

## Biological Control of *Alternaria* spp. Using the Aqueous Extracts of Certain Medicinal Plants under In Vitro Conditions

**Yesna Abbasi**

Tenth-Grade Student, Allameh Iqbal High School, District 4, Shiraz, Iran (fraei181158@gmail.com)\*

**Setayesh Farhang**

Tenth-Grade Student, Hazrat Masoumeh High School, District 4, Shiraz, Iran

**Sheida Shakeri**

M.Sc. in Plant Pathology and Plant Systematics, District 4, Shiraz, Iran

**Sara Bostanian**

M.Sc. in Molecular Developmental Biology, Dr. Hesabi Research Center, District 4, Shiraz, Iran

### Abstract

**Introduction:** The control of plant pests and diseases is considered one of the fundamental challenges in agriculture, and the widespread use of chemical pesticides to combat these agents entails negative environmental and health consequences. In recent years, biotechnology and the use of natural plant compounds as suitable alternatives to chemical pesticides have attracted increasing attention. Numerous studies have demonstrated that plant extracts and essential oils can play an effective role in controlling plant pathogenic agents.

**Materials and Methods:** The objective of this study was to comparatively evaluate the effects of

### Introduction

The use of medicinal plants in the treatment of diseases has a history spanning centuries. Each medicinal plant contains one or more bioactive compounds, and its application is defined according to these active constituents. Although many pharmaceuticals are synthetic, at least one-third of medicines are of plant origin or are modified after extraction from plants. Chemical drugs are

aqueous extracts of chicory, chamomile, ajwain, Shirazi thyme, *Peganum harmala*, black pepper, red pepper, Damask rose, and garlic, in comparison with two control treatments (distilled water and a chemical pesticide), on the control of the growth of the fungus *Alternaria* under in vitro conditions. Aqueous plant extracts were prepared at a concentration of 1 g in 500 mL of water, and 1000  $\mu$ L of each extract was added to the WA1 culture medium in 6-cm Petri dishes. Subsequently, fungal colony growth was assessed.

**Results:** The results of analysis of variance and mean comparisons using SAS software indicated that the aqueous extracts of *Peganum harmala*, ajwain, and black pepper showed no significant difference from the chemical pesticide treatment in controlling the growth of *Alternaria* at an error level of less than 1% and exhibited performance comparable to that of the chemical pesticide.

**Conclusion:** Based on the results of this study, the aqueous extract of *Peganum harmala* was identified as the most effective plant-based treatment and was recognized as a suitable and acceptable option for replacing chemical pesticides in the control of *Alternaria* fungus, and it can be utilized in biological management programs for plant diseases.

**Keywords:** *Alternaria*; Biological Control, Pest; Plant Extracts; Medicinal Plants

associated with side effects, which has contributed to the continued value and importance of medicinal plants even after many centuries. One of the most critical global challenges is plant protection. The continuous use of chemical pesticides to control plant pests and diseases poses serious risks to the health of farmers, food consumers, and animals. The negative effects of pesticides have led to renewed interest in plant-derived compounds.[1]

<sup>1</sup> Water Agar

According to the authors of the book Introduction to the Applications of Biotechnology (Biotechnology Development Headquarters), plant protection is one of the agricultural sectors in which biotechnology has played a significant role in recent years. Plant extracts and essential oils contain compounds that can act against a wide range of microorganisms, and their antimicrobial effects against bacteria, fungi, and pathogenic yeasts have been demonstrated. These effects are associated with plant secondary metabolites.[2]

Iran, due to its climatic conditions and geographical location, is considered one of the most important regions in the world for the growth of medicinal plants and has historically been a major source of their production and use. In recent years, the application of plant-derived products in the control of pathogenic agents such as viruses, bacteria, fungi, and parasites has received increasing attention from researchers. Many fungi are classified as pathogenic agents that cause diseases or disorders in humans, animals, and plants. Plant extracts are among the materials used as antifungal agents.[3]

Most current studies focus on identifying effective natural compounds that lack harmful environmental effects and are economically feasible. In this regard, natural plant extracts, in addition to their antipathogenic effects, are environmentally safe and have therefore attracted considerable attention.[4] The aim of this study was to identify a natural alternative to antimicrobial agents in order to prevent their adverse effects and reduce antibiotic resistance in various microorganisms. Considering the climatic conditions of Fars Province, a large area of this region is annually cultivated with various agricultural crops, among which vegetables particularly tomato account for a substantial proportion. According to Mehr News Agency, in July 2023, more than 10,000 hectares of agricultural land in Fars Province are annually devoted to tomato cultivation. In the present study,

several medicinal plants with antifungal properties were evaluated for the control of one of the most important fungal plant pathogens affecting tomato and other vegetable crops, namely *Alternaria* spp., in comparison with the chemical fungicide copper oxychloride, as a biological control strategy.

