



Evaluation of the Biocontrol Potential of *Morina persica* L. Extract against *Ditylenchus dipsaci* (Kühn) Filipjev and Some Plant Pathogenic Fungi

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Abstract

In this study, antifungal and nematocidal effects of methanol extracts of *Morina persica* L. were determined. In antifungal activities, plant extract was evaluated against *Alternaria solani* (Ell. & Mart.), *Fusarium oxysporum* f. sp. *lycopersici* (FOL) (Sacc.) W.C. Snyder & H. N. Hans and *Verticillium dahliae* Kleb. The experiment was carried out by the agar plate methods at a concentration of 0.625, 1.25, 2.5, 5, 10 and 20 mg mL. All concentration of *M. persica* plant extracts was showed a different level of antifungal activities against all the three test fungi depend on concentrations. Nematocidal activity was evaluated against stem nematode *Ditylenchus dipsaci* (Kühn) Filipjev under in vitro condition. Five different concentrations (31.25, 62.5, 125, 250 and 500 ppm) of *M. persica* plant extracts were tested. The nematodes exposed to 24, 48, 72 and 96 hours in plant extracts and kept at 25 °C. *M. persica* plant extracts were found highly effective on *D. dipsaci*. The plant extracts of *Morina persica* L. was the first time determined the antifungal and nematocidal activities in this study, and has potential effect as a biopesticide against plant pathogens and plant parasitic nematodes.

Keywords: *Morina persica*, *Alternaria solani*, *Fusarium oxysporum* f. sp. *lycopersici*, *Verticillium dahliae*, *Ditylenchus dipsaci*, Plant extract.

Morina persica L. Ekstraktının *Ditylenchus dipsaci* (Kühn) Filipjev ve Bazı Bitki Patojeni Funguslara Karşı Biyolojik Mücadelede Kullanım Potansiyelinin Belirlenmesi Öz

Bu çalışmada, *Morina persica* L. metanol ekstraktının antifungal ve nematisidal etkinliği belirlenmiştir. Antifungal etkinliğini belirlemede bitki ekstraktı *Alternaria solani* (Ell. & Mart.), *Fusarium oxysporum* f. sp. *lycopersici* (FOL) (Sacc.) W.C. Snyder & H. N. Hans ve *Verticillium dahliae* Kleb. patojenlerine karşı denenmiştir. Deneme, *M. persica* ekstraktının 0,625, 1,25, 2,5, 5, 10 ve 20 mg mL'lik konsantrasyonlarında agar petri yöntemi ile gerçekleştirilmiştir. *M. persica* ekstraktının tüm konsantrasyonları doza bağlı olarak farklı seviyelerde antifungal etkinlik göstermiştir. Nematocidal aktivitesi *Ditylenchus dipsaci* (Kühn) Filipjev'e karşı in vitro koşullarda gerçekleştirilmiştir. *M. persica* ekstraktının 5 farklı konsantrasyonu (31,25, 62,5, 125, 250 ve 500 ppm) *D.dipsaci*' ye karşı denenmiştir. Nematodlar, 25°C'de 24, 48, 72 ve 96 saat bitki ekstraktına maruz bırakılmıştır. *M. persica* ekstraktının *D.dipsaci*'ye karşı oldukça etkili olduğu tespit edilmiştir. *Morina persica* L. ekstraktı'nın antifungal ve nematisidal etkinliği ilk kez bu çalışma ile belirlenmiş ve ele alınan nematod ve patojenlere karşı biyopestisit olarak potansiyel bir etkiye sahip olduğu saptanmıştır.

Anahtar Kelimeler: *Morina persica*, *Alternaria solani*, *Fusarium oxysporum* f.sp.*lycopersici*, *Verticillium dahliae*, *Ditylenchus dipsaci*, Bitki ekstraktı.

