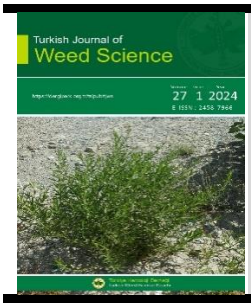

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Araştırma Makalesi/Research Article

Assessment of Antifungal and Phytotoxic Properties of *Peganum harmala* L. Extracts on Cultivated Plants And Pathogenic Fungi

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Abstract

This study aimed to assess the antifungal potential of *Peganum harmala* L. against significant plant pathogenic fungi and its phytotoxic impact on select cultivated plants. The aerial parts (flowers, shoots, leaves, and seeds) of *P. harmala* were harvested from Kırşehir Province during the 2023 growing season. Subsequently, the collected plants were shade-dried in the laboratory and finely powdered using an electric grinder. One liter of methanol was added to 100 grams of the dried plant material, followed by a 48-hour maceration period. After methanol evaporation, the residue was dissolved in DMSO to achieve concentrations of 0.5, 1, 2, and 4 mg mL^{-1}. Nine-centimeter diameter Petri dishes were prepared with two layers of filter paper, and 25 seeds each of the test plants (wheat, cress, and clover) were evenly distributed. The Petri dishes were then sealed and incubated at 24°C for a period ranging from 1 to 4 weeks. After the incubation period, seed germination rates and the lengths of roots and shoots were measured for the test plants. Additionally, the impact of the methanol extract on the mycelial growth of pathogenic fungi including *Alternaria alternata* (A.A), *Verticillium dahliae* (V.D), *Sclerotinia sclerotiorum* (S.S), *Alternaria solani* (A.S), and *Monilinia fructigena*(M.F) at concentrations of 0.5, 1, 2, and 4 mg mL^{-1} was evaluated. The results indicated varying effects of the plant methanol extract on the cultivated plants, with *Sclerotinia sclerotiorum* displaying inhibition rates of 0.0%, 0.0%, 19.68%, 39.59%, and 62.97% against *Alternaria solani*, *Monilinia fructigena*, *Verticillium dahliae*, *Alternaria alternata*, and *Sclerotinia sclerotiorum*, respectively. Consequently, the study identified the biological activity of *P. harmala* and recommended further validation through pot and field experiments.

Key Words: *Peganum harmala* L., phytotoxic effect, antifungal activity, germination, root and shoot growth

INTRODUCTION

There are abiotic and biotic factors in the agricultural ecosystem that cause problems in products. These biotic factors include diseases, pests and weeds. Synthetic fungicides are used extensively due to the inadequacy of environmentally friendly methods such as cultural control in the control against plant pathogenic diseases. Unconscious and excessive use of these chemicals can cause harm to the environment and people. Studies are being carried out to identify natural products that can replace them in order to reduce this negative effect. On the other hand, weeds, which are another plant protection problem, have negative effects on agricultural products. The most significant among these negative effects is the competition with cultivated plants for water, nutrients and light. In addition, the allelopathic effects of weeds on cultivated plants may cause loss of yield. Every plant within the agricultural ecosystem contains secondary compounds and plays a role in allelopathic interactions between plants. These compounds can be released from the plants themselves or released into the environment from their by-products. Although these components released into the agricultural ecosystem are called allelochemicals, they can cause phytotoxic effects on other plants (Alam and Islam, 2002; Yilar et al., 2020). The compounds that cause this phytotoxic effect can be found in different parts of plants (Zeng et al., 2008) and when released into the environment, they cause negative effects on other plants. For the reasons mentioned above, weeds can cause serious yield losses in cultivated plants.

