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Original article (Orijinal araştırma)

Control of *Fusarium oxysporum* f. sp. *radicis lycopersici* Jarvis & Shoemaker (Ascomycota: Hypocreales) and *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nemata: Meloidogynidae) with *Aspergillus niger* Tiegh. (Ascomycota: Eurotiales) culture filtrate on tomato[1]

Domateste *Fusarium oxysporum* f. sp *radicis lycopersici* (Jarvis & Shoemaker) (Ascomycota: Hypocreales) ve *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nemata: Meloidogynidae)'nın *Aspergillus niger* Tiegh. (Ascomycota: Eurotiales) kültür filtratı ile kontrolü

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Abstract

The effects of *Aspergillus niger* Tiegh. (Ascomycota: Eurotiales) culture filtrate on *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (N) and *Fusarium oxysporum* f. sp. *radicis lycoper*sici Jarvis & Shoemaker (Ascomycota: Hypocreales) (FORL) were investigated under controlled conditions on tomato between April and August 2022 in the Isparta province. The study consisted of 8 treatments; 1: N, 2: FORL, 3: N+*A. niger*, 4: FORL+*A. niger*, 5: N+FORL, 6: N+FORL+*A. niger*, 7: N+FORL+nematicide, 8: N+FORL+fungicide. In inoculation, 1000 *M. incognita* second juvenile larvae/1ml and $3X10^6$ spore/ml FORL were used for each seedling according to treatment. Two days after inoculation, 10 ml of undiluted *A. niger* culture filtrate was applied to each potting soil. After 60 days, 0-9 gall and egg mass index, and 0-4 disease severity scale were evaluated. While the suppressive effect of *A. niger* culture filtrate on the gall and egg mass of *M. incognita* was found over 55%, disease severity was found to be over 25%. The highest suppressive effect on gall and egg mass was determined in N+FORL+nematicide, followed by N+FORL+*A. niger*. The disease severity of N+FORL+*A. niger*, N+FORL+nematicide, and N+FORL+fungicide has been determined to be lower than N+FORL and FORL.

Keywords: *Aspergillus niger*, fermentation fluid, fungicidal effect, Fusarium wilt, nematicidal effect

Öz

Aspergillus niger Tiegh. (Ascomycota: Eurotiales) kültür filtratının *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (N) ve *Fusarium oxysporum* f. sp. *radicis lycopersici* Jarvis & Shoemaker (Ascomycota: Hypocreales) (FORL) üzerindeki etkileri Isparta ilinde Nisan-Ağustos 2022 tarihleri arasında domateste kontrollü koşullarda araştırılmıştır. Çalışma 8 uygulamadan oluşmaktadır; 1: N, 2: FORL, 3: N+*A. niger*, 4: FORL+*A. niger*, 5: N+FORL, 6: N+FORL+*A. niger*, 7: N+FORL+nematisit, 8: N+FORL+fungusit. İnokulasyonda her fide için uygulamaya göre 1000 *M. incognita* ikinci dönem larva/1ml ve $3X10^6$ spor/ml FORL kullanılmıştır. İnokulasyondan iki gün sonra, her saksı toprağına 10 ml seyreltilmemiş *A. niger* kültür filtratı uygulanmıştır. Altmış gün sonra 0-9 gal ve yumurta paketi indeksi ve 0-4 hastalık şiddeti skalası değerlendirilmiştir. *Aspergillus niger* kültür filtratı uygulamasının *M. incognita*'nın gal ve yumurta paketi üzerindeki baskılayıcı etkisi %55'in üzerinde bulunurken, hastalık şiddeti üzerindeki baskılayıcı etkisi %25'in üzerinde bulunmuştur. Gal ve yumurta paketi üzerindeki baskılayıcı etki en yüksek N+FORL+nematisit'de belirlenmiştir, ardından N+FORL+*A. niger* uygulamasının geldiği belirlenmiştir. N+FORL+*A. niger*, N+FORL+nemasit ve N+FORL+fungisit uygulamalarının hastalık şiddeti N+FORL ve FORL uygulamalarına göre daha düşük saptanmıştır.

