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Antifungal Activity of Extracts From the *Ferulago Pauciradiata in Vitro* Against *Botrytis Cinerea* Pers

Sinem KARAKUŞ[1*], Tuba Nur SUYURDU[2], Ekrem KÖKSAL[2], Cemalettin ALP[3]

Highlights:

- *Ferulago pauciradiata*'nın antifungal özellikleri ilk kez belirlendi
- *Ferulago pauciradiata*'nın metanol, etanol ve heksan özleri, *Botrytis cinerea*'nın miselyum büyümesini, tohum tüpünün uzamasını ve spor çimlenmesini önemli ölçüde engellemiştir

ABSTRACT:

This is the first study to reveal the antifungal properties of *Ferulago pauciradiata* plant. In this context investigated the effects of methanol (FPM), ethanol (FPE), hexane (FPH) and water (FPW) extracts of the *F. pauciradiata* plant on the prevention of losses caused by gray mold (*Botrytis cinerea* Pers) *in vitro*. The effects of FPM, FPE, and FPH 10, 25, 50, 100, 300, 500, 1000, and 2000 µL doses were determined by mycelium growth, germ tube elongation, and spore germination *in vitro*. The water extract didn't show antifungal activity against *B. cinerea*. Compared to the control, both FPM and FPH caused 100% inhibition at the dose of 2000 µL by suppressing mycelial growth due to dose increases, while FPE had a 97.3% effect on the same parameter at the dose of 2000 µL. While there was no elongation at the 2000 µL dose of FPM and FPH, there was an elongation of 8.4 µm at the same dose of FPM. In spore germination, 0% germination was observed in FPM and FPH 2000 µL dose, while 17.5% germination was observed in FPE. These results show that *F. pauciradiata* extracts, which are of biological origin and are not environmentally toxic, are a good alternative for use in the control of *B. cinerea.*

Keywords:

- Biocontrol
- Antifungal effect
- Gray mold
- Plant extract

[1]Sinem KARAKUŞ (**Orcid ID:** 0000-0002-6698-153X), Hakkâri University, Çölemerik Vocational School, Hakkâri, Türkiye

[2]Tuba Nur SUYURDU (**Orcid ID:** 0009-0003-4772-7581), Ekrem KÖKSAL (**Orcid ID:** 0000-0003-0853-566X), Erzincan Binali Yıldırım University, Faculty of Science and Arts, Department of Chemistry, Erzincan, Türkiye

[3]Cemalettin ALP (**Orcid ID:** 0000-0001-6213-7297), Erzincan Binali Yıldırım University, Çayırlı Vocational School, Department of Medical Services and Techniques, Erzincan, Türkiye

Corresponding Author: Sinem KARAKUŞ, e-mail: sinemkarakus@hakkari.edu.tr

INTRODUCTION

Gray mold (*Botrytis cinerea* Pers) is one of the fungal pathogens that cause significant losses in many plants (Šernaitė et al., 2020). This pathogen, which infects many plant species before and after harvest, causes severe economic losses in the agricultural sector (Elad et al. 2016; Paňitrur-De La Fuente et al. 2018). Various methods have been developed to combat this pathogen. At the beginning of these methods are fungicides used in chemical control. (Singh and Sharma, 2007). The applied fungicides have teratogenic, carcinogenic, and highly acute toxigenic effects. These chemicals are used to cause environmental pollution as they have long corruption times. In addition, many phytopathogenic fungi are gaining resistance to synthetic insecticides (Lingk, 1991; Unnikrishnan and Nath, 2002; Gisi and Sierotzki, 2008). Various synthetic chemicals such as sterol, benzimidazoles, aromatic hydrocarbons, and biosynthesis inhibitors have long been used as antifungal agents to inhibit the growth of phytopathogenic fungi (Pavela, 2007). Secondary metabolites produced naturally in plants that can replace these synthetic insecticides have been identified. In recent years, researchers have been examining wild or cultivated plants that breed varieties of compounds and investigating ways to obtain and apply these natural secondary compounds in plants. These metabolites are known to be healthier for both consumers and the environment as they are easily biodegraded by natural processes (Vyvyan 2002; Weston and Duke 2003).