*Rosa damascena* (damask rose) is a dense shrub characterized by numerous small, compact, uniform, hook-shaped thorns. Its flowers are pink (occasionally red) and aromatic. The medicinal properties of its petals include antibacterial, antiviral, anti-inflammatory effects, and strengthening of the heart and kidneys. The extract contains three flavonol glycosides: quercetin-3-O-glycoside, kaempferol-3,5-rhamnoside, and kaempferol-3-O-arabinoside. The essential oil of *R. damascena* and its major constituents, including citronellol, geraniol, and nerol, exhibit strong antimicrobial activity against several bacteria and fungi.[5]

*Garlic* (*Allium sativum* L.) belongs to the family Liliaceae and is a perennial herbaceous plant with a stem reaching up to 40 cm in height. Its underground bulb consists of 5–12 cloves enclosed in thin, whitish-gray membranes. The leaves are narrow, dark green, and linear, and the flowers are small, pink, and arranged in an umbel at the end of the stem. In recent years, considerable attention has been paid to the medicinal effects of garlic extracts and the isolation of their bioactive compounds. Numerous pharmacological properties have been reported, including antibiotic activities such as antibacterial, antiviral, antiparasitic, and antifungal effects, as well as beneficial effects on circulatory and cardiovascular disorders and anticancer properties.[6]

*Chicory* (*Cichorium intybus* L.) belongs to the family Asteraceae and is considered one of its most important species. The plant has a relatively thick, vertical root, a flowering stem bearing numerous blue flowers, and basal leaves that are lobed, while the upper leaves are simple and alternate. Major

bioactive constituents of chicory include flavonoid and terpenoid compounds. This plant exhibits notable medicinal properties, particularly strong antifungal and antibacterial activities, and has been recognized as a potent natural antibiotic.[7]

*Ajwain (Trachyspermum ammi [syn. T. capticum])* is a glabrous, aromatic herb with an erect stem reaching 20–50 cm in height and compound umbels with 6–8 rays. Its fruits (seeds) are small, ovoid, yellowish-brown, aromatic, and rich in thymol-like fragrance, and the dried, mature seeds constitute the medicinal part of the plant.[8] Ajwain seeds are rich in fiber, minerals, vitamins, and antioxidants.[9] Thymol, the principal chemical constituent, is well known for its antibacterial, antispasmodic, and antifungal properties.[10] Studies on plants of the Apiaceae family have demonstrated moderate to strong antimicrobial activity.[11]

*Zataria multiflora Boiss. (Shirazi thyme)* is an aromatic spice plant belonging to the family Lamiaceae and is distributed in Iran, Pakistan, and Afghanistan. The essential oil of this plant exhibits various biological activities, including antiseptic, anesthetic, anticonvulsant, and antibacterial effects. Its main constituents are phenolic compounds such as carvacrol and thymol. This plant is considered one of the most widely used and effective natural sources for the treatment of infectious diseases. Chemical analyses of *Z. multiflora* essential oil have shown the presence of significant amounts of monoterpenes, including thymol, carvacrol, linalool,  $\gamma$ -terpinene, and p-cymene. Recent studies have confirmed its antiparasitic and antifungal properties.[12]

*Peganum harmala (Syrian rue)* is a perennial medicinal plant belonging to the family Zygophyllaceae and is widely used for various therapeutic purposes. The seeds contain approximately 2–6% pharmacologically active alkaloids, predominantly  $\beta$ -carboline compounds such as harman, harmine, harmaline, and harmalol. Harmaline is known for its toxic, fungicidal, and

bactericidal effects. In Iran, *P. harmala* has long been recognized as an important medicinal plant in traditional medicine. Several pharmacological properties have been reported for this plant, including antimicrobial, antitumor, and monoamine oxidase inhibitory activities.[13]

*Chamomile (Matricaria chamomilla L.)* is a member of the family Asteraceae, with a height ranging from 30 to 70 cm and a branched stem terminating in capitula with a diameter of 1–2.5 cm. The flowers consist of both ligulate and tubular florets. Chamomile extract contains more than 120 chemical compounds, including chamazulenes, flavonoids, and coumarins, among which chamazulene, apigenin, and bisabolol are considered the most important bioactive constituents. Previous studies have demonstrated the antimicrobial, antifungal, and antibacterial activities of various chamomile species.[14]