Anahtar sözcükler: *Aspergillus niger*, fermente sıvı, fungisidal etki, Fusarium solgunluğu, nematisidal etki

[1] Part of this study presented as a poster presentation at 8th International Entomopathogens and Microbial Control Congress (October 06th-08th, 2022-Antalya, Türkiye).

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Introduction

Tomato, *Solanum lycopersicum* L. is an important Solanaceae species and is widely grown in tropical and temperate regions of the world. Many diseases and pests, including plant-parasitic nematodes, damage tomato plants and cause yield losses (Ji et al., 2019). Root-knot nematodes feed on roots and vascular tissues, disrupting water and nutrient flow, and cause symptoms such as stunning, slow growth, yellowing of leaves, wilting and early plant death in infected plants (Asaturova et al., 2022). In addition, root-knot nematodes suppress the host plant's defense mechanism, making the plant more susceptible to attacks by other plant pathogens (Goverse & Smant, 2014). In presence of nematodes, pathogens appear earlier in plants, disease effect increases and the plant dies completely (Lobna et al., 2016, 2017; Göze Özdemir et al., 2022a). *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 is the most important root-knot nematode species due to its aggressive, wide host spectrum which may infect almost all plants, causing significant economic damage in all subtropical-tropical regions and can be seen in high prevalence in the world and including Türkiye (Sikora & Fernández, 2005; Uysal et al., 2017; Gürkan et al., 2019; Aslan & Elekçioğlu, 2022; Evlice et al., 2022; Tapia-Vázquez et al., 2022). It is stated that the damage by the root-knot nematodes and symptoms of large galls in the roots are more severe in tropical areas than in temperate areas. It is reported that crop loss in different tomato varieties is between 25 and 100% in the world (Kaşkavalcı, 2007; Seid et al., 2015; Aydınlı & Mennan, 2019). It is seen that nematicides and resistant varieties are generally used to control root-knot nematodes in tomatoes (Hajihassani et al., 2022). Although nematicides can effectively suppress nematodes, their use is limited due to their short-term effects. In addition, high costs, resistance development in nematodes, health and environmental hazards, residues, adverse effects on soil fauna and beneficial microflora, and phytotoxic effects on plants limit the use of nematicides (Haydock et al., 2013; da Silva et al., 2019). With the widespread use of resistant varieties, *Mi* virulent root-knot nematode populations have been reported in many countries (Devran & Söğüt, 2010; Aydınlı & Mennan, 2019; Hajihassani et al., 2022).

Fusarium oxysporum f. sp. *radicis lycopersici* Jarvis & Shoemaker (Ascomycota: Hypocreales) (FORL), which causes tomato root rot, is an important pathogen species that causes more than 60% yield loss in an open field and greenhouse tomato production (Can et al., 2004; Özbay et al., 2004; Hibar et al., 2007; Arıcı et al., 2013; Manzo et al., 2016). While it causes stunting and yellowing in tomato seedlings, root rot, wilting and death occur in plants in later periods. Rot in the root area and necrosis in the stem vascular bundles are around 15-30 cm from the soil surface at most (Singh et al., 2022). FORL has been reported from the Mediterranean region, Europe (the UK, the Netherlands, Belgium and France), where the tomato is intensively grown, USA, Japan (Katan & Katan, 1999; Baysal et al., 2008). First detected in Türkiye by Can et al. (2004), this pathogen causes significant yield losses in tomato growing regions (Baysal et al., 2008; Çolak & Biçici, 2013). The resistant varieties as well as cultivation methods and chemical control programs are used in *Fusarium* diseases (Aydın, 2019; Bilici et al., 2021). However, cultural methods are insufficient due to the saprophytic viability and limited development of resistant varieties (Çolak & Biçici, 2011; Jiménez-Díaz et al., 2015). In some studies, root-knot nematodes were found to break the plant's resistance even in resistant cultivars developed against Fusarium wilt (Lobna et al., 2016; Colak-Ates et al., 2018; Göze Özdemir et al., 2022a). Some chemicals such as metam sodium, and dichloro-propene used as soil fumigants are effective and recommended against soil-borne pathogens to alleviate disease severity before planting in infested areas. However, the uninformed chemical application against soil-borne pathogens causes environmental pollution and toxic effects on human health, as well as the possibility for pathogens to develop resistance to chemicals (Baysal et al., 2008). Soil fumigation with methyl bromide has been used successfully and extensively to control FORL in tomatoes for several years. However, methyl bromide has been phased out in developed countries (Myresiotis et al., 2012).

