium* in the culture filtrates obtained from different isolates, and their proportions have not been fully determined in our current study.

In this studies, the content of the culture filtrate may have different amounts of mycotoxins and some *F. culmorum* isolates may have suppressed the reproduction of the nematode due to the effect of these toxins. In a study conducted by Goze Ozdemir*et al.*(2023) on wheat, despite using the same *F. culmorum* isolates and the same *P. thornei* isolate, no antagonistic relationship was identified. However, a negative effect on *P. thornei* was observed in barley varieties. The difference in cereal type and variety appears to be a significant factor in the nematode-fungus interaction. These findings underscore the importance of further investigating the chemical composition of culture filtrates and their role in suppressing nematode activity. Identifying specific compounds responsible for antagonistic effects could contribute to the development of novel biocontrol strategies. In summary, while disease severity was consistently elevated in co-inoculated treatments, nematode reproduction was frequently suppressed, particularly by low-pathogenic *F. culmorum* isolates. This unexpected inverse relationship challenges traditional assumptions and highlights the complex dynamics of pathogen-pathogen interactions in plant systems.

Conclusion: This study demonstrated that the presence of *P. thornei* increases disease severity caused by *F. culmorum* culture filtrates in barley, with the degree of interaction varying by variety and fungal isolate. Culture filtrates of *F. culmorum* isolates directly or indirectly interacted with root lesion nematodes in an antagonistic and mutualistic interaction, reducing the nematode reproduction rate. Most fungal culture filtrates, particularly from less aggressive isolates, had suppressive effects on nematode reproduction, suggesting complex interactions that are not solely dependent on pathogenicity. Further research should focus on characterizing the secondary metabolites of *Fusarium* involved and assessing their potential in sustainable plant protection.

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