Plants in the *Apiaceae* family have been used medicinally for thousands of years as a natural product (Evergetis and Haroutounian, 2015). A significant part of this family is rich in phenolic compounds, essential oils, and coumarins (Cavanagh, 2007; Ntalli et al., 2010; Dorman and Deans, 2000; Lang and Buchbauer, 2012; Siddiqui and Zaki, 2017). *Ferulago*, which belongs to the *Apiaceae* family, is known as "Çağşir" and "Çakşir" in the local language, and it is also known as coriander, lamb's head, lamb gnaw (Kürkçükoğlu et al., 2010). *Ferulago* species are used in the treatment of spleen, headache, and ulcer diseases (Baser et al., 2002; Reza et al., 2007). In addition, *Ferulago* species are known for their antioxidant, antimicrobial (Maggi et al., 2009), cytotoxic, and immunomodulatory (Maxia et al., 2009) effects (Karabulut Uzuncakmak et al., 2023).

This species *Ferulago pauciradiata* Boiss & Heldr is a perennial rhizome endemic plant (Cumhur, 2019). Despite intensive studies on the biological activity of *F. pauciradiata*, no information was found about the antifungal activity of *F. pauciradiata* extract. This study aimed to evaluate the *in vitro* antifungal activities of extracts prepared from the aerial part of *F. pauciradiata* using different solvents.

MATERIALS AND METHODS

Collection of Plant

Plant samples were collected on the Erzincan-Kemah road in June 2023. The species was identified by Prof. Dr Ali Kandemir, and it was deposited in Erzincan Binali Yıldırım University Herbarium with the collector number TKS1.

Preparation of plant extract

The plant samples, which were dried at room temperature without sunlight, were turned into powder with a herb grinder. 10 g of plant material was extracted with 50 mL each of methanol, ethyl acetate, water, and hexane in an ultrasonic bath for 30 minutes x2 at room conditions. Solvents were removed by evaporator and stock solution was prepared from the extracts with a final concentration of 50 mg/mL.

Determination of antifungal effect on mycelial growth inhibitions and minimum inhibitory concentration

B. cinerea was isolated from infected grapes (*Vitis vinifera* cv. Karaerik). The strain isolates numbered MF7413141, MH997908, MK562062, and MH782039 obtained from the Genbank (GenBank; http://ncbi.nlm.nih.gov) database were used for molecular identification in this study. The fungus was grown on potato dextrose agar (PDA) medium at 25 ˚C in the dark. The water (FPW), methanol (FPM), ethanol (FPE), and hexane (FPH) extracts of *F. pauciradiata* were mixed with sterile molten PDA to obtaining the final concentrations (10, 25, 50, 100, 300, 500, 1000, and 2000 µL). 20 mL of each medium was poured into 90 mm Petri plates and then were inoculated with 4 mm plugs from 7-day-old cultures. From the second day of incubation, the petri dishes were checked daily, and the diameters of fungal mycelium were measured and recorded daily. The experiment was performed in triplicate, and percentage mycelial growth inhibitions (MGI) were calculated using the following formula (Yahyazadeh et al., 2008).

MGI (%) = [(dc-dt)/dc] x 100

MGI-inhibition (%), dc-mycelium diameter in the control Petri dish (mm), dt-mycelium diameter in the experimental Petri dish (mm). Minimum inhibitory concentration (MIC) was defined as the minimum concentration that completely inhibits *B. cinerea* (Talibi et al., 2012).

Spore germination and germ tube elongation

The effects of FPW, FPM, FPE, and FPH extracts of *F. pauciradiata* on *B. cinerea* spore germination and germ tube elongation were determined as described by Qin et al. (2010). After ten days of incubation at 25°C, spores from the fungal cultures were collected, and 5 mL of sterilized pure water was added to the culture. The suspension was passed through 3-layer cheesecloth, and the mycelial particles were removed. Pathogen suspension at 1×10^5 conidial/mL was prepared. The resultant suspensions were shaken using a vortex mixer for 30 s before inoculation. 10 µL of spore suspension was spread in the petri plates containing different concentrations (10, 25, 50, 100, 300, 500, 1000, and 2000 µL) of plant extract. The Petri were incubated in the dark at 25 ± 1 ˚C for 24 h. After incubation, spore germination was determined microscopically (40×10) by counting 100 spores, and the length of germ tubes was measured with an ocular micrometer.