Management of root-knot nematode and FORL disease complex in tomatoes has proved to be difficult. Therefore, attention has been focused on its biological control through beneficial microorganisms

that can act on both factors. Numerous reports of several fungal and bacterial antagonists suppressing their reproduction and growth have been reported in separate studies on root-knot nematodes and FORL (Omar et al., 2006; Baysal et al., 2008; Myresiotis et al., 2011; Arici, 2015; Moosavi & Zare, 2020; Göze Özdemir et al., 2022b, c). Important species belonging to the genus Aspergillus are included in toxin-producing fungi (Sandoval et al., 2020). *Aspergillus niger* Tiegh. (Ascomycota: Eurotiales) produced extracellular enzymes like citric acids, amylases, lipases, cellulases, xylanases and proteases. It is also used for waste management and transformations (Patil et al., 2017). Also, *A. niger* causes black mould in onions and certain fruits like grapes, peanuts, vegetables etc. (Sharma, 2012). Therewithal, researchers report that different *Aspergillus* species have nematicidal effects on root-knot nematodes (Bhat & Wani, 2012; Devi & Bora, 2018; He et al., 2020; Xiang et al., 2020; Naz et al., 2021). *Aspergillus niger* LOCK 62 produces an antifungal chitinase enzymes (Brzezinska & Jankiewicz, 2012). Application of dextrose base formulation of *A. niger* significantly reduced Fusarium wilt of brinjal (Patil et al., 2017). The extracts of *Aspergillus fumigatus* Fresenius, 1863 and *Rhizopus oryzae* Went & H. C. Prinsen Geerligs, 1895 exhibited promising antifungal activity against *F. oxysporum in vitro* (Attia et al., 2022). Alwathnani & Perveen (2012) reported that the effectiveness of the *A. niger* was 35.6% to *F. oxysporum* f. sp. *lycopersici* (FOL).

Göze Özdemir et al. (2022b) investigated the inhibitory activity of different concentrations of culture filtrates of some fungi against *M. incognita in vitro* and found a high inhibitory activity of *A. niger*. At 100% concentration of the culture filtrate of *A. niger*, it suppressed the hatching from the egg masses of *M. incognita* by 81%, while mortality rate on second juvenile larvae (J2) was found to be 85. 3%. Subsequently, the same concentrations of *A. niger* in tomatoes and peppers were investigated under controlled conditions. The percent control effect on gall, egg mass number, and soil J2 density of *M. incognita* at 100% and 75% concentrations of *A. niger* culture filtrate was determined to be over 70% in tomatoes and peppers (Göze Özdemir et al. 2022c).

There are limited studies using *A. niger* against root-knot nematodes and FORL disease. The suppression of at least one or both of these interacting organisms is of great importance in terms of preventing the formation of the disease complex. Therefore, it is important to study the effect of *A. niger* in controlling *M. incognita* and FORL disease complex. This study aims to investigate the culture filtrate of *A. niger* local isolate which is isolated from Türkiye for antinematicidal and antifungal effects in tomatoes under controlled conditions.

