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The effect of root extracts of the *Peganum harmala* L. plant on inhibiting the growth of the fungus *Rhizoctonia solani* Kuhn: An In Vitro Study

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Abstract

This study aimed to evaluate the inhibitory and lethal effects of root extracts of the Wild Rue plant (*Peganum harmala* L.) against the pathogenic fungus *Rhizoctonia solani* under laboratory conditions. Two types of extracts (aqueous and alcoholic) were used at concentrations of (5%, 10%, 15%, and 20%). The results indicated that the effectiveness increased proportionally with the concentration. The inhibition rate of the aqueous extract reached 64.8% at a concentration of 20%, while the alcoholic extract was significantly superior, reaching 84% at the same concentration. Statistical analysis confirmed highly significant differences between concentrations ($P < 0.001$) as determined by one-way ANOVA, while two-way ANOVA showed extract type, concentration, and their interaction were significant factors, explaining approximately 85% of the total variance. The study concluded that the alcoholic extract is more effective than the aqueous one in inhibiting *R. solani*, making it a promising option for fungal control as a safe alternative to chemical pesticides.

Keywords: Alcoholic extracts, Chemical pesticides, Dimethyl sulfoxide, *Rhizoctonia solani, Peganum harmala*.

Introduction:

Plant extracts are important natural sources of bioactive compounds with diverse biological and therapeutic properties. Interest in these extracts has increased in recent decades due to their antimicrobial potential, particularly against fungi that cause plant diseases [1]. With growing concerns over chemical fungicides, including resistance development, environmental contamination, and risks to human health, plant-derived compounds are increasingly considered sustainable alternatives for disease management. Fungi comprise a diverse group of microorganisms, many of which are pathogenic and responsible for significant economic and health-related losses [2]. Plant-pathogenic fungi cause major agricultural diseases, including late blight (*Phytophthora infestans*), vascular wilts (*Fusarium* spp.), leaf spots (*Alternaria* spp.), and *Verticillium* wilt [3]. In addition to field losses, fungi contribute to post-harvest deterioration [4] and produce mycotoxins such as aflatoxins, which are highly carcinogenic and contaminate food supplies worldwide [5]. It is estimated that approximately 25% of global crops are affected by mycotoxins [6]. Among soil-borne pathogens, *Rhizoctonia solani* is particularly important due to its wide distribution, broad host range, and ability to cause diseases such as root rot, stem canker, and damping-off [7]. Its persistence in soil and survival through sclerotia make it difficult to control using conventional methods, highlighting the need for environmentally safe management strategies.

Peganum harmala L. is a medicinal plant widely used in traditional medicine and known to contain bioactive alkaloids such as harmine, harmaline, and harmalol, which exhibit antimicrobial and antifungal properties [8]. These compounds are believed to inhibit fungal growth by disrupting cell membrane integrity, interfering with enzymatic activity, and affecting nucleic acid synthesis. Previous studies have demonstrated the antifungal potential of *P. harmala* and other plant extracts against fungi such as *Fusarium*, *Aspergillus*, and *Alternaria*, although their effectiveness varies depending on extraction methods and concentrations. However, studies specifically evaluating their activity against *Rhizoctonia solani* remain limited.

Therefore, this study aimed to extract bioactive compounds from *Peganum harmala* roots and evaluate their antifungal activity against *Rhizoctonia solani* at different concentrations. The findings are expected to contribute to the development of safe and sustainable alternatives for controlling plant pathogenic fungi.

Materials and Methods:

Peganum harmala roots were collected from the Al-Qadhama region (south of Gharyan, Libya) in May 2024. They were washed with distilled water twice, air-dried in the shade for two weeks, and ground into a dry powder stored at 4°C. The pathogenic fungus *Rhizoctonia solani* was isolated from potato tubers infected with black scurf disease, sterilized using sodium hypochlorite, and cultured on PDA (Potato Dextrose Agar) to obtain a pure isolate stored at 4°C [9] (Figures 1 and 2).

