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Bitki patojeni funguslara karşı *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* ve *Alchemilla vulgaris* L. ekstraktlarının antifungal aktivitelerinin değerlendirilmesi**

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ÖZET:
Amaç: Mevcut çalışma, *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* ve *Alchemilla vulgaris* L. ekstraklarının *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea, Macrophamina phaseolina*, *Rhizoctania solani*, *Alternaria alternata*, *Cylindrocarpon destructans* ve *Phytopthora infestans'a* karşı antifungal aktivitelerini değerlendirmek için gerçekleştirildi.
Gereç ve Yöntem: Üç farklı bitki türünden üç ekstrakt formunun antifungal potansiyeli, Potato Dextrose Agar media (PDA) üzerinde Agar difüzyon yöntemi kullanılarak *in vitro* olarak gerçekleştirildi. Üç farklı ekstraktın bir dozu (%1.0, %1.5 ve %2.0) test edildi. Deneysel sonuçlar üç tekrarlı elde edildi.
Bulgular: Çalışmada hazırlanan tüm bitki ekstraktları, bitki patojeni funguslara karşı önemli aktivite göstermiştir. Özellikle *Tanacetum praeteritum* subsp. *Praeteritum*, beş farklı fungus türünde %73,5 - 100 arasında miselyum büyümesini engellerken, *Grindelia robusta* Nutt, dört farklı fungus türünden ikisi üzerinde %100 etki göstermiştir.
Sonuç: Bitki ekstraktları, test edilen tüm funguslara karşı antifungal aktivite sergiledi. Analizler, tüm ekstraktların fungus miselyum büyümesini önemli ölçüde engellediğini göstermektedir. Bu çalışma, çevre dostu ve ekonomik olarak uygulanabilir bitki sağlığı uygulamaları için yeni yöntemlerin geliştirilmesinin yolunu açmaktadır.
Anahtar Kelimeler: *Anti-fungal aktivite, bitki ekstraktı, bitki patojenleri, fungus ilacı*

Evaluation of antifungal activities of *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. extracts against plant pathogenic fungi

ABSTRACT:
Purpose: Present study, we were carried out to evaluate the antifungal activities of extracts of *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. against *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea, Macrophamina phaseolina*, *Rhizoctania solani*, *Alternaria alternata*, *Cylindrocarpon destructans* and *Phytopthora infestans*.
Material and Methods: The screening of the antifungal potential of three extract forms from three different plant species was performed *in vitro* by using Agar-diffusion method on Potato Dextrose Agar media (PDA). One doses (1.0%, 1.5% and 2,0%) of the three different extracts were tested. Experimental results were obtained in three replicates.
Results: All plant extracts prepared in the study exhibited significant activity against plant pathogenic fungi. In particular, *Tanacetum praeteritum* subsp. *praeteritum* prevented the growth of micelles between 73.5 - 100% in five different fungal species, while *Grindelia robusta* Nutt. had a 100% effect on two of the four different fungus species.
Conclusion: The plant extracts exhibited an antifungal activity against all the tested fungi. The analysis shown that the all extracts caused significantly inhibited the mycelium growth of fungi. This study paves the way for the development of new methods for environmentally friendly and economically viable phytosanitary practices.
Keywords: *Anti-fungal activity, plant extract, plant pathogens, fungicide*

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INTRODUCTION

Grindelia robusta Nutt. is one of the species growing naturally especially in North and South America and rarely in Europe (Ferreres et al., 2014; Nowak and Rychlińska, 2012). *Grindelia robusta* Nutt. belongs to the *asteraceae* family and is rich in diterpenes as well as flavonoids, acetylenes, saponins, essential oils, tannins and phenolic acids. *Grindelia robusta* Nutt. extracts have been reported to have anti-inflammatory, anti-microbial, anti-asthmatic, sedative and expectorant properties (Krenn et al., 2009; El-Shamy et al., 2000; Zerbe et al., 2015). In addition, it has been reported that the essential oils obtained from the *Grindelia robusta* Nutt. plant have antioxidant properties (Fraternale et al., 2007). *Tanacetum praeteritum* subsp. *praeteritum* is endemic in Turkey (Gören, 1995) and, annual and perennial plants belonging to the *Tanecetum* species are very rich in essential oils, sesqui-terpene lactones (Bagci, 2009; Brown et al., 1997). The species belonging to the genus *Tanecetum* have a wide range of medical importance, and the extracts and essential oils obtained from these species have been reported to have anti-inflammatory, anti-bacterial, anti-fungal and insecticidal effects (Gören et al., 2001; Bagci et al., 2008).