Materials and Method

Material

The FORL isolate used in this study was isolated from a tomato plant in Antalya / Serik district in Türkiye, and its diagnosis was made according to Gerlach & Nirenberg (1982) and Davis & Raid (2002) (Göze Özdemir et al., 2022a). *Meloidogyne incognita* isolate DR17 was used (Uysal et al., 2017), whose mass production continues under climate room conditions (24±1°C, 60%±5% humidity). The local *A. niger* culture used in this study was obtained from Isparta University of Applied Sciences (ISUBU), Faculty of Agriculture, Biotechnology and Tissue Culture Laboratory (Arıcı & Tuncel, 2020). Isolate of *Aspergillus niger* (AnIB18) was isolated from vermicompost using red California worms, *Eisenia fetida* (Savigny, 1826) (Annelida: Lumbricidae) in the Isparta province, Türkiye. The study was carried out on a tomato cultivar Gulizar F1, which is known to be susceptible to root knot nematode and FORL (Göze Özdemir et al., 2022a). Two positive controls were used in the study, one chemical nematicide (Velum®, Fluopyram, Bayer Crop Production Inc., Türkiye) and one fungicide (Cebir®, Fludioxonil + Metalaxyl, Hektaş Crop Protection Inc., Türkiye). The maximum field recommendation doses of Velum and Cebir were used 0.16 ml/L and, 0.25 ml/L, respectively. Only plants with simultaneous application of *M. incognita* and FORL were evaluated as the negative control.

Methods

Preparation of culture filtrate of *Aspergillus niger*

Aspergillus niger isolate (AnIB18) was cultured on Potato Dextrose Agar (PDA) medium in 6 cm diameter petri dishes at 27°C for 7 days. Three mycelial discs (5 mm in diameter) of this isolate were transferred into 50 mL of Potato Dextrose Broth in 250 mL Erlenmeyer flask and incubated for 15 days at 27±1°C. In this way, 5 erlenmeyer flasks were prepared to be used in the study. At the end of this period, the fungal cultures were filtered twice through Whatman filter paper (Naz et al., 2021). The culture filtrate was used at 100% concentration without dilution in the study (Göze et al., 2022b, c) and stored at 4°C.

Preparation of *Meloidogyne incognita* inoculum

Since root-knot nematodes are obligate parasites, their mass production on live plants was continued and renewed every 2-3 months in the Tueza F1 tomato variety. Mass production of *M. incognita* was carried out on the Tueza F1 tomato variety with 20 replicates under climatic room conditions (24±1°C, 60±5% humidity-RH). After mass-produced tomato roots of the Tueza F1 tomato variety were washed in tap water, egg masses were removed from the roots under a stereomicroscope and incubated in water at 25±2ºC for three days in a petri dish containing a sterile sieve of 3 cm diameter. After three days, the J2s hatched from the eggs were counted under a light microscope and placed in 1 ml tubes, adjusted to the number to be used in the experiment. Approximately 1000 J2 of *M. incognita* were used as the nematode inoculum (Lobna et al., 2017).

Preparation of *Fusarium oxyspo*rum f. sp. *radicis lycopersici* Inoculum

FORL isolate was incubated at 25°C for 7 days in sterile petri dishes (9 cm) containing PDA. Then, 5 fungal disc pieces (1 cm^2) were cultured in autoclaved 250 ml flasks containing 50 ml of PDB (potato dextrose broth agar) and incubated at 25°C in the dark for 7 days. Handshaking was performed daily during the incubation period. After seven days, the culture filtrate was first filtered through two layers of filter paper (Whatman No.1) and then refiltered through a 0.45 µm pore size filter to remove fungal spores and mycelium. The filtrate was kept at +4°C until the experiment was established (Lobna et al., 2016).