RESULTS AND DISCUSSION

B. cinerea is one of the most harmful pathogens worldwide, causing economic losses in fresh and post-harvest fruits and vegetables (Yan et al., 2010). Although chemical fungicides are thought to be the most effective treatment method against the pathogen, their long-term toxicity to the environment and human health causes great harm. Therefore, the search for new environmentally friendly alternatives has increased (Contreras et al., 2022). Recently, researchers have focused on the development of other natural chemicals such as essential oil, plant extracts, and natural preservatives for the safe control and management of gray mold (Zhao et al., 2021). We, therefore, investigated the effect of antifungal activities of FPM, FPE, and FPH extracts of *F. pauciradiata* against *B. cinerea* infections. In the literature, no findings were found on the antifungal properties of *F. pauciradiata* extracts used in the study. However, there are studies showing that different plant extracts have antifungal effects against *B. cinerea* (Dėnė and Valiuškaitė, 2021; Hadadi et al., 2020; Karakuş et al., 2021; Latinović et al., 2019; Šernaitė et al., 2020). According to our results, it was determined that FPM, FPE, and FPH extracts of *F. pauciradiata* plant had a significant antifungal activity on the mycelial growth of *B. cinerea*. FPM, FPE, and FPH extracts of F. pauciradiata plant (10, 25, 50, 100, 300, 500, 1000, and 2000 µL) inhibited the mycelial growth of B. cinerea in a dose-dependent manner

(Table 1). The FPM, FPE, and FPH extracts of *F. pauciradiata* plant MIC values were determined as 10 µL and 25 µL, respectively. FPM and FPH application resulted in a reduction in *B. cinerea* diameter compared with the control group, and mycelium growth, germ tube elongation, and spore germination were completely inhibited at the highest concentrations of FPM and FPH tested (2000 µL) (Table 1-3-4). We indicated that growth inhibition was dependent on plant extract concentrations and that the antifungal activity of plant extract was dose-dependent. In the literature, no findings were found on the antifungal properties of *F. pauciradiata* extracts used in the study. However, studies are showing that some *Ferulago* species, such as *Ferulago longistylis, Ferulago asparagifolia, Ferulago galbanifera, Ferulago angulata subsp. carduchorum, Ferulago thyrsiflora, Ferulago bernardii, Ferulago nodosa, Ferulago sylvatica,* and *Ferulago humilis* have antibacterial and antifungal activity of plant essential oil (Khalighi-Sigaroodi et al., 2005; Taran et al., 2010). Therefore, our study is the first to investigate the antifungal properties of plant extracts of *Ferulago* species.