The aqueous extract was prepared by mixing 20g of powder with 500ml of distilled water, shaken for 48 hours and then left to stand for 24 hours at ambient temperature. The solution was filtered and centrifuged to obtain a clear, pure solution [10]. The alcoholic extract was prepared by mixing 20g of powder with 80ml of 95% methanol, kept for 72 hours, filtered, and evaporated in a rotary evaporator at 40°C [11]. The active substance was dissolved in 10% DMSO (Dimethyl sulfoxide) to prepare the concentrations used [12]. PDA medium was prepared according to standard procedures, and the extract was mixed with the medium before solidification at concentrations of (5%, 10%,15%,20%). Only these low concentrations were chosen to avoid affecting the solidity of the nutrient medium. Plates were incubated at (25–28°C) for 5–7 days for five replicates per concentration, and fungal inhibition was evaluated by measuring colony diameters compared to control plates [13].The Minimum Inhibitory Concentration (MIC) was tested using serial dilution in PDB liquid in a 96-well microtitre plate [14]. The Minimum Fungicidal Concentration (MFC) was determined by re-culturing tubes with no growth on PDA and monitoring growth after 48–72 hours [15].

Results:

The study results showed varying effectiveness of the aqueous and alcoholic extracts of *P.harmala* roots against *R.solani*, where the percentage of fungal growth inhibition increased with the extract concentration.

The aqueous extract showed relatively weak effects at low concentrations (5% -10%), with inhibition rates ranging between 4.22% and 6%. The 20% concentration recorded the highest effectiveness with an inhibition rate of 64.8% and an average colony diameter of 3.16 cm compared to the control (9cm) (Figure2) & (Table1). The alcoholic extract clearly outperformed the aqueous extract. At a 5% concentration, the inhibition rate was 27%, while concentrations of 10%, 15%, and 20% recorded inhibition rates of 60%, 80.6%, and 84%, respectively, with a significant decrease in colony diameter to 1.44 cm at the 20% concentration. Results showed the alcoholic extract had an MIC of 12.5 mg/ml and an MFC at 25 mg/ml. In contrast, the aqueous extract recorded an MIC of 25 mg/ml and an MFC at 100 mg/ml, indicating the alcoholic extract is more efficient in inhibiting and killing the fungus (Figure 3) & (Figure 4).