Effect of *Aspergillus niger* culture filtrate on *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *radicis lycopersici* disease severity on tomato

In this study, the effects of undiluted culture filtrate of local *A. niger* isolate and positive controls of nematicide and fungicide on nematode and fungus in the simultaneous inoculation of *M. incognita* (N) and FORL in the Gülizar F1 tomato cultivar were investigated between April and August 2022 in Isparta province, Türkiye. Only plants with simultaneous application of *M. incognita* and FORL were evaluated as a negative control. The study was set up in a climate room under controlled conditions (24±1°C, 60%±5% humidity-RH) in plastic pots and in a randomized plot design for each treatment with 5 replications. A single tomato plant was used in each replication, and a total of five tomato plants were used in five replications. The study consists of 8 treatments; 1: N, 2: FORL, 3: N+*A. niger*, 4: FORL+*A. niger*, 5: N+FORL, 6: N+FORL+*A. niger*, 7: N+FORL+nematicide, 8: N+FORL+fungicide. Three-week-old tomato seedlings were transplanted into plastic pots with a diameter of 14 cm containing approximately 1500 g of sterile soil (68% sand, 21% silt and 11% clay). In the initial inoculum density per pot, 1000 *M. incognita* J2/1ml and $3X10^6$/10 ml FORL were used, and simultaneous inoculation was performed. The nematode inoculum was evenly dispersed with a pipette into three 2-3 cm deep holes drilled around the seedling stem and deep enough to contact the roots in the soil. FORL inoculum was poured into these holes and opened on the soil surface of the pots with the help of a measuring tape (Lobna et al., 2016, 2017). Two days after nematode and FORL inoculation, 10 ml of undiluted *A. niger* culture filtrate was applied to the holes drilled around the seedling in each potting of soil. The maximum field recommendation doses of Velum® and Cebir® were used at 0.16 ml/L and 0.25 ml/L, respectively.

The study was terminated 60 days after the culture filtrate applications of *A. niger*. Tomato plants related to each application were carefully removed from the soil and their soils were washed with tap water. Evaluation was 1-9 root gall scale for nematodes (1= no gall, 2= 5% root gall, 3= 6-10% root gall, 4= 11-18% root gall, 5= 19-25% root gall, 6= 26-50% root gall, 7= 51-65% root gall, 8= 66-75% root gall, 9= 76-100% root gall) and egg mass production rate scale (1= no egg mass, 2 = 1 or 2 egg masses, 3= 3-6 egg masses, 4= 7-10 egg masses, 5= 11-20 egg masses, 6= 21-30 egg masses, 7= 31-60 egg masses, 8= 61-100 egg masses, 9= more than 100 egg masses) (Mullin et al., 1991). The severity of disease caused by FORL was evaluated according to the 0-4 scale (0: No damage to the seedling, 1: Discoloration and small lesions at the junction of the seedling with the soil surface, 2: Larger lesions turned stem, 3: Large lesions surrounding the stem, resulting in a concave appearance, 4: Dead plant due to fungal damage) (Chandler & Santelman, 1968; Erberk, 2020). The percentages of suppressing gall, egg mass and disease severity were calculated with the formula % = (nematode or FORL alone – treatment/Nematode or FORL alone) X 100 (Xiang et al., 2020).

Statistical analyses

The SPSS Version 20 software (IBM Corporation, Armonk, New York, USA) was used for the statistical analysis. The results were presented as mean±standard error. All the data were checked for normality of distribution by using the Shapiro-Wilk tests. In the data conforming to normal distribution, one-way ANOVA and Tukey multiple comparison tests were performed ($p \leq 0.05$).

Result and Discussion

The highest gall and egg mass index were found in N and N+FORL treatments. These two treatments were followed by the N+FORL+fungicide treatments with 6.0 gall index and 6.2 egg mass indexes. The lowest gall (1.4) and egg mass (1.4) index was determined in the N+FORL+nematicide treatment. The gall and egg mass index of the N+*A. niger* treatment was lower than the N treatment. It was determined that the gall and egg mass index of the N+FORL+*A. niger* treatment was lower than the N+FORL+fungicide treatment, but higher than the N+FORL+nematicide treatment and the difference between these three treatments was found to be statistically significant ($p \leq 0.05$) (Table 1).