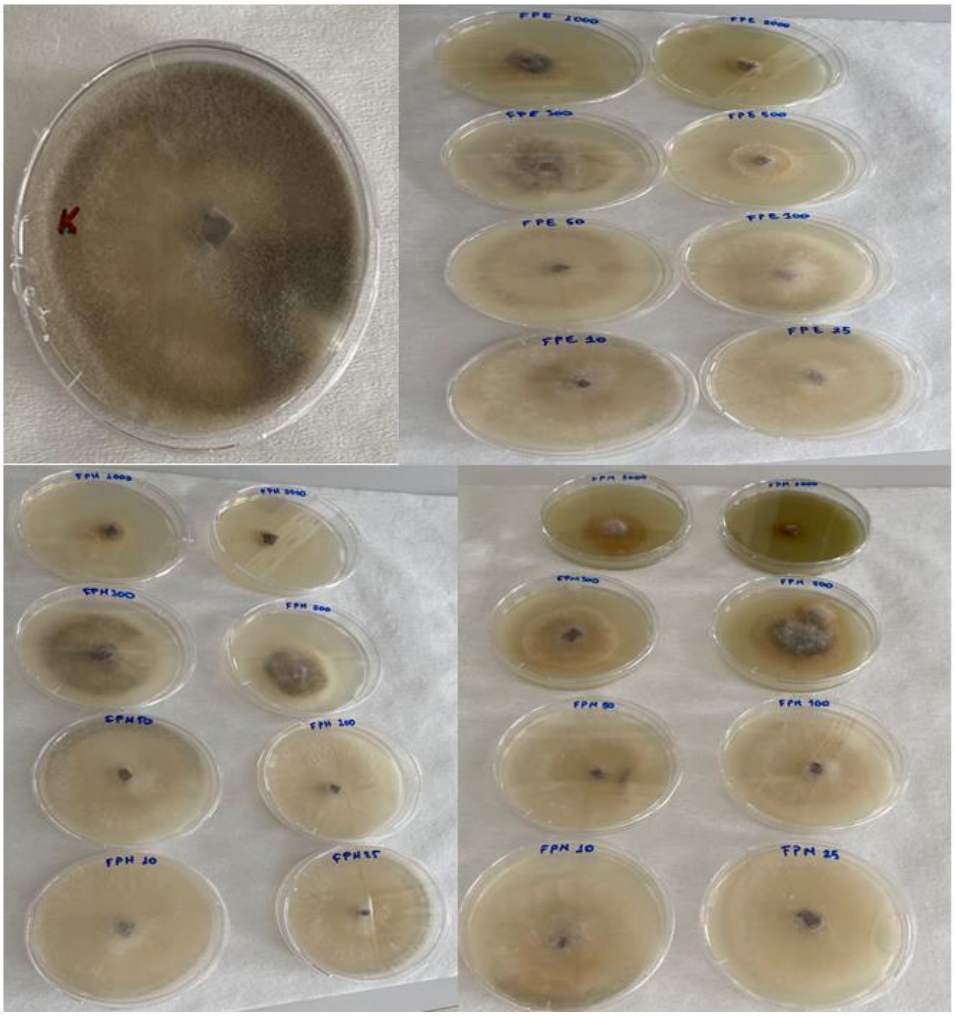

Figure 1. Control and Effects of the FPM, FPE, and FPH Extracts of the *F. Pauciradiata* Plant on the Mycelial Growth of Pathogen

When the effect of FPM, FPE, and FPH extracts of *F. pauciradiata* plant on the mycelial growth of *B. cinerea in vitro* was appraised, FPM, FPE, and FPH extracts, in addition to inhibiting mycelial growth in connection with the rise in concentration, 2000 µL doses of FPM and FPH extracts inhibited mycelial growth by 100% (Table 1). FPE extract inhibited the pathogen growth by 97.3% at 2000 µL concentration (Table 1). At 1000 µL concentration, FPH was 96.7% inhibited, whereas FPM was 90.6%, and FPE was 82.7% inhibition (Table 1). At the lowest concentrations (10 µL) of the extracts,

FPH, FPM, and FPE showed inhibition of 19.8%, 19.7%, and 17.3%, respectively. The results are shown in Figure 1, where, FPM, FPE, and FPH extracts of F. pauciradiata showed antifungal activity against *B. cinerea* at different concentrations. FPM, FPE, and FPH extracts of *F. pauciradiata* (>500 µL) considerably inhibited the mycelial growth of *B. cinerea in vitro*. The findings in this work were similar to those of Šernaitė et al. (2020), who reported that separately mixing plant extracts of the *Syzygium aromaticum* L., *Laurus nobilis* L., *Rosmarinus officinalis* L. inhibited the *B. cinerea* growth 100% at 600–2000 µL concentration. Moreover, it was also similar to the findings of other studies confirming that the plant extracts showed antifungal activity against *B. cinerea* (Hadizadeh et al., 2009; Hammani et al., 2011; Vio-Michaelis et al., 2012). Moghaddam et al. (2018) stated that the essential oils (with 20 µL mL−1 MIC and 30< MBC) of *Ferulago angulata* have antifungal effects against *Fusarium oxysporum* (100.0 ± 0.00) and *Colletotrichum tricbellum* (52.50 ± 1.67%) fungi. The antifungal effect of the extract obtained from the same plant is lower than the essential oil. This is believed to be due to the effect level of the active ingredient stability and the amount that is included in the extract (Tripathi et al., 1985).

Table 1. Inhibitory effects of Extracts of *F. Pauciradiata* on Mycelial Growth İnhibitions (MGI)

Application dose (µL/Petri dish)	FPE MGI (%)	FPH MGI (%)	FPM MGI (%)
10	17.3	19.8	19.7
25	18.9	24.3	21.5
50	23.2	26.1	38.6
100	36.7	38.9	43.7
300	44.5	42.4	63.7
500	72.3	84.7	68.2
1000	82.7	96.7	90.6
2000	97.3	100	100