Alchemilla vulgaris L., a perennial herbaceous plant belonging to the *Rosaceae* family, grows in moist areas, pastures and wet meadows in America, Europe and Asia (Jurić et al., 2020). *Alchemilla vulgaris* L., is widely used in traditional medicine for the treatment of eczema, ulcers, heal inflammations, wounds, digestive problems, gynecological and gastrointestinal diseases (Boroja et al., 2018; Tasić-Kostov et al., 2019). It has also been reported that *Alchemilla* species have antiviral, antioxidant, anti-diarrheal, antiproliferative and antibacterial activities (Neagu et al., 2015).

Plant fungal diseases cause very serious problems such as blight, wilting, black spot, root rot, white mold and gray mold in many fruits and vegetables and significantly reduce annual agricultural yield (Iqbal et al., 2019). Several drugs are widely used for the treatment of diseases caused by these fungal pathogens; however, these chemicals lead to toxic residues in food. Recently, researchers have focused on the use of environmentally-friendly biological methods for the protection of plants from damages (Özbek et al., 2020; Copping and Menn, 2000). In the current study, we determined anti-fungal properties of *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. plant extracts dissolved in different solvent against plant pathogenic fungi species (*Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea, Macrophamina phaseolina*, *Rhizoctania solani*, *Alternaria alternata*, *Cylindrocarpon destructans* and *Phytopthora infestans*).

MATERIAL and METHODS

Plant Material and Solvents

Grindelia robusta Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. were provided from the collection of medicinal plants at Hekim Sinan Botanical Garden of Medicinal and Aromatic Plants (Kütahya). Ethyl acetate, hexane and methanol were purchased from Sigma Aldrich.

Microorganisms

Microorganisms used in this study (*Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea, Macrophamina phaseolina*, *Rhizoctania solani*, *Alternaria alternata*, *Cylindrocarpon destructans* and *Phytopthora infestans*) were obtained from stock cultures in Phytopathology Laboratory at Faculty of Agriculture, Tokat Gaziosmanpasa University.

Preparation of plant extracts

100 g dried *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. plants were mixed with 500 mL methanol, hexane and ethyl acetate solutions separately after grinding, and stirred for 48h with a magnetic stirrer. Then, plant particles in solvents were filtered, solvents were evaporated at approximately 40 °C and extracts were obtained in a viscous form. These extracts were diluted with 10 % acetone and kept at 4 °C as 50% stocks for further use.

Anti-fungal studies

Grindelia robusta Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. extracts prepared with hexane, ethyl acetate and methanol, respectively, were used in *in vitro* anti-fungal tests. *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. extracts dissolved in solvents were added to autoclaved Potato Dextrose Agar (PDA) in different concentrations (1.0%, 1.5% and 2.0%). 5 mm mycelium disks for *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea, Macrophamina phaseolina*, *Rhizoctania solani*, *Alternaria alternata*, *Cylindrocarpon destructans* and *Phytopthora infestans* were inoculated on solidified PDA growth media and placed in a 25 °C incubator. PDA without plant extract was used as a control in the study. After 1 week incubation period, fungi mycelium radius measurements were carried out.

Evaluation of anti-fungal properties

In this study, anti-fungal tests percent inhibition values of three different plant extracts against different pathogenic fungi species were determined by comparing mycelium radial growth measurements of pathogens with that of controls. Inhibition rates were determined using the following equation:

$$\mathrm{MGI}(\%) = \frac{(dc - dt)}{dc} \times 100$$

where, **MGI** = inhibition (%), **dc** = radial growth in control petri dish (mm) and, **dt** = radial growth in petri dish with plant extract (mm) (Deans and Sobada, 1990).