*Red pepper (Capsicum annum L.)* is an annual plant belonging to the family Solanaceae and contains high levels of flavonoids and phenolic compounds. Antimicrobial compounds isolated from red pepper include capsanthin, terpinene,  $\alpha$ - and  $\beta$ -pinene, linalool, and terpineol.[15]

*Black pepper (Piper nigrum L.)* is a flowering climbing plant of the family Piperaceae and is commonly used in dried form as a spice and condiment. It exhibits antimicrobial effects against molds, primarily due to the presence of piperidine-related compounds in the fruit and pericarp. The inhibitory effects of these compounds at various concentrations against a range of fungi and bacteria have been well documented.[16]

Fungi are among the most important agents responsible for food spoilage during storage and preservation. They not only reduce the quality and quantity of food products but also pose serious health risks to consumers due to their potential to produce mycotoxins. *Alternaria* spp., comprising over 275 species, has *A. alternata* as the

predominant species in most soils and plant tissues. This species exhibits a global distribution and infects cereals, ornamental plants, oil crops, vegetables including broccoli, eggplant, carrot, potato, tomato, and beans and fruits such as citrus, apple, berry, and peach. *Alternaria* induces disease by causing leaf and green tissue lesions, reducing photosynthesis. The fungus degrades cell walls through cellulase and pectin methyl-galacturonase enzymes and kills host cells via alternariol production, thereby absorbing essential nutrients.[17]

*Alternaria* is among the most common plant pathogens worldwide, particularly in tomatoes, including in Iran. It can form lesions on leaves, stems, flowers, and fruits across various hosts, with the highest damage occurring on fruits. Leaf lesions are dark brown to black, often numerous and large. Pathogenic *Alternaria* species overwinter within dead plant tissues as mycelium and conidia, and may persist inside or on seeds, potentially infecting seedlings.[18]

### **Biological Control**

Biological control is one of the most successful non-chemical pest management strategies. Numerous organisms and substances naturally feed on or infect pests, pathogens, and weeds, often preventing populations from reaching economically damaging levels. Broadly, biological control involves manipulating parasites, predators, and pathogens to maintain pest and pathogen populations below economic thresholds.[19]

Pest management remains a major agricultural challenge, and widespread chemical pesticide use has adverse effects, including the destruction of beneficial insects, risks to human and animal health, environmental pollution, and the development of pesticide resistance. Consequently, scientists are exploring alternatives, such as medicinal plants.[20]

Over the past 50 years, many microbes, including bacteria and fungi, have been shown to suppress pathogens of both above- and below-ground plant organs. Recently, hundreds of commercial formulations have been developed for the biological control of diverse plant pathogens. Researchers have demonstrated that plant-derived materials—powders, essential oils, or extracts—can serve as effective practical tools for inhibiting plant pathogens.[21] This study focuses on evaluating some of these plant-based agents. Researchers have investigated the antifungal effects of aqueous extracts of *Thymus zygis*, hairy thyme, savory, and Alpine thyme at various concentrations against *Alternaria solani*. They reported that aqueous extracts of the two thyme species exhibited the strongest inhibitory effect on the fungus and could serve as practical agents for the biological control of *A. solani*, the causal agent of tomato leaf blight.[22] Accordingly, the present study also aimed to evaluate the aqueous extract of Shirazi thyme.

Regarding the biocontrol potential of plant-derived compounds, studies have shown that Shirazi thyme essential oil inhibits *Aspergillus flavus*, suggesting its application as a natural preservative in food industries.[23] Further phytochemical analyses indicated that thymol possesses strong antifungal properties, while carvacrol exhibits broad-spectrum antimicrobial activity.[24]

Green mold of citrus, caused by *Penicillium digitatum*, has also been targeted using plant-based antifungal agents. Laboratory and storage trials revealed that garlic and thyme extracts are effective natural inhibitors of this pathogen.[25] Additional studies on aqueous extracts of medicinal plants against *Alternaria* demonstrated that chives and basil extracts have high antifungal activity. Similarly, aqueous extracts of mint and oregano at concentrations above 50% significantly reduced *Fusarium* growth in vitro.[26] Other studies

reported that extracts of thyme, tobacco, and catnip can act as fungicides against *Alternaria*. [27]

