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Research Article

IN VITRO ANTIFUNGAL ACTIVITY OF *CAPPARIS SPINOSA* EXTRACT AGAINST SOIL-BORNE PLANT PATHOGENS

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ABSTRACT

Soil-borne pathogenic fungi are a significant concern in agriculture, as they greatly reduce crop yield and performance while increasing production costs. In many parts of the world, chemical control has been widely used to manage these pathogens. However, the high cost of fungicides, the development of fungicide resistance, climate change, and growing concerns about their environmental and health impacts on humans and animals have necessitated the search for alternative solutions. The present study, therefore, aimed to evaluate the antifungal activity of alcoholic leaf extract of *Capparis spinosa* against various soil-borne fungi *in vitro*, including *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium graminearum*, and *Alternaria alternata