Worldwide, weed control is estimated to result in a loss of approximately 13.2% in the eight most crucial food and commercial crops (Oerke et al., 1995). Mechanical methods such as hand weeding and hoeing demand significant labor and time. Consequently, chemical control remains the most widely used approach. However, chemical herbicides can pose environmental risks due to their toxicity (Batish et al., 2007c; Kordali et al., 2009). Integrated strategies aimed at enhancing weed management are necessary to reduce reliance on herbicides. Recent efforts have focused on investigating the allelopathic effects of various plants to sustainably control weeds (Singh, 2003c; Macías et al., 2006). Residues from natural products released by allelopathic and medicinal plants can aid in decreasing the use of synthetic herbicides, thereby reducing pollution and promoting safer agricultural practices (Singh et al., 2003a; Khanh et al., 2007). Various plant families are being explored for this purpose, and research on these species is ongoing.

Peganum harmala L. is a perennial, glabrous plant belonging to the Zygophyllaceae family, typically growing between 30-70 cm in height. It is an invasive species in meadow pastures, spreading in uncultivated and barren lands (Anonymous, 2008; Ertuğrul et al., 2022). Commonly referred to as "peganum" in our region, it was initially named "Moly" by Dioscorides, while Syrians called it "Besasa," and Cappadocians referred to it as "Moly" (Ratsch, 1992). This plant possesses antimicrobial (Arshad et al., 2008), anti-inflammatory, and analgesic properties (Monsef et al., 2004). In folk medicine, *P. harmala* has been utilized as an emmenagogue and abortifacient in North Africa and the Middle East (Monsef et al., 2004). Additionally, boiling *P. harmala* leaves is employed in treating rheumatism (Chopra et al., 1986). Carboline alkaloids such as harmine, harmaline, harmalol, peganine, vasicin, vasicin, deoxyvasicin, peganone-1 (3-6-dihydroxy-8-methoxy-2methyl anthraquinone), and peganone-2 (8-hydroxy – 7 methoxy – 2 methyl anthraquinone) obtained from various parts of this plant are utilized against various diseases (Aarons, 1977; Sobhani et al., 2002).

Medicinally, the fruits and seeds of *P. harmala* are known to possess digestive, diuretic, hallucinogenic, hypnotic, antipyretic, antispasmodic, antiemetic, narcotic, and uterine stimulant properties (Chatterjee, 1997; Kiritikar, 1995; Sharma, 1988). The red dye derived from the seeds is extensively used for coloring carpets in Turkey and Iran (Baytop, 1999). Leaves of *P. harmala* are beneficial in treating asthma, colic, dysmenorrhea, hiccups, hysteria, neuralgia, and rheumatism (Chatterjee, 1997; Kiritikar, 1995; Sharma, 1988). The plant has also been explored for its antimicrobial (Adday et al., 1989; Alkofahti et al., 1990; Prashanth et al., 1999) and antitumor properties (Prashanth et al., 1999), as well as its potential in treating malaria (Kiritikar et al., 1995) and as an insecticide (Ahmed et al., 1981). While studies highlighting the allelopathic and antifungal activity of this plant are limited, existing research demonstrates its effectiveness against various crops, weed species, and plant pathogenic fungi (Sodaeizadeh et al., 2010; Farajollahi et al., 2013; Shao et al., 2013; Bitchagno et al., 2022; Al-Jalili et al., 2022).

This study aims to assess the phytotoxic impact of *P. harmala*, a plant abundant in secondary compounds, on various cultivated plants from diverse families, as well as its antifungal activity against significant plant pathogenic fungi.

MATERIALS and METHODS

2.1. Plant Materials: Plant material of *Peganum harmala* used in the experiments was collected during the flowering stage from Kırşehir Province, throughout the 2023-2024 vegetation period. The collected plant materials were dried in the shade under laboratory conditions.

2.2. Fungal Cultures: The plant pathogenic fungi utilized in this study (*Alternaria alternata*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Alternaria solani*, and *Monilinia fructigena*) were obtained from stock cultures maintained at Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Phytopathology Laboratories. Fungal cultures were utilized after incubating for 7 days at 23±2°C on Potato Dextrose Agar (PDA) plates. The plant pathogens used in the study are given in Table 1.

Table 1. Plant pathogens used in the study.