Besides, the inhibitory effects of the extracts of the *F. pauciradiata* plant on spore germination and germ tube elongation were consistent with those on mycelial growth (Table 2-4). Likewise, FPH and FPM were more successful in the inhibition of spore germination and germ tube elongation of pathogens, compared to those of FPE, FPH and FPM at concentrations of 2000 µL completely inhibited germ tube elongation and spore germination. For example, the control group germinated 100%, while at the concentration of 1000 µL, the spores germinated of FPM, FPH, and FPE were %11.8, %14.6, and %27.8, respectively (Table 2-4). Moreover, at 10 µL of the concentration, FPE, FPH, and FPM indicated spore germination of 91.2%, 90.2%, and 84.4%, respectively (Table 2-4). The germ tube elongation was also determined as 119.8 µm in the control group, while there was no elongation of FPH and FPM at 2000 µL (Table 3-4). At the concentration of 1000 µL, the germ tube elongation of FPE, FPH, and FPM was 12.1, 7.1, and 15.2 µm, respectively. Likewise, the germ tube elongation decreased significantly compared to the control group depending on the concentration ratio. FPM demonstrated the best results compared to the two other applications. According to our results, FPM, FPH, and FPE have high antifungal effects. In another study examining the effectiveness of polar extracts of the plant of *Colobanthus quitensis* Kunth. (Bartl) against *B. cinerea*, they showed that the conidia density of the extract was strongly inhibited (Contreras et al., 2022). Moreover, it has been stated that some plant extracts do not inhibit the growth of microorganisms (Singh et al., 1980). Investigations with different plants show that the effects of extracts on the type and target organism may differ (Karakuş et al., 2021).

Table 2. Inhibitory Effect of FPE Extracts of *F. Pauciradiata* on the Germ Tube Elongation and Spore Germination of Pathogen

Application dose (µL/Petri dish)	Germ tube elongation (µm)	Spore germination (%)
Control	119.8	100
10	101.3	91.2
25	94.6	90.3
50	75.8	84.5
100	58.7	78.9
300	41.3	64.7
500	24.6	48.7
1000	12.1	27.8
2000	8.4	17.5

Table 3. Inhibitory Effect of FPH Extracts of *F. Pauciradiata* on the Germ Tube Elongation and Spore Germination of Pathogen

Application dose (µL/Petri dish)	Germ tube elongation (µm)	Spore germination (%)
Control	119.8	100
10	98.7	90.2
25	90.1	86.4
50	89.6	78.9
100	70.3	69.7
300	61.4	54.6
500	24.6	39.7
1000	7.1	14.6
2000	0	0

Table 4. Inhibitory Effect of FPM Extracts of *F. Pauciradiata* on the Germ Tube Elongation and Spore Germination of Pathogen

Application dose (µL/Petri dish)	Germ tube elongation (µm)	Spore germination (%)
Control	119.8	100
10	98.9	84.4
25	85.4	78.6
50	74.1	62.1
100	64.7	55.7
300	50.1	47.8
500	41.7	22.5
1000	15.2	11.8
2000	0	0

Since the water extract of the *F. pauciradiata* plant did not show antifungal activity, the results are not given in the text. In a similar study, in which the effect of different extracts of *Nepeta meyeri* plant against *B. cinerea* was investigated, it indicated that EOs showed high antifungal activity on the other, and water, methanol, and hexane extracts did not show antifungal effect (Karakuş et al., 2021). Other studies showed that the boiling water extraction of *Urtica dioica* L was effective against *B. cinerea*, however, *Apium graveolens* Mill and *Sinapis arvensis* L did not have any effect on *B. cinerea* (Torun et al., 2018). As a result of using different extracts of the same plant in our study, while water extract was not effective, ethanol, methanol, and hexane extracts were effective. We think that this difference is due to the different concentrations of the antifungal substances contained in the extracts.

CONCLUSION

In recent years, researchers have turned to developing safer antifungals instead of chemicals against plant pathogens. The plant extracts are promising natural ingredients that can be applied in agricultural systems against phytopathogenic microorganisms. The study presented here on the effect

of FPM, FPE, and FPH extracts of the *Ferulago pauciradiata* plant on *B. cinerea* antifungal activity is the first antifungal study with *F. pauciradiata*. This study revealed that FPM, FPE, and FPH extracts inhibited the mycelial growth of *B. cinerea*. The results of our investigation indicated that extracts of the *F. pauciradiata* plant have promising antifungal agent properties. It can be used to control *B. cinerea* caused by *F. pauciradiata* gray mold, and it is environmentally friendly and could be a potential alternative to synthetic pesticides.

Additionally, further studies are needed to investigate the effects of *F. pauciradiata* plant extracts against other major bacteria and fungi to develop new natural antibacterial and antifungal agents to prevent fungal and bacterial diseases in plants.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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