Investigations also showed that *Aspergillus* and *Alternaria* species are susceptible to garlic extract, which inhibited *A. flavus* and aflatoxin B1 in wheat flour, although its efficacy under variable environmental and biological conditions requires further study. [28]

Studies on peppers revealed significant antifungal effects: red pepper extract at 0.5% concentration was more effective than black pepper in reducing aflatoxin production and inhibiting *A. flavus* and *A. parasiticus*, suggesting its potential use in food safety. [29] Concentrations up to 1% inhibited *A. flavus* growth by up to 70%. [30]

Chicory and chamomile have also demonstrated biocontrol potential. For instance, both plants inhibited *Candida albicans*, with chamomile showing stronger antifungal activity. Their essential oils have been considered safe alternatives to antibiotics. [31,32,33,34] *Zataria multiflora* (Shirazi thyme) extracts showed moderate antifungal activity compared to standard antifungal drugs like nystatin and miconazole, with essential oil demonstrating inhibitory zones of 18–60 mm against *Candida* species. [35,36]

*Rosa damascena* (rose) has been shown to inhibit *C. albicans* using aqueous extracts. [37] *Peganum harmala* (esfand), traditionally used in Iran for disinfection and pest control, exhibited 83.3% inhibition of *Fusarium graminearum* and *F. culmorum* growth with methanolic extracts. [38] Similar antifungal activity was reported against *F. oxysporum* and *F. sp. melonis*, highlighting the global relevance of Iranian medicinal plant research. [39,40]

The primary aim of this study was to evaluate the use of aqueous extracts of locally available, inexpensive, and traditional medicinal plants, whose efficacy against various plant pathogens has been documented, as a promising approach for the

biological control of *Alternaria*. Although the antifungal potential of these extracts has been established, comparative studies among different plant species remain limited. Therefore, this research provides a valuable basis and incentive for further studies in the biological control of plant-pathogenic fungi.

## **Materials and Methods**

### **Preparation of Culture Media**

The culture media used in this study were PDA (Potato Dextrose Agar) and WA (Water Agar).

### **Sterilization of Equipment**

All equipment, including culture media, water, plant extract flasks, scalpels, water samplers, and cork borers, were sterilized in an autoclave at 121 °C for 20 minutes.

### **Preparation of Aqueous Plant Extracts**

Aqueous extracts were prepared using the traditional boiling method. [41] Briefly, 20 g of dried, chopped medicinal plants obtained from certified herbal suppliers were placed in plastic bags, labeled, and transferred under a biological safety hood. The plant material was then exposed to UV light for 10 minutes, followed by boiling in 400 mL of distilled water for 20 minutes over medium flame. After boiling, the extracts were filtered through sterile containers and, to ensure the absence of initial contamination, exposed to UV light under a laminar flow hood for an additional 10 minutes. [42]

### **Preparation of Fungal Isolates**

*Alternaria* sp. isolates were obtained from the Fars Province Plant Preservation Center and transferred to the laboratory at Shahid Ghasem Soleimani Research Center, Shiraz for propagation and preparation of the initial inoculum (Figure 1).

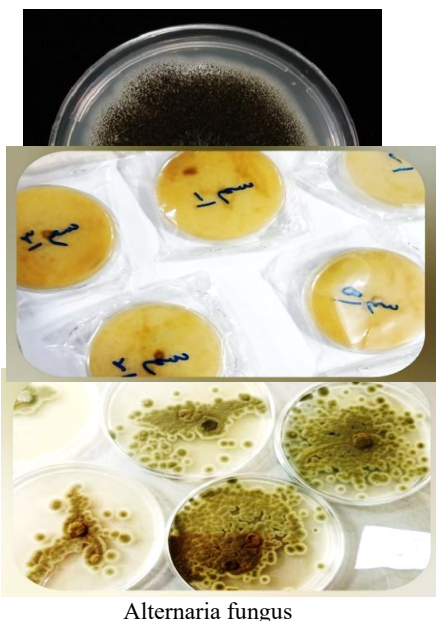


Figure 1.  
inoculum

Initial  
of

Alternaria fungus

### Preparation of fungal inoculum

To propagate a sufficient amount of the primary fungal inoculum, a small block was excised from the margin of young mycelia of *Alternaria* fungal colonies and transferred to the center of 8-cm Petri dishes. The plates were then incubated for 7 days in an incubator at 25 °C.[43] Experimental design: A completely randomized design with 9 treatments and 6 replicates was employed (Figure 2).