Latin Name	Abbreviation	Plant from which it was isolated.	Turkish Name
Alternaria alternata	*A.A*	Apple	Apple fruit rot
Verticillum dahlie	*V.D*	Aubergine	Verticillium wilt
Alternaria solani.	*A.S*	Tomato	Early blight
Monilinia fructigena	*M.F*	Pear	Mummy disease
Sclerotinia sclerotiorum (Lib.) de Bary	*S.S*	Cucumber	white rot

2.3. Preparation of Plant Methanol Extracts: 100 g of each ground plant material was weighed and placed in 1 L Erlenmeyer flasks, followed by the addition of 600 ml methanol. The mixture was agitated at 120 rpm in an orbital shaker at room temperature for 24 hours. Subsequently, the extract was filtered, and methanol was evaporated at 32°C until a solid material was obtained. Different concentrations (0 (control), 0.5, 1, 2, and 4 mg mL^{-1}) were prepared using the residual extract dissolved in dimethyl sulfoxide (DMSO) (Yılar and Bayar, 2023).

2.4. *In vitro* Antifungal Activity Study: The agar plate method was modified and employed to evaluate the antifungal effect of the methanol extract of *Peganum harmala* L. (Nwosu and Okafor, 1995). Prepared PDAs were autoclaved and cooled to 40-50°C, then mixed with sterile PDA at various concentrations (0 (control), 0.5, 1, 2, and 4 mg/mL). The PDAs were transferred to 60 mm diameter Petri dishes (~10 mL/petri). Mycelium disks (5 mm in diameter) from 7-day-old fungal cultures were placed onto the Petri dishes. Following inoculation, the fungal cultures were incubated at 23±2°C for 10 days. Fungal growth was monitored daily for 7 days, and colony diameter was calculated by measuring the fungal colony diameter in orthogonal directions.

Percentage inhibition of mycelial growth was calculated by comparing the inhibition in growth with the control development. Thiram 80% (wt/vol) (commercial fungicide) was used as a positive control, while 5% (v/v) DMSO was used as a negative control. The experiment was replicated thrice with three replications each, and percentage inhibition of mycelial growth was calculated according to the formula specified by Pandey et al. (1982):

Percent Inhibition (%)=(Growth in control−Growth in extract/ Growth in control)x100

2.5. Phytotoxic Effect of Plant Methanol Extract: Phytotoxicity studies on seed germination and seedling development of cultivated plants were conducted in 9 cm diameter Petri dishes. Two layers of blotting paper were placed in the Petri dishes, and the seeds of the test plants (25 each) were evenly distributed. Methanol extracts prepared at different concentrations (50, 100, 200, and 400 mg/mL) and pure water-DMSO for control purposes were added to the Petri dishes (6 ml/petri dish) and moistened. Petri dishes were tightly sealed with paraffin and incubated at an average temperature of 24°C for 1-3 weeks. At the end of the incubation period, seed germination rate and the lengths of radicle and shoot were measured (Önen, 2003).

2.6. Data Analysis: The significance levels of differences between treatments in the experiments were determined by analysis of variance (ANOVA), and means were compared using the Duncan test. Statistical analyses were performed using the SPSS 15.0 computer program.

RESULTS and DISCUSSION

3.1. Phytotoxic Effect of *Peganum harmala* Methanol Extract

The phytotoxicity of *Peganum harmala* methanol extract exhibited varying degrees of impact on different cultivated plants. While the methanol extract did not statistically affect the seed germination of *Lepidium sativum*, it significantly inhibited the seed germination of *Triticum aestivum* and *Medicago sativa* compared to the control (Table 2).

Table 2. Percentage Effect of *Peganum harmala* Methanol Extract on Seed Germination and Seedling Development of Cultivated Plants.