Introduction

Vegetables are one of the important sources of many nutrients for human and the major consumption products all around the world. Turkey is ranked 4th in the world in vegetable products such as tomatoes and eggplant (FAO, 2017). Plant pests and diseases cause significant yield losses on vegetable production every year. *Alternaria solani* (Ell. & Mart.) is one of the important diseases on tomatoes and known as an early blight (Yazıcı et al., 2011). *Fusarium oxysporum* f.sp.*lycopersici* (FOL) (Sacc.) W.C. Snyder & H. N. Hans. is known as a fusarium wilt and caused significant yield losses on tomatoes (Can et al., 2004). *Verticillium dahliae* Kleb causes verticillium wilt in many plants (Dervis et al., 2010). *Ditylenchus dipsaci* (Kühn) Filipjev is known stem nematode and causes yield losses over 450 plant species (Anonymous, 2017). *D.dipsaci* caused up to 80% yield loss on onion (Sturhan and Brzeski, 1991).

Synthetic chemicals are often preferred to control these pests and diseases. Although the chemical control is effective and cheap, there is a negative effect on human health, environment, and non-target organisms. Therefore, environmentally friendly, harmless for human health and non-target



organisms new control methods are being investigated. One of the effective methods is to use plant extracts. Several plant species have the potential for using as biopesticide due to that contained nematicidal and antifungal compound (Nwosu and Okafor, 1995; Chitwood, 2002; Gupta et al., 2011). *Morina persica* L. (Morinaeaceae) is an endemic, a herbaceous perennial plant. Each plant has 5-8 flowers. This plant is found in the terrestrial region in Turkey (except the South-eastern Anatolia). *M. persica* has distributed Greece, Lebanon, Syria, Iran and Central Asia (Anonymous, 2016). Before, *M. persica* has not studied biopesticide effect on plant pest and diseases in Turkey.

The aim of this study was to determine alternative control methods for important plant diseases such as *A.solani*, *F. oxysporum f.sp.lycopersici* and *V.dahliae*, and nematode, *D.dipsaci* by plant extract of *M. persica*. For this purpose, the methanol extracts of *M.persica* tested, and antifungal and nematicidal activities were determined.

Materials and Methods

Plant Material

The upper part of ground from *Morina persica* L. (Morinaeaceae) was collected from Tokat in 2016. They were washed with sterile water and kept the room temperature until drying. The plant parts were ground in the grinder. They were stored in the jar until used.

Plant Extracts

Powdered plant material (100 g) was extracted with methanol by incubated orbital shaker (Lab. Corporation Group, Model-SI-300) at 150 rpm for 72 h (30 °C). After that, it was evaporated to dryness in a rotary evaporator (Heildolph Group, Model-Hei-Vap Presicion). For antifungal activities, the extract was diluted by 5% of Dimethyl sulfoxide (DMSO) and for nematicidal activity 1% of DMSO.

Fungi Culture

The plant pathogenic fungi (Table 1) used in the research were obtained from the stock cultures in the laboratory of Plant Pathology, Department of Plant Protection, Faculty of Agriculture, University of Gaziosmanpaşa University, Turkey. Plants pathogens were grown on Petri dishes (90 mm) containing 20 ml of PDA and incubated at 22±2°C for 7 days, these fungi cultures were used in the study.

Table 1. List of plant pathogens

Plant pathogens	Abbreviation	Origin	Place of Isolation
<i>Alternaria solani</i>	AS	Tomato	Antalya, Turkey
<i>Fusarium oxysporum f.sp.lycopersici</i>	FOL	Tomato	Antalya, Turkey
<i>Verticillium dahliae</i>	VD	Eggplants	Antalya, Turkey

In Vitro Antifungal Activity

The antifungal activities of the plant extract were determined by agar plate method. Plant extract was added to PDA at 40 °C to give the concentration of 0.625, 1.25, 2.5, 5, 10 and 20 mg/mL for the extract and then the PDA with extract was poured (~10 ml/plate) each alone in Petri plates (60mm in diameter). Seven-day-old agar discs (5mm in diameter) bearing the desired fungus growth was transferred to the Petri plates. These fungus cultures were incubated at 22±2 °C for 7 days. Fungus growths were recorded daily. Commercial fungicide (Thiram 80%) was used as a positive control and 5% DMSO was used as a negative control. The experiment was set up 3 replications and repeated twice.