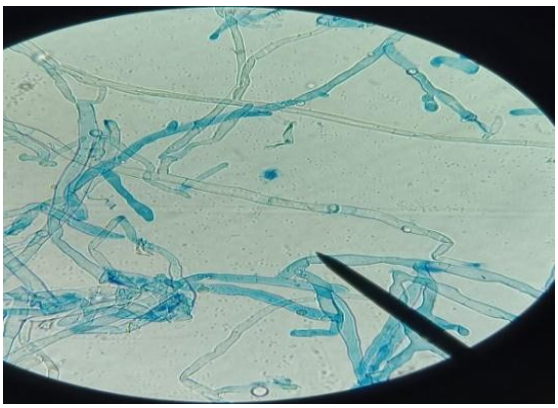

Fig 1: Microscopic image of *R. solani*

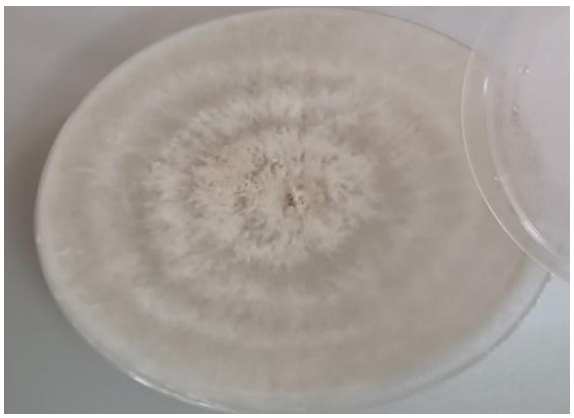

Fig 2: Pure culture of *R. solani*

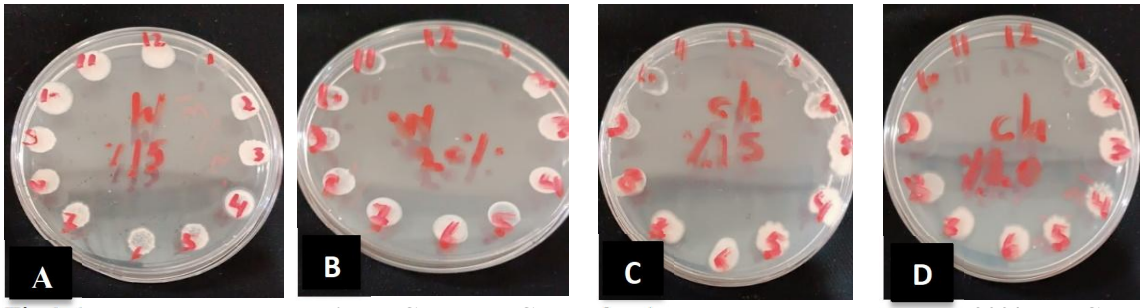

Fig 3: Fungal growth results in MIC and MFC tests for A: aqueous extracts 15%, B: 20% and C: alcoholic extracts 15%, D: 20%

Table 1: Shows the average lengths of diameters of fungal colonies in centimeters at different concentrations of the aqueous extract and the percentages of inhibition of fungal growth.

Concentration	Plate number					Mean	Inhibition%
	1	2	3	4	5		
5%	9	8.9	8	8.5	8.7	8.62	4.22 %
10%	8.3	8.7	8.4	8.5	8.4	8.46	6%
15%	8	8.4	8.9	8.5	7.9	8.34	7.33%
20%	2.9	3.5	3.1	3.3	3.0	3.16	64.8%
Control	9	9	9	9	9	9	0

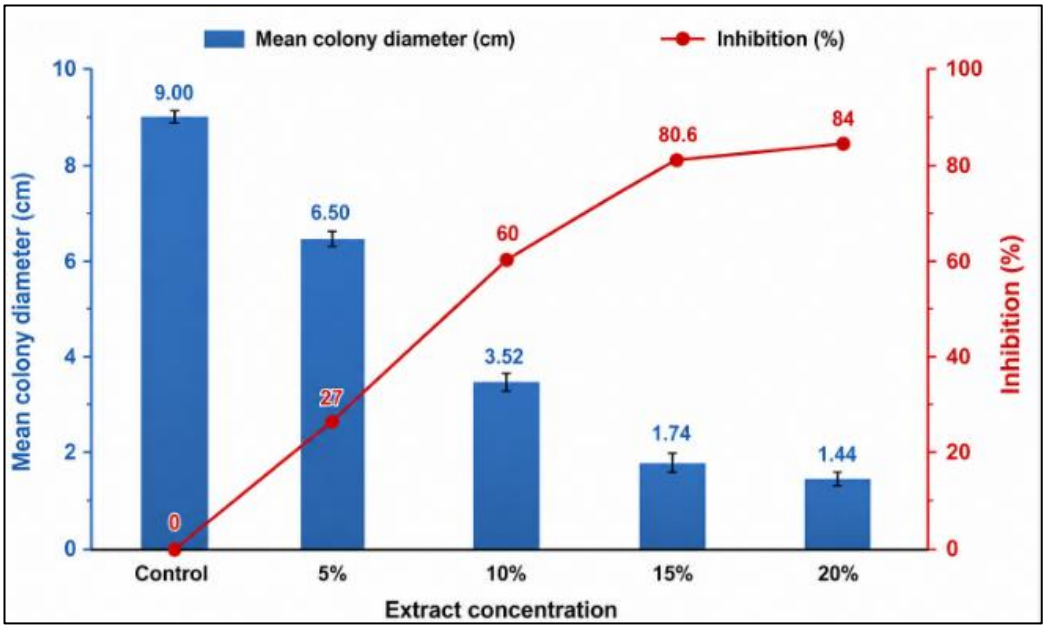

Fig4. Shows the average lengths of diameters of fungal colonies in centimeters at different concentrations of the alcoholic extract and the percentages of inhibition of fungal growth.