The suppressive effect of *A. niger* culture filtrate treatment on gall and egg mass of *M. incognita* was found to be 59.9% and 57.7%, respectively. In simultaneous treatments, the highest suppressive effect was determined in the N+FORL+nematicide treatment, followed by the treatment of N+FORL+*A. niger*. While the suppressive effect of the N+FORL+nematicide treatment on gall and egg mass was determined over 75%, of that N+FORL+*A. niger* treatment was found above 60%. On the other hand, the N+FORL+fungicide treatment showed a suppressive effect of 33.3% and 31.0% on gall and egg mass, respectively (Table 1).

While the disease severity was found to be highest in the N+FORL treatment with 3.6, it was found 3.0 in the FORL treatment. However, the difference between them is statistically insignificant ($p \geq 0.05$). The disease severity of the FORL+*A. niger* treatment (2.4) was determined to be lower than the FORL and N+FORL treatments. The FORL+*A. niger*, N+FORL+*A. niger*, N+FORL+nematicide and N+FORL+fungicide treatments were found to have similar disease severity and no statistical difference were observed between them ($p \geq 0.05$) (Table 1).

The suppressive effect of *A. niger* culture filtrate treatment on the disease severity of FORL was found to be 26.6%. The suppressive effect of the fungicide, nematicide and *A. niger* treatments on disease severity in simultaneous inoculations was found to be similar. Although the suppressive effect on disease severity in simultaneous inoculations was the highest at 33.3% in the N+FORL+*A. niger* treatment, the difference between this and the FORL+*A. niger*, N+FORL+nematicide (26.6%) and N+FORL+fungicide (26.5%) treatments were not statistically significant ($p \geq 0.05$) (Table 1).

Generally, the nematicidal and fungicidal effects of *A. niger* are studied by different researchers separately (Alwathnani & Perveen, 2012; Bhat & Wani, 2012; Devi & Bora, 2018; Arici &Tuncel, 2020; Attia et al., 2022). In the current study, it was revealed that *A. niger* has both nematicidal and fungicidal properties. In

simultaneous inoculations, the suppressive effect of *A. niger* on gall and egg mass was found to be lower than nematicide treatment, while *A. niger* culture filtrate partially suppressed FORL disease in the current study (Disease severity scale 2.4). Also, there are several studies on the biological efficacy of *A. niger* as Plant Growth-Promoting Fungi against Fusarium wilt in tomato. Similar results were obtained in earlier studies (Kerkeni et al., 2007; Nikhat et al., 2019; Abdel-Motaal et al., 2020; Jamil et al., 2021; Abd Alhakim et al., 2022).

Table 1. Effect of *Aspergillus niger* culture filtrate on *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *radicis lycopersici* disease severity on tomato

	Treatments	Gall index (1-9)[1]	Percent suppressive effect on gall (%)	Egg mass index (1-9)[2]	Percent suppressive effect on egg mass (%)	Disease severity scale[3]	Percent suppressive effect on disease (%)
1	N	9.0±0.0 a [4]	-	9.0±0.0 a	-	-	-
2	FORL	-	-	-	-	3.0±0.4 ab	-
3	N+*A. niger*	3.6±0.2 c	59.9±2.7 b	3.8±0.2 c	57.7±2.2 b	-	-
4	FORL+*A. niger*	-		-		2.4±0.2 b	26.6±6.6 a
5	N+FORL+*A. niger*	3.2±0.2 c	64.3±2.2 b	3.2±0.3 c	64.3±4.1 ab	2.0±0.3 b	33.3±10.5 a
6	N+FORL (negative control)	8.6±0.2 a	4.4±2.7 d	8.8±0.2 a	4.4±2.7 d	3.6±0.2 a	0.0±0.0 b
7	N+FORL+ Nematicide (Positive control)	1.4±0.2 d	84.3±2.7 a	1.4±0.2 d	77.7±6.0 a	2.2±0.2 b	26.6±6.6 a
8	N+FORL+Fungicide (Positive control)	6.0±0.4 b	33.3±4.9 c	6.2±0.3 b	31.0±4.1 c	2.2±0.2 b	26.5±6.6 a

[4] The lowercase letters in the same column indicate significant differences between treatments ($p \leq 0.05$).