The percentage of mycelial growth inhibition was calculated accordingly the formula mentioned by Pandey et al., 1982.

$$I = 100 \times (dc - dt) / dc$$

I; Mycelial growth inhibition

dc; Is the mycelial growth in control

dt; Is the mycelial growth in treatment

Nematode Culture

Ditylenchus dipsaci (Kühn) Filipjev was collected on garlic from Taşköprü, Kastamonu in 2017. *D. dipsaci* was identified based on morphological and morphometric characters (Barracough and Blackith, 1962). The nematode was cultured in the onion according to Mennan (2005). *D. dipsaci* was extracted by using petri dish methods.

In Vitro Nematicidal Activity

Ten µl of the nematode suspension (50±5 nematode/ µl) were transferred to 1ml each concentration in well of 24 well-plates in five replicates and repeated twice, while distilled water containing 1% DMSO used as a control. Five different concentrations (31.25, 62.5, 125, 250 and 500 ppm) of *M. persica* plant extracts were tested. The mortality of nematodes was evaluated after 24, 48, 72 and 96 hours. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol et al., 1989).

Statistical Analysis

For antifungal activities; Data were analyzed by using analysis of variance (ANOVA) test. Differences between means were determined by the Tukey test (at the 0.05 probability level). LD doses were calculated by POLO 1.0. For nematicidal activities; Data were analyzed by analysis of variance, and means were compared using Duncan's multiple range test. The software SPSS 16.0 was used to conduct all the statistical analysis.

Result and Discussion

Antifungal Activity

In this study, the methanol extracts of *M.persica* was tested against *A.solani* (AS), *F. oxysporum f.sp.lycopersici* (FOL) and *V. dahliae* (VD). All concentrations of the methanol extracts of *M.persica* displayed varied antifungal activities on AS, FOL, and VD. The effect increased as the concentrations increased (Figure 1). The concentration of 20 mg/ml plant extract, the development of mycelium has been suppressed by 55% of AS, 60% FOL and 39% of VD. (Table 2). Antifungal and antibacterial properties were worked by some researchers. The n-hexane, dichloromethane and water extracts of *M.persica* were tested against human pathogens [fungus (*Candida albicans*) and bacteria (*Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*)]. *C. albicans*, *E. coli*, and *P. aureginosa* were not showing any activities however other pathogens were showed a different level of inhibition (Taşdemir et al., 2004). Similar work was done by Mocan et al., (2016), the methanol, acetone and water extracts of *M.persica* was studied against human pathogens and all extracts were showed antifungal and antibacterial effects. Tosun et al., (2005) was tested *M.persica* extracts against *Mycobacterium tuberculosis* and found a lower antimycobacterial effect.