Discussion:

The results of the present study demonstrated that both aqueous and alcoholic extracts of *Peganum harmala* exhibited inhibitory effects against *Rhizoctonia solani*, with a clear superiority of the alcoholic extract. This finding is consistent with previous studies reporting the effectiveness of plant-derived extracts against phytopathogenic fungi.Several investigations have shown that extracts from medicinal plants possess strong antifungal activity against species such as

Fusarium, *Aspergillus*, *Alternaria*, and *Rhizoctonia,* for example, Zhao *et al*. (2022)[16] reported complete (100%) inhibition of *R. solani* using *Moutan Cortex* extracts, while recent studies (2020–2025) have highlighted the antifungal potential of various plant extracts as eco-friendly alternatives to synthetic fungicides. Similarly,Vimalaveera *et al*. (2025)[17], demonstrated low minimum inhibitory concentrations (MICs) for several medicinal plant extracts, supporting their strong antifungal efficacy.

The superior performance of the alcoholic extract observed in this study can be attributed to the higher efficiency of organic solvents in extracting bioactive compounds, particularly alkaloids and flavonoids. *Peganum harmala* is known to contain β-carboline alkaloids such as harmine and harmaline, which possess potent antifungal properties. These compounds are reported to disrupt fungal cell wall and membrane integrity, interfere with nucleic acid synthesis [18], and inhibit key enzymatic processes essential for fungal growth. In addition, flavonoids and phenolic compounds may induce oxidative stress in fungal cells through the generation of reactive oxygen species (ROS), leading to cellular damage and growth inhibition. Therefore, the enhanced inhibitory effect of the alcoholic extract may be explained by its higher content and better solubility of these active constituents compared to aqueous extracts.

The observed increase in antifungal activity with higher concentrations (15% and 20%) indicates a dose-dependent relationship between extract concentration and fungal inhibition. This trend agrees with earlier findings, such as those reported by Sarpeleh *et a*l. (2009) [19], and is further supported by recent studies (2020–2024) demonstrating that increasing concentrations of plant extracts result in greater antifungal effects due to higher availability of active compounds. Environmental factors may also influence the effectiveness of plant extracts. Parameters such as temperature, humidity, and pH can affect both fungal growth and the stability of bioactive compounds. High humidity generally promotes fungal proliferation, which may reduce the relative effectiveness of antifungal agents, whereas elevated temperatures may enhance or reduce extract activity depending on compound stability. In arid environments, such as the study area, high temperatures and low moisture levels may naturally limit fungal growth, potentially enhancing the apparent effectiveness of plant extracts. However, under field conditions, fluctuations in environmental factors could influence the persistence, degradation, and overall performance of these extracts. Therefore, further studies are required to evaluate their stability and efficacy under different environmental conditions. The findings of this study have important implications for sustainable agriculture. The demonstrated antifungal activity of *Peganum harmala* extracts suggests their potential use as natural biopesticides for controlling soil-borne pathogens such as *Rhizoctonia solani*. Plant-based antifungal agents are biodegradable, environmentally friendly, and pose lower risks to human health compared to synthetic fungicides. Recent studies (2020–2025) emphasize the increasing role of plant extracts in integrated pest management (IPM) systems[20], organic farming, and the development of green pesticides. These extracts could be applied as seed treatments, soil amendments, or foliar sprays to reduce fungal infections and improve crop productivity. Furthermore, the multi-target mode of action of plant-derived compounds reduces the likelihood of resistance development in pathogens [21].

Statistical analysis (ANOVA, $p \leq 0.05$) confirmed significant differences between extract types and concentrations, with post-hoc tests indicating that the alcoholic extract achieved the highest average inhibition. This further supports the conclusion that extraction method plays a critical role in determining antifungal efficacy.

Conclusions:

The study demonstrated that aqueous and methanolic extracts of *P. harmala* roots at various concentrations (5%-20%) have inhibitory and lethal effects on *R. solani* in vitro, with a positive correlation between concentration and inhibition. The alcoholic extract proved to be more potent. These findings highlight the potential of *P. harmala* root extracts as a natural antifungal agent and support further investigation for agricultural applications. The study recommends further experiments under greenhouse and open field conditions, along with chemical analysis of the active compounds to identify the components responsible for this effect.

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