RESULTS and DISCUSSION

The effect of *Grindelia robusta* Nutt. extracts dissolved in hexane on *Phytopthora infestans*, *Macrophamina phaseolina*, *Rhizoctania solani* and *Fusarium oxysporum*. f. sp. *lycopersici* mycelium growth were shown in Figure 1. The anti-fungal effect of *Grindelia robusta* Nutt. extract against *Phytopthora infestans* and *Rhizoctania solani* mycelium growths were the highest (100.0%) with plant extract dissolved in hexane at 2.0% dose. Very good inhibition rates were obtained for *Fusarium oxysporum*. f. sp. *lycopersici* and *Macrophamina phaseolina* at the same dose, with 73.84% and 57.3%, respectively. In addition, the exprimental images of the growth rate of fungi in the *Grindelia robusta* Nutt. extracts on the disc surface compared to the control group is shown in Table 1. The percent inhibition values for *Phytopthora infestans*, *Macrophamina phaseolina*, *Fusarium oxysporum*. f. sp. *lycopersici*, *Rhizoctania solani* and *Botrytis cinerea* mycelium mycelium growth were given in Figure 2. The anti-fungal effect of *Tanacetum praeteritum* subsp. *praeteritum* extract against *Phytopthora infestans* mycelium growth was the highest (100.0%) with plant extract dissolved in ethyl acetate at 1.5% dose. At the same dose (1.5%), very high inhibition values were obtained for *Macrophamina phaseolina*, *Fusarium oxysporum*. f. sp. *lycopersici*, *Rhizoctania solani* and *Botrytis cinerea* mycelium growths. At this dose, inhibition rates were calculated to be between 73.47–87.82%.

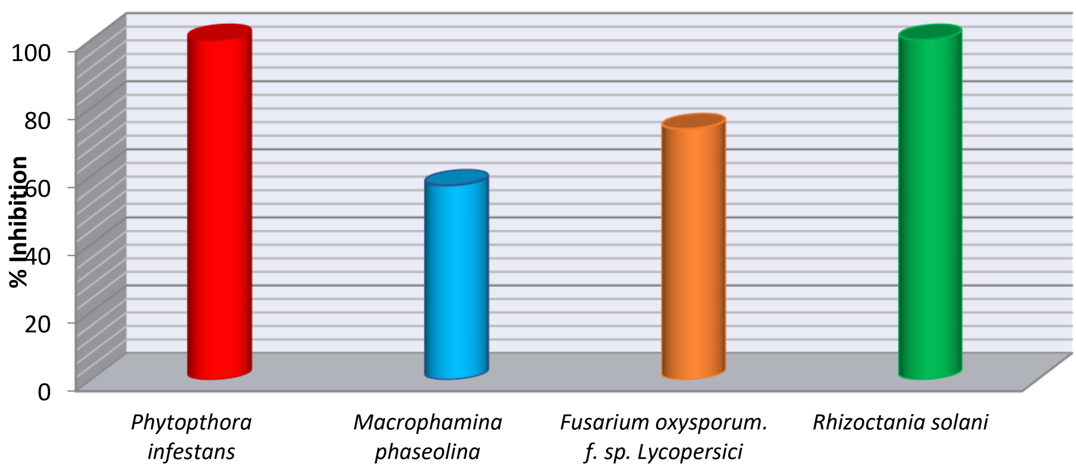
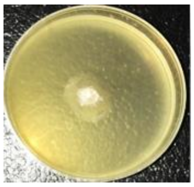
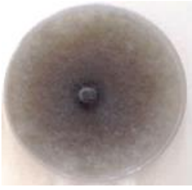
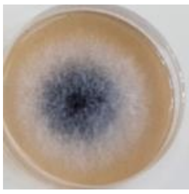
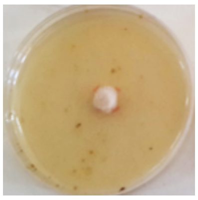


Figure 1. Percent inhibition of *Phytopthora infestans*, *Macrophamina phaseolina*, *Fusarium oxysporum*. f. sp. *lycopersici* and *Rhizoctania solani* mycelium growth of *Grindelia robusta* Nutt. Extracts.

Table 1. Comparison of the growth rate of fungi in *Grindelia robusta* Nutt. extracts with the control group on the disc surface.