Test plants	Doses (mg mL^{-1})	Germination (%)	Root (cm)	Shoot (cm)
Lepidium sativum	**Control**	100^{a*}±0,0	$3,90^{a}$±0,37	$3,89^{a}$±0,17
	0.5	99^{a}±1,0	$3,56^{a}$±0,25	$3,57^{a}$±0,18
	1	99^{a}±1,0	$3,42^{a}$±0,32	$3,55^{a}$±0,25
	2	99^{a}±1,0	$2,10^{b}$±0,26	$3,48^{a}$±0,43
	4	99^{a}±1,0	$1,85^{b}$±0,31	$3,22^{a}$±0,40
Triticum aestivum	**Control**	100^{a}±0,0	$15,05^{a}$±1,38	$12,08^{a}$±10,1
	0.5	98^{ab}±1,15	$8,86^{b}$±0,68	$9,30^{ab}$±0,21
	1	98^{ab}±2,0	$7,96^{b}$±1,0	$9,18^{ab}$±0,67
	2	98^{ab}±2,58	$6,24^{bc}$±0,60	$8,78^{b}$±1,70
	4	94^{b}±0,84	$4,87^{c}$±0,36	$7,72^{b}$±0,75
Medicago sativa	**Control**	$98,0^{a}$±2,0	$2,32^{a}$±0,21	$3,15^{a}$±0,45
	0.5	$91,0^{b}$±1,0	$2,19^{a}$±0,04	$2,53^{a}$±0,31
	1	$90,0^{b}$±1,15	$2,13^{a}$±0,33	$2,50^{a}$±0,28
	2	$90,0^{b}$±1,0	$1,76^{a}$±0,02	$2,30^{a}$±0,11
	4	90.0^{b}±1,15	$1,70^{a}$±0,31	$2,21^{a}$±0,13

While the *Peganum harmala* methanol extract exhibited a significant inhibitory effect on cress root development compared to the control, it was found to be ineffective in altering plant shoot development. Furthermore, unlike the effect observed on cress plants, the extract demonstrated statistically significant inhibitory effects on both wheat root and shoot development at the P<0.05 significance level compared to the control (Table 2).

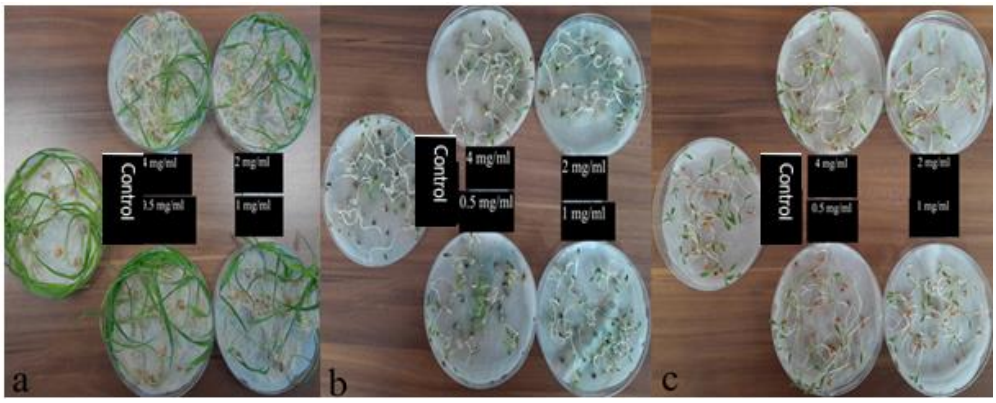

Figure 1. Phytotoxic effect of *P. harmala* methanol extract on test plants (a: wheat, b: clover, c: cress)

The genus Peganum encompasses various classes of metabolites with significant pharmacological effects. Plants within this genus contain phenolic compounds, terpenes, and nitrogen-containing compounds, with alkaloids being identified as the main components of the obtained extracts (Ratsch, 1992). Additionally, it has been reported that *P. harmala* leaves contain phytochemicals such as saponins, steroids, and tannins (Pahlavia et al., 2018). Aqueous extracts of *P. harmala* leaves have been found to contain phenolic acids including gallic, vanillic, caffeic, syringic, and trans-ferulic acids, as well as derivatives of benzoic acid (Sodaeizadeh et al., 2009).