Figure 2. Petri dishes containing the aqueous extract treatments of the tested plants

Distilled water was used as the negative control, and copper oxychloride fungicide was used as the positive control (Figure 3).

A)

B)

Figure 3: A) Positive control (fungicide treatment), B) negative control (water treatment)

Initially, 1000  $\mu$ L of the aqueous extracts of chamomile, thyme, garlic, chicory, ajwain, black pepper, red pepper, esfand, and Damask rose were dispensed under a laminar flow hood under sterile conditions, adjacent to a flame, into 6-cm Petri dishes using a micropipette, and WA culture medium was added. The plates were exposed to UV treatment for 20 minutes, during which the culture media were kept completely covered. After 24 hours, following confirmation of the absence of contamination, fungal blocks were transferred to the center of the Petri dishes using a cork borer and



scalpel. Subsequently, colony diameters were measured for a period of 5 days, and during the

measurement period, the plates were maintained in an incubator at 25 °C (Figure 4).

Figure 4: Measurement and data collection

Distilled water was used as the negative control, and the fungicide at a concentration of 1 g in 500 mL of water was used as the positive control.[44]

### Data Analysis Method

In this study, the obtained data were analyzed using SAS 9.2 for Windows. The experiment was designed as a completely randomized design, and a one-way analysis of variance (ANOVA) was conducted. Mean comparisons were performed using Duncan’s multiple range test. Subsequently, tables and charts were generated using Microsoft Excel in a Windows environment (Table 1; Figure5).

Table 1: Analysis of variance for the effect of different plant extracts on Alternaria colony diameter

Source of Variation	Degrees of Freedom (DF)	Sum of Squares (SS)	Mean Square (MS)	F-Value (F)
Treatment	10	377.12	37.71	93.67**
Error	55	22.14	0.40	
Total	65	399.26		

\*\*Significant at the 1% probability level

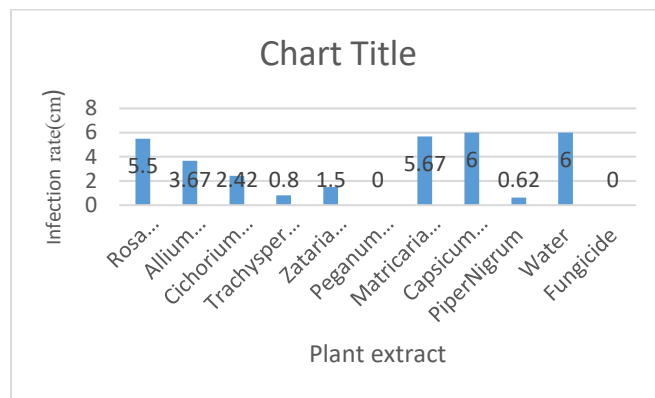


Figure5: Comparison of the mean effects of different plant extracts on the control of Alternaria infection

### Results

Evaluation of colony diameter growth in treatments containing plant extracts compared to the water control indicated that chamomile, red pepper, and rose extracts did not differ significantly from the control, suggesting that these treatments had no inhibitory effect on Alternaria colony growth. In contrast, treatments with ajwain, garlic, Peganum harmala, black pepper, chicory, and thyme extracts showed significant reductions in colony growth compared to the water control, indicating their potential to inhibit Alternaria growth.

Comparisons with the fungicide control revealed that chamomile, garlic, chicory, thyme, red pepper, and rose extracts were significantly less effective than the chemical treatment, demonstrating that the fungicide remains a more efficient option for controlling Alternaria. Among the plant extracts, ajwain, Peganum harmala, and black pepper showed inhibitory effects comparable to the fungicide, with Peganum harmala exhibiting performance statistically equivalent to the chemical control, indicating its high potential as a botanical alternative.

## Discussion and Conclusion

In this study, *Peganum harmala* (espond) extract demonstrated a significant inhibitory effect on *Alternaria* growth, showing performance comparable to the chemical fungicide. Consistent with previous research, rose and chamomile extracts were ineffective in controlling *Alternaria* and *A. solani*. [45] Among the tested extracts, espond, black pepper, and ajwain exhibited the strongest antifungal activity against *Alternaria*. [46]

The antifungal effect of espond aligns with prior studies reporting its inhibitory activity against various fungi, including *Aspergillus niger*, *Candida* species, and *Fusarium oxysporum*. [47,48] The plant's bioactive alkaloids contribute to its antimicrobial properties, including beta-carboline, which has demonstrated significant antibacterial and antifungal effects. [49,50,51] These findings support espond extract as a promising botanical alternative to chemical fungicides for controlling *Alternaria*.