Pathogenic fungi	Growth rate of control	Growth rate of fungus
Phytopthora infestans		
Macrophamina phaseolina		
Fusarium oxysporum. f. sp. *lycopersici*		
Rhizoctania solani		

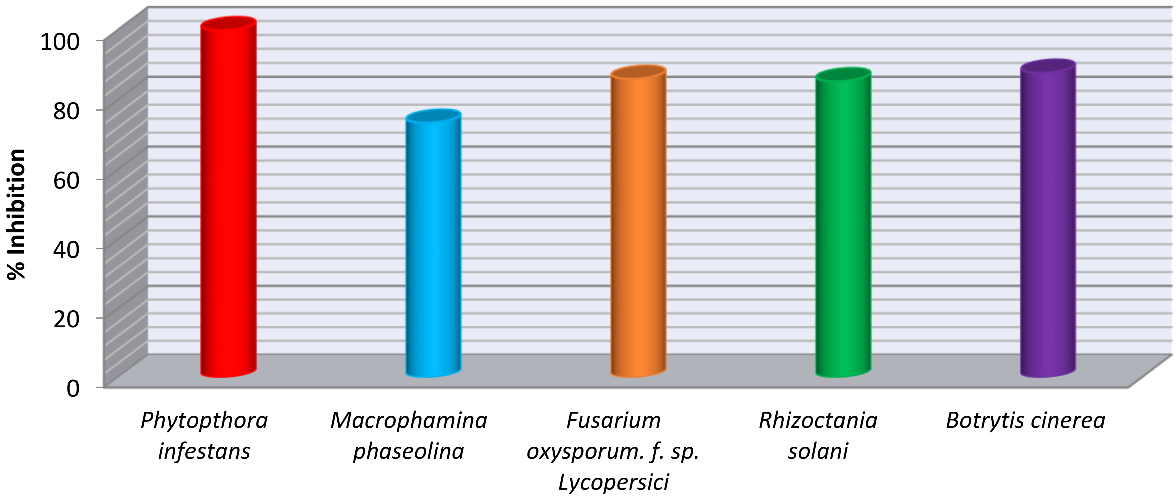


Figure 2. Percent inhibition of *Phytopthora infestans*, *Macrophamina phaseolina*, *Fusarium oxysporum*. f. sp. *lycopersici, Rhizoctania solani* and *Botrytis cinerea* mycelium growth of *Tanacetum praeteritum* subsp. *praeteritum* extracts.

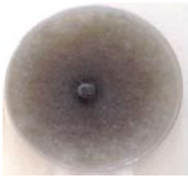
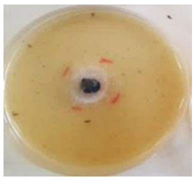
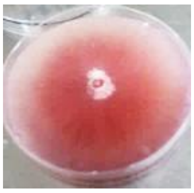
The exprimental images of the growth rate of fungi in the *Tanacetum praeteritum* subsp. *praeteritum* extracts on the disc surface compared to the control group is shown in Table 2.

Alchemilla vulgaris L. extracts were prepared with methanol at a dose of 1.0%. The anti-fungal effect of *Alchemilla vulgaris* L. extracts against *Phytopthora infestans*, *Macrophamina phaseolina*, *Rhizoctania solani, Alternaria alternata* and *Cylindrocarpon destructans* mycelium growth were given in Figure 3.

Alchemilla vulgaris L. extracts showed a fairly good rate of inhibition against *Phytopthora infestans*

(86.98%) and *Macrophamina phaseolina* (81.57%), while moderately inhibition against *Alternaria alternata* (63.11%) and *Cylindrocarpon destructans* (45.20%). The moderate effect in *Alternaria alternata* and *Cylindrocarpon destructans* fungus types might be solvent and dose related. In addition, *Alchemilla vulgaris* L. extracts had no effect on *Rhizoctania solani* mycelium growth. Especially for *Rhizoctania solani*, better inhibition rates can be observed at higher extract doses. The exprimental images of the growth rate of fungi in the *Alchemilla vulgaris* L. extracts on the disc surface compared to the control group is shown in Table 3.

Table 2. Comparison of the growth rate of fungi in *Tanacetum praeteritum* subsp. *praeteritum* extracts with the control group on the disc surface.