Several studies have investigated the biological activity of *P. harmala*, a plant rich in phytochemical composition. It has been observed that phytotoxins released when plant material is incorporated into soil have varying effects on *Avena fatua* L. (Poaceae) and *Convolvulus arvensis* L. (Convolvulaceae) plants, with a notable reduction observed in *C. arnesis* plants (Sodaeizadeh et al., 2010). Moreover, the germination of *Chenopodium album*, *Amaranthus retroflexus*, and *Avena fatua* seeds was inhibited by a 1% dose of *P. harmala* dried and ground fruit hydroalcoholic extract, resulting in reductions of 60%, 50%, and 40%, respectively, compared to the control (Tafti et al., 2011).

P. harmala has also been found to exhibit an allelopathic effect on *Bromus tectorum* when grown in soils where plant materials obtained from different parts (root, shoot, leaf, and seed) are mixed. In this study, a high concentration of *P. harmala* demonstrated a strong inhibitory effect on the germination and early development period of *B. tectorum* (Farajollahi et al., 2013).

Under *in vitro* bioassay conditions, ethanol extracts of *P. harmala* showed significant inhibitory effects on both monocotyledonous (*Triticum aestivum* L.) and dicotyledonous (*Lactuca sativa* L.) plants at a dose of 0.05g/mL. Similar studies have suggested that the essence and extracts of numerous plants, including *P. harmala*, could serve as biological herbicides due to their inhibitory effects on mitochondrial activity and fat oxidation in plants (Robles et al., 1999; Ehlers and Thompson, 2004).

3.2. Antifungal Activity Study Results

In our investigation, we examined the biofungicidal effects of methanol extract derived from *Peganum harmala* L. against significant plant pathogens including *Alternaria alternata*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Alternaria solani*, and *Monilinia fructigena*. The rates of mycelial growth inhibition exhibited by these plant pathogenic fungi against the extract are presented in Table 3 and Figure 2. Statistical analysis revealed significant differences between the doses at the P<0.05 level. Comparing to the negative control, the 4 mg mL^{-1} dose of the plant extract inhibited the mycelial growth of *Sclerotinia sclerotiorum*, *Alternaria solani*, *Monilinia fructigena*, *Verticillium dahliae*, and *Alternaria alternata* by 0.0%, 0.0%, 19.68%, 39.59%, and 62.97%, respectively. The extract demonstrated similar inhibitory effects on the mycelial growth of the plant pathogens.

The most sensitive pathogen to the plant extract was *Alternaria alternata*, followed by *Verticillium dahliae*, *Monilinia fructigena*, *Sclerotinia sclerotiorum*, and *Alternaria solani*, respectively (Table 3, Figure 2).

Table 3. Mycelium growth inhibition of methanol extract of *Peganum harmala* against plant pathogens (%)

Doses (mg mL^{-1})	A.A	V.D	S.S	A.S	M.F
P+	100 ^{a} ±0,00	100 ^{a} ±0,00	100 ^{a} ±0,00	100 ^{a} ±0,00	100^{a} ±0,00
N-	0.00^{e} ±0.00	0.00^{c} ±0.00	0.00 ^{b} ±0.00	0.00 ^{b} ±0.00	0.00^{c} ±0.00
0,5	33.93^{d} ±4.83	0.00^{c} ±0.00	0.00 ^{b} ±0.00	0.00 ^{b} ±0.00	0.00^{c} ±0.00
1	40.20^{d} ±2.46	12.28^{c} ±6.19	0.00 ^{b} ±0.00	0.00 ^{b} ±0.00	0.00^{c} ±0.00
2	48.66^{c} ±3.02	13.11^{c} ±7.15	0.00 ^{b} ±0.00	0.00 ^{b} ±0.00	0.00^{c} ±0.00
4	62.97^{b} ±1.63	39.5 9^{b} ±9.54	0.00 ^{b} ±0.00	0.00 ^{b} ±0.00	19.68^{b} ±1,68