Given that this research was conducted under laboratory conditions, future studies should evaluate the effects of aqueous extracts of espond, ajwain, and black pepper at varying concentrations on different pathogenic fungi. Additionally, besides assessing colony diameter reduction, future research should examine spore inhibition. Combined treatments could also enhance control efficacy to below economic damage thresholds. Finally, *in vivo* studies are recommended to validate the extract's effectiveness under more realistic, field-like conditions.

## References

1- El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, et al. Medicinal plants: Bioactive compounds, biological activities, combating multidrug-

resistant microorganisms, and human health benefits - A comprehensive review. *Front Immunol.* 2025;16:1491777.

2- Lotfi A, Milani S, Jahanbakhshian Z. Introduction to the applications of biotechnology. Tehran: Danesh Bonyan Fanavar; 1396.

3- Kumar A. Plant-Derived Antifungal Agents: Challenges at the Level of Clinical Trials. *Nat Prod Commun.* 2025.

4- Jiang CJ, Sun Y, Xu S, Liu X, Xie X. The potential of medicinal plant waste extracts for eco-friendly crop protection: A review. *Front Sustain Food Syst.* 2025;9:1556604.

5- Zhao X, Jiang Y, Qiao M, Lin F, Miao B. Recent advances in bioactive compounds, health functions, and utilization of rose (*Rosa* spp.). *Molecules.* 2025;30(19):3869.

6- Verma T, Aggarwal A, Dey P, Chauhan AK, Rashid S, Chen KT, Sharma R. Medicinal and therapeutic properties of garlic, garlic essential oil, and garlic-based snack food: An updated review. *Front Nutr.* 2023;10:1120377.

7- Duda Ł, Kłosiński KK, Budryn G, Jaśkiewicz A, Kołat D, Kałuzińska-Kołat Ż, et al. Medicinal use of chicory (*Cichorium intybus* L.). *Scientia Pharm.* 2024;92(2):31.

8- Hedge IC, Lamond JM. *Trachyspermum*. *Flora Iranica.* 1987;162:396–398.

9- Singh G, Maurya S, de Lampasona MP, Catalan C. Chemical constituents, antifungal and antioxidative effects of ajwain essential oil and its acetone extract. *J Agric Food Chem.* 2004;52(11):3292-3296.

10- Shankaracharya NB, Nagalakshmi S, Naik JP, Rao IJM. Studies on chemical and technological aspects of Ajowan (*Trachyspermum ammi* Syn *Carum copticum*) seeds. *J Food Sci Technol.* 2000;37(3):277–281.

11- Siddiquie F, Ahsan F, Mahmood T, Ahmad MA, Singh A, Bano S. Unlocking the food treasures: *Trachyspermum ammi* – A comprehensive exploration from field to pharmacology. *Food Saf Health.* 2024;2(2):106–121.

12- Moradinezhad F, Hedayati S, Ansarifard E. Assessment of *Zataria multiflora* essential oil-incorporated electrospun polyvinyl alcohol fiber mat as active packaging. *Polymers.* 2023;15(4):1048.

13- Moloudizargari M, Mikaili P, Aghajanshakeri S, Asghari MH, Shayegh J. Pharmacological and therapeutic effects of *Peganum harmala* and its main alkaloids. *Pharmacogn Rev.* 2013;7(14):199–212.