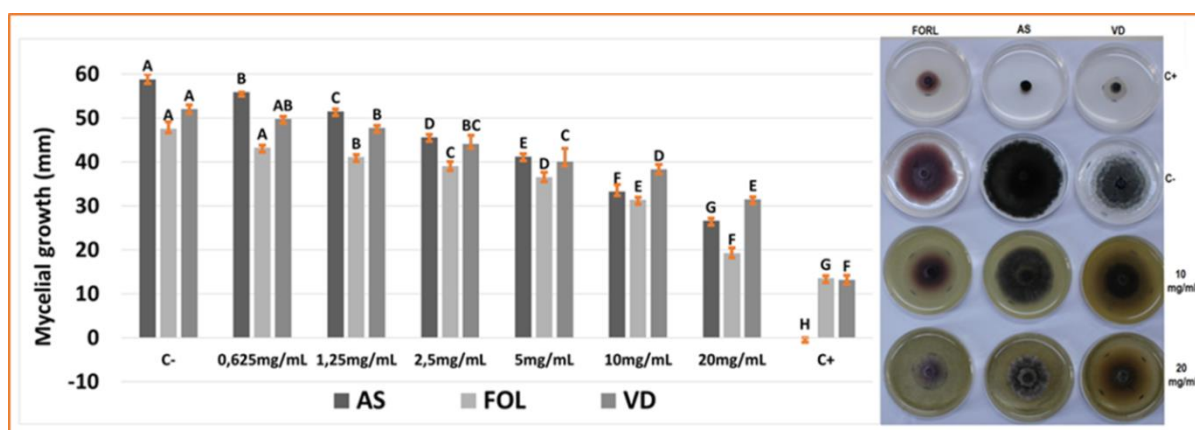


Figure 1. Mycelial growth (mm) of *Morina persica* L. against test fungi (The picture is showing 10 and 20 mg/ml concentrations of plant extract effect after 7 days)

According to the result, depending on the pathogen species, some physical changes have been observed such as the developments of the mycelium of all pathogens suppressed and sporulation decreased (Figure 1). We believe that 100% suppression can be done by increasing the concentrations (Table 2).



Table 2. Mycelial growth inhibition (%) of *Morina persica* L. against test fungi

Doses mg/mL	Test Fungi (%)		
	AS	FOL	VD
C-	0	0	0
0,625	5	9	4
1,25	12	14	8
2,5	22	18	15
5	30	23	23
10	43	34	26
20	55	60	39
C+	100	72	75

The LD₁₀, LD₅₀ and LD₉₀ values of the effect of the *M.persica* extract against the test fungi were calculated (Table 3).

Table 3. *Morina persica* L. extracts against the test fungi have calculated the value of LD.

Test Fungi	Effective Concentrations (mg/mL)			
		95% limits		
		LD	Low.	Upr.
AS	LD10	0.98	0.68	1.30
	LD50	14.31	11.41	19.02
	LD90	208.14	120.08	441.79
	Slope	1.102±0.091		
	Heterogeneity	0.14		
	Chi-square	2.22		
FOL	LD10	1.33	0.93	1.74
	LD50	16.22	12.65	22.37
	LD90	198.40	111.70	444.98
	Slope	1.178±0.106		
	Heterogeneity	0.70		
	Chi-square	11.23		
VD	LD10	1.51	0.96	2.08
	LD50	41.52	26.53	81.39
	LD90	1145.84	412.33	5692.06
	Slope	0.889±0.102		
	Heterogeneity	0.36		
	Chi-square	5.79		

Nematicidal Activity

In this study, the methanol extracts of *M.persica* was tested against *D. dipsaci*. *M.persica* showed highly nematicidal activity on *D. dipsaci*. Mortality effect was increased by the time. The concentrations of 250 and 500 ppm were observed 100% mortality on nematode in 24 hours. After 96 hours exposure to plant extract, all nematodes were recorded the dead.

Most of the plant extract study has done on *Meloidogyne* spp. A few studies were found on *D.dipsaci* to control by biopesticides. Valenzuela (1995) were evaluated 18 Chilean plants to control *D.dipsaci*. Extracts of *Aristotelia chilensi* (Mol.) Stuntz, *Chenopodium ambrosioides* L., and *Ovidia pilloillo* (Gay) Meisn were found effective on *D. dipsaci*. Zouhar et al., (2009) were obtained by exposure to essential oils of *Eugenia caryophyllata* L. Merr. & Perry, *Origanum compactum* Benth, *Origanum vulgare* L., *Thymus vulgaris* L., and *Thymus matschiana* L., with which only the concentrations of 5000 and 7500 ppm were found effective. Hassan et al., (2015) were tested plant extracts and commercial synthetic pesticides against *D. dipsaci*. They found that ethanol and water extracts of leaves of *Inula viscosa* (L.) Aiton and dry fruits of *Melia azedarach* L., showed nematicidal activity against *D.dipsaci*. Our result also showed the highly effective nematicidal effect on *D.dipsaci*.