Aspergillus niger shows that the disease severity can be reduced by suppressing the growth or reproduction of either nematode or fungus when inoculated at the same time. It was determined that disease severity was highest in the negative control. However, it was observed that disease severity decreased when nematode was controlled. Yeon et al. (2022) reported that *A. niger* F22 formulation (Nemafree, 20% SC) and oxalid acid can reduce nematode populations and promote tomato plant growth by increasing the activities of defense enzymes in tomato plants. Jin et al. (2019) reported that culture filtrate application of *A. niger* NBC001 isolate can control *Heterodera glycines* Ichinohe, 1952, cyst nematode in soybean seedlings, in both pot and field conditions. It has been stated that *A. niger* and *A. candidus* are potential fungal agents that can be used against plant parasitic nematodes (Khan & Anwer, 2008; Yin et al., 2015; Jang et al., 2016; Shemshura et al., 2016; Maishera et al., 2019).

Although the application of fungicide to N+FORL did not show a large suppressive effect on gall and egg mass, it reduced the disease severity. Additionally, the nematicidal and fungicidal effect of *A. niger* is higher than the fungicide against N+FORL. However, the lack of suppression of the nematode may result in the formation of new offspring and the emergence of new infections from these offspring to the plant. Since the exposure of the plant to nematode attack will increase the susceptibility of the plants to secondary microorganisms, the disease may recur in the plant or the severity of the disease may increase (Back et al., 2002; Göze Özdemir et al., 2022a). It has been reported in different studies that simultaneous infection with root-knot nematode and FORL causes more severe damage to the host plant than infection with each nematode and fungus alone (McGawely, 2001; Hajji et al., 2016). All these results show that nematode control is a priority in preventing disease formation. There are problems experienced in chemical control on both organisms and a lack of resistance is observed in some commercial tomato varieties. It is important to prioritize alternative control methods in the prevention of disease complexes. Antifungal and antinematicidal effects of *A. niger* against root-knot nematode and FORL were determined, and it provides disease control as a good biocontrol agent.

The fact that this isolate is native to Türkiye increases the importance of the study. Metabolite production may differ depending on the type of fungal isolates and culture medium or its composition (Wang et al., 2004; Mohanty et al., 2008; Kim et al., 2013). This effect may be caused by toxic compounds such as secondary

enzymes or toxins secreted by *A. niger* (Maria & Urszula, 2012; Patil et al., 2017; He et al., 2020; Xiang et al., 2020; Naz et al., 2021). It is thought that toxic enzymes and toxins are more in culture filtrates and nematicidal effect increases as a result of synergistic or antagonistic interactions with each other (Kim et al., 2013).

In previous studies, we applied *A. niger* separately only to fungi and nematodes. This is the first study conducted in Türkiye to evaluate the use of *A. niger* culture filtrate in the control of FORL on tomatoes. According to the results, undiluted *A. niger* culture filtrate were determined to be a highly promising potential source of microbial nematicide or fungicide on tomato. Also, its compounds will be a resource for the development of new chemicals to manage root-knot nematodes and FORL. For this reason, chemical compounds of *A. niger* isolate (AnIB18) should be identified. It is necessary to investigate the environmental conditions (temperature, RH, culture medium, etc.) that will affect the effectiveness of *A. niger*. Since this study was carried out in pots containing sterilized soil, the effectiveness of *A. niger* in field conditions needs to be investigated because when applied to the field, its interaction and competitiveness with other soil microorganisms are unknown. As a conclusion, it can be said that application of *A. niger* to the soil will be an effective alternative control method to reduce pesticide use and increase yield in root-knot nematode and FORL control.

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Reference

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