- 14- Sharifi-Rad M, Per PP, Martins N, Behl T, Quispe C, Imran M, et al. Chamomile (*Matricaria chamomilla* L.): A review of ethnomedicinal use, phytochemistry and pharmacological uses. *Genes*. 2022;13(4):684.
- 15- Di Salle A, Esposito A, Cerulli A, Masullo M, Festa A, Palumbo A, et al. Exploring the metabolome and antimicrobial properties of *Capsicum annuum* L. (Baklouti and Paprika) dried powders from Tunisia. *Molecules*. 2024;29(22):5236.
- 16- Stojanović-Radić Z, Vukić M, Branković J, Vuković NL, Galovičová L, Čmiková N, et al. The antimicrobial and antibiofilm potential of the *Piper nigrum* L. essential oil: in vitro, in situ, and in silico study. *Ind Crops Prod*. 2024;208:118919.
- 17- Woudenberg JHC, Seidl MF, Groenewald JZ, de Vries M, Stielow JB, Thomma BPHJ, Crous PW. *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes? *Stud Mycol*. 2015;82:1–21
- 18- Schmey J, Vandecasteele C, De Saeger S. *Alternaria* diseases on potato and tomato. *Mol Plant Pathol*. 2024;25(4):e13435.
- 19- Heimpel GE, Mills NJ. *Biological control: Ecology and applications*. Wiley-Blackwell; 2017.
- 20- Riyaz M, Mathew P, Zuber SM, Rather GA. Botanical pesticides for an eco-friendly and sustainable agriculture: New challenges and prospects. In: Bandh SA, editor. *Sustainable agriculture*. Springer; 2022. p. 107–125.
- 21- Collinge DB, Jensen DF, Rabiey M, Sarrocco S, Shaw MW, Jørgensen HJL. Biological control of plant diseases – What has been achieved and what is the direction? *Plant Pathol*. 2022;73(S1):3–23.
- 22- Homae A, Moslehi A, Asili J. Antifungal activity of some Lamiaceae plants' aqueous extracts against *Alternaria solani*, the causal agent of tomato early blight. *J Plant Prot Res*. 2019;59(3):285–292.
- 23- Sharma R, Yadav N. Antifungal properties and inhibitory effects upon aflatoxin production of *Thymus vulgaris* L. by *Aspergillus flavus* Link. *Food Chem*. 2015;168:208–213.
- 24- Khosravi AR, Minoocianhaghighi MH, Shokri H. Chemical composition and antifungal activity of *Thymus vulgaris* essential oil against some pathogenic fungi. *J Med Plants*. 2011;10(38):131–139.
- 25- Sharma RK, Singh R. Antifungal activity of garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) extracts against *Penicillium digitatum*, the causal agent of green mold in citrus fruits. *J Plant Pathol Microbiol*. 2015;6(5):1–6.
- 26- Goussous SJ, El-Samraa FM, Tahhan RA. Antifungal activity of several medicinal plant extracts against the early blight pathogen (*Alternaria solani*). *Arch Phytopathol Plant Prot*. 2010;43(17):1745–1757.
- 27- Bajpai VK, Baek KH, Kang SC. Control of *Alternaria alternata* by solvent extracts of plant origin. *Int J Food Saf*. 2012;14:89–97.
- 28- Santiago WD, de Oliveira MF, de Souza MA. Antifungal activity of garlic (*Allium sativum*) aqueous extract against *Aspergillus* and *Alternaria* species isolated from wheat flour. *Food Control*. 2019;105:106–112.
- 29- Khamoushi M, Khani MR, Ghasemi Pirbalouti A. Comparing the effects of red, black and bell pepper extracts on aflatoxin production, peroxide value and sensory properties in pistachio. *Iranian J Food Sci Technol*. 2021;18(115):163–194.
- 30- Ebrahimi F, Rohanii K, Jamalou F. Comparison of *Cichorium intybus* L. and *Matricaria chamomilla* L. hydroalcoholic extracts with nystatin on isolated *Candida albicans* from vaginal infection in vitro. *J Ilam Univ Med Sci*. 2018;25(6):63–72.
- 31- Das S, Horváth B, Šafranko S, Jokić S, Széchenyi A, Kőszegi T. Antimicrobial activity of chamomile essential oil: Effect of different formulations. *Molecules*. 2019;24(23):4321.
- 32- Höferl M, Nostro A, Paparella A, Krutmann J, Schmidt M, Anz D, et al. Fluconazole-resistant and susceptible *Candida albicans* strains from HIV-positive patients with oropharyngeal candidiasis are inhibited by chamomile oil. *Antibiotics*. 2021;10(12):1473.
- 33- Shaikh T, Rub RA, Sasikumar S. Antimicrobial screening of *Cichorium intybus* seed extracts. *Arab J Chem*. 2012;5(4):425–430.
- 34- Rahman H, Khan UA, Qasim M, Muhammad N, Khan MD, Asif M, et al. Ethnomedicinal *Cichorium intybus* seed extracts: An impending preparation against multidrug resistant bacterial pathogens. *Jundishapur J Microbiol*. 2016;9(11):e35436.
- 35- Ebrahim Nataj N, Rezaei D, Ansari S, Amirian Chayjan K, Sepidarkish M, Jafarzadeh J. Investigation of the antifungal effects of alcoholic extracts of *Achillea* and *Trachyspermum ammi* on *Candida albicans* isolated from denture stomatitis. 1401.