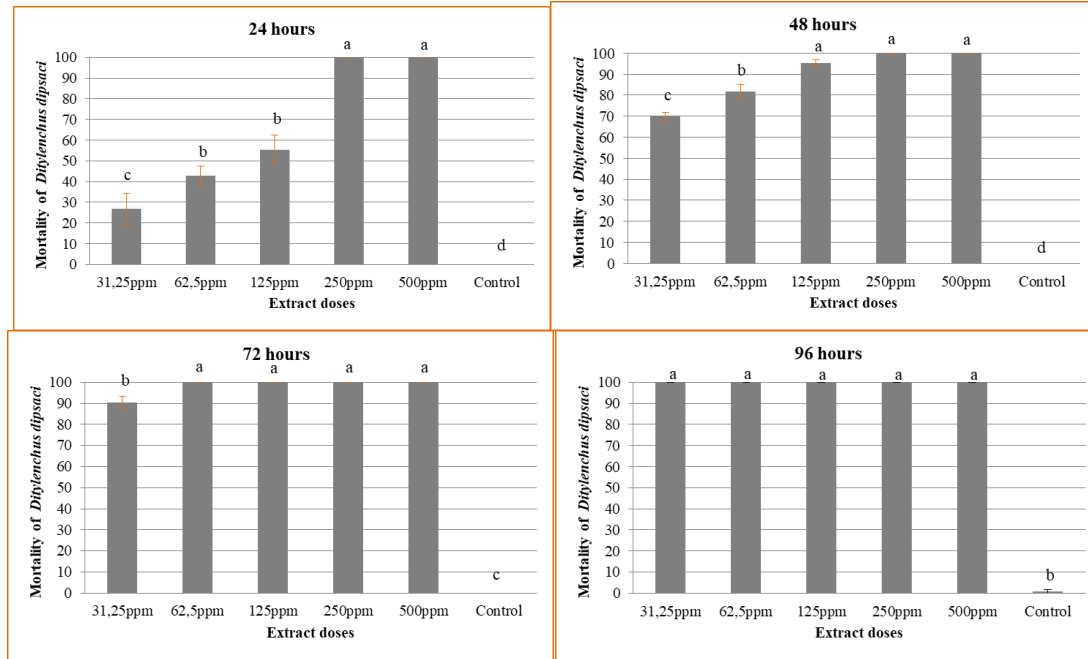


Figure 2. Nematicidal activity of *Morina persica* L. against *Ditylenchus dipsaci* (Kühn) Filipjev

Conclusion

Turkish plant fauna is very rich and many researchers are worked on properties of their antioxidant, insecticidal, antifungal and nematicidal (Onaran and Yılar, 2012; Emniyet et al., 2014; Erdoğan, 2015; Kepenekci, 2016, Tunaz, 2017). *M. persica* has potential health beneficial properties and uses tea in the traditional Anatolian medicine. The aerial part of *M. persica* has a rich source of phenolic compounds. Methanolic extracts of *M.persica* revealed good inhibitory properties on acetylcholinesterase, and antibacterial and antifungal properties (Mocan et al., 2016). The plant extract of *M. persica* which used in our study was showed a different level of antifungal activities in a dose depend manner. Also, it was found highly effective on *D. dipsaci*. The extracts determined activities showed that can be used as biopesticides. In addition, the first time displayed antifungal and nematicidal properties of *M. persica* by this study.

Note: This work was presented in the ICAFOF International Conference on Agriculture, Forest, Food Sciences and Technologies 15-17 May 2017 in Cappadocia, Nevşehir/Turkey. The abstract was published in the abstract book.

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