Pathogenic fungi	Growth rate of control	Growth rate of fungus
Phytopthora infestans		
Macrophamina phaseolina		
Fusarium oxysporum. f. sp. *lycopersici*		
Rhizoctania solani		

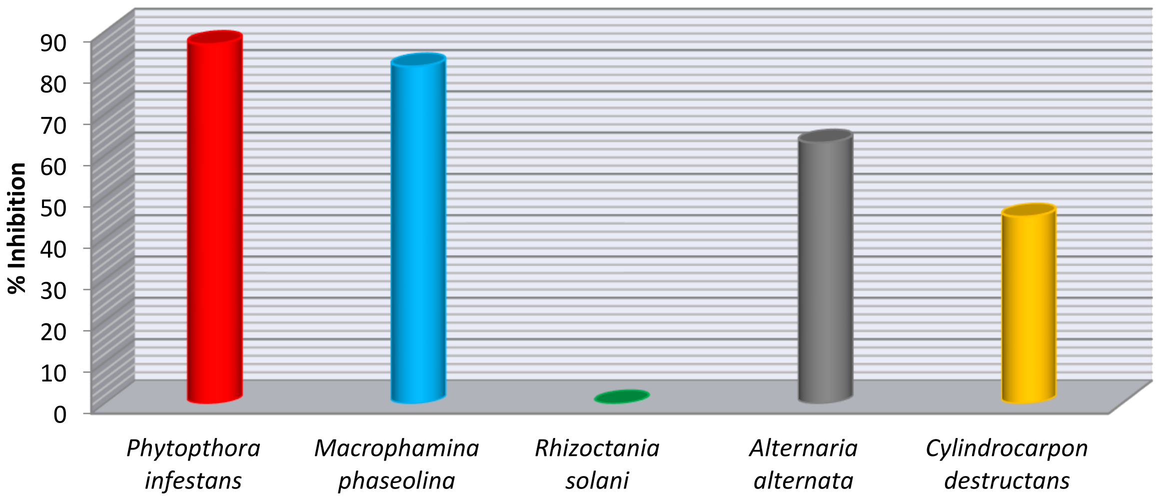
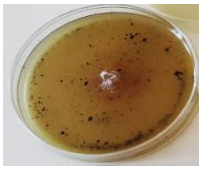
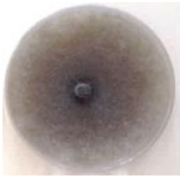
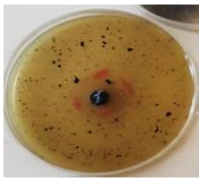
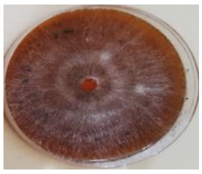
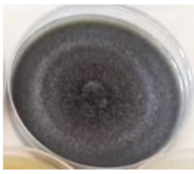
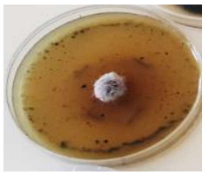
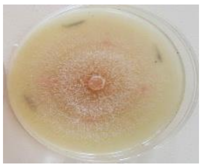
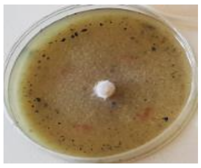


Figure 3. Percent inhibition of P*hytopthora infestans*, *Macrophamina phaseolina*, *Rhizoctania solani, Alternaria alternata* and *Cylindrocarpon destructans* mycelium growth of *Alchemilla vulgaris* L. extracts.

Table 3. Comparison of the growth rate of fungi in *Tanacetum praeteritum* subsp. *praeteritum* extracts with the control group on the disc surface.

Pathogenic fungi	Growth rate of control	Growth rate of fungus
Phytopthora infestans		
Macrophamina phaseolina		
Rhizoctania solani		
Alternaria alternata		
Cylindrocarpon destructans		

CONCLUSION

In the current study, anti-fungal properties of *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. extracts in different solvents against certain plant pathogenic fungi were determined. Today, different kind of organic molecules were synthesized in the laboratory conditions with different biological properties, including antifungal properties (Özbek et al., 2017; Gürdere et al., 2020; Özbek and Gürdere, 2020). However, these synthesized organic molecules lead to toxic residues in food. For this reason, researchers focused on the use of environmentally friendly biological methods to protect plants from different damages. In the present study, natural plant-based extracts were analyzed in terms of their anti-fungal activities. All plant extracts mentioned in the study exhibited significant activity against plant pathogenic fungi. Finally, it can be stated that *Grindelia robusta* Nutt., *Tanacetum praeteritum* subsp. *praeteritum* and *Alchemilla vulgaris* L. extracts dissolved in different solvents have anti-fungal properties against certain plant pathogens and can be used as biofungicides in the future to prevent agricultural fungal infections.

Conflict of Interest

No conflict of interest was reported by the authors.

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