Means with different letters in the same column are different at the P<0.05 significance level according to LSD.
N-: Negative control; P+: Positive control; A.A(*Alternaria alternata*), V.D(*Verticillum dahlie*, S.S(*Sclerotinia sclerotiorum*, AS(*Alternaria solani*), M.F(*Monilinia fructigena*)

In a study, water, ethanolic, and methanolic extracts derived from the leaves, flower tissues, and seeds of *Peganum harmala* demonstrated effectiveness against various plant pathogens including *Phytophthora drechsleri*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Cladosporium cucumerinum*, *Corynespora cassiicola*, *Alternaria* sp., *Ulocladium* sp., *Botrytis cinerea*, *Monosporascus cannonballus*, *Fusarium oxysporum f. sp. melonis*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Trichoderma harzianum*. These extracts were tested for their impact on mycelial development and spore germination of the pathogens. The study reported significant antifungal activity against several of these plant pathogens (Sarpeleh et al., 2009).

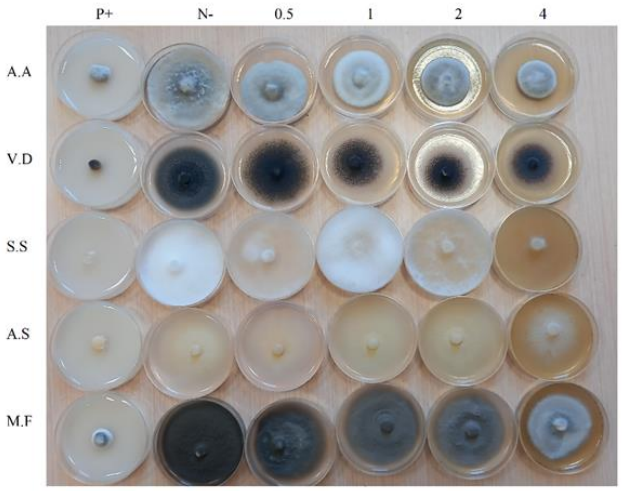

Figure 2. Effect of methanol extract of *Peganum harmala* on mycelium development of plant pathogens

N-: Negative control; P+: Positive control; A.A(*Alternaria alternata*), V.D(*Verticillum dahlie*, S.S(*Sclerotinia sclerotiorum*, AS(*Alternaria solani*), M.F(*Monilinia fructigena*)

Hajji et al. (2020) investigated the antifungal activity of *Peganum harmala* seed oil against 10 plant pathogenic fungi (*R. solani*, *M. phaseolina*, *Pythium* sp. 1, *Pythium* sp. 2, *Alternaria* sp., *Colletotrichum* sp., *M. cannonballus*, *F. solani* f. sp. *cucurbitae*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *niveum*) by incorporating different concentrations of the oil into melted agar. They found that 50% seed oil of *P. harmala* exhibited notable activity against *Pythium* sp., showing mycelium inhibition rates ranging from 56% to 82%, followed by *F. solani* f. sp. *cucurbitae* with inhibition rates of 15% to 55.7%. Abdal et al.(2023) assessed the antifungal activity of *P. harmala* seed water extract against fungi such as *Phytophthora infestans*, *Oidium oxysporum*, *Botrytis cinerea*, and *Alternaria solani*, known to infect tomatoes. Their results indicated that *P. harmala* seed water extract inhibited the development of plant pathogens to varying degrees. These findings align with previous studies.

CONCLUSION

In vitro bioassays demonstrated the phytotoxic effects of methanol extracts of *P. harmala* on the mycelial development of significant plant pathogenic fungi and on the tested cultivated plants. This suggests that *P. harmala* can produce phytochemical compounds that inhibit seed germination and growth in cultivated plants, as well as the mycelial development of pathogenic fungi. However, considering potential variations between laboratory, greenhouse, and field conditions, further investigations are warranted to validate the phytotoxic and antifungal activity of *P. harmala* under different environmental settings.

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