- 36- Shokri H, Sharifzadeh A, Khosravi AR. Antifungal activity of the *Trachyspermum ammi* essential oil on some of the most common fungal pathogens in animals. *Iranian J Vet Med*. 2016;10(3):173–180.
- 37- Attaee Bojd MS, Hanafi Bojd R, Ghanad Kafi M, Namaei MH. Comparison of antimicrobial effects of industrial rose water and aqueous and oil extracts of rose (*Rosa damascena*). *J Birjand Univ Med Sci*. 2014;21(4):445–456.
- 38- Yazdani R, Pourmirzaei P, Hosseini SJ, Ghaffari SMM. Effect of methanolic extract of *Peganum harmala* L. on in vitro growth of *Fusarium graminearum* and *Fusarium culmorum*, the causal agents of wheat head blight. *J Plant Dis*. 2014;50(2):169–181.
- 39- Nefzi A, Abdallah RB, Jabnoun-Khiareddine H, Saidana-Medimagh S, Haouala R, Daami-Remadi M. Antifungal activity of aqueous and organic extracts from *Withania somnifera* L. on two *Fusarium* spp. infecting watermelon and melon in Tunisia. *J New Sci Agric Biotechnol*. 2016;27(5):1493–1503.
- 40- Diba K, Hosseinpour L, Sharbatkhori M, Geramishoar M. Antifungal activity of alcoholic extract of *Peganum harmala* in vitro. *J Urmia Univ Med Sci*. 2010;20(4):271–276.
- 41- Mthembu SX, Ncube B, Ndhlala AR. Extraction and processing of bioactive phytoconstituents from widely used South African medicinal plants for the preparation of effective traditional herbal medicine products: A narrative review. 2025.
- 42- Pourebad M, Rezaei M, Naghipour F, Jafari A. Antimicrobial activity of aqueous and ethanolic extracts of native Iranian medicinal plants against some human pathogenic bacteria. *J Biol Agric Sci*. 2024;12(3):45–58.
- 43- Karimi K, Tahmasbi S, Rahimian H, Karami M. Antifungal effects of some plant extracts on phytopathogenic fungi. *J Plant Prot*. 2015;29(4):481–492.
- 44- Rezaei S, Mousavi SM, Karimi K. Antifungal activity of aqueous extracts of several medicinal plants (including thyme, chamomile, garlic, chicory, ajwain, black pepper, red pepper, *Peganum harmala*, and *Rosa damascena*) against *Alternaria alternata* in vitro. *Iranian J Plant Pathol*. 2016;8(2):185–198.
- 45- Karimi K, Tahmasbi S, Rezaei S. Comparison of antifungal effects of aqueous extracts of *Peganum harmala*, *Rosa damascena*, and *Matricaria chamomilla* on *Alternaria alternata* in vitro. *J Plant Prot*. 2023;37(1):73–85.
- 46- Alwadani KS, Abo-Elyousr KAM, Sallam NMA. Antifungal activity of some plant extracts against tomato early blight pathogen (*Alternaria solani*). *J Agric Sci Technol B*. 2014;4(8B):1–10.
- 47- Diba K, Geramishoar M, Sharbatkhori M, Hosseinpour L. Anti-fungal activity of alcoholic extract of *Peganum harmala* seeds. *J Med Plant Res*. 2011;5(23):5550–5554.
- 48- Abdollah RB, Jabnoun-Khiareddine H, Nefzi A, Mokni-Tlili S, Daami-Remadi M. Evidence of antifungal activity of wild rue (*Peganum harmala* L.) on phytopathogenic fungi. *J Plant Dis Prot*. 2016;123(3):131–139.
- 49- Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria and fungi: A review on mode of action and antifungal activity. *Pharmaceuticals*. 2013;6(12):1451–1474.
- 50- Arshad N, Zitterl-Eglseer K, Hasnain S, Hess M. Effect of *Peganum harmala* or its  $\beta$ -carboline alkaloids on certain antibiotic-resistant strains of bacteria and protozoa from poultry. *Phytother Res*. 2008;22(11):1533–1538.
- 51- Saad A, Fadli M, Bouaziz M, Benharref A, Mezrioui NE, Hassani L. Antibacterial activity of *Peganum harmala* seed and root extracts against some multidrug-resistant bacteria. *Afr J Microbiol Res*. 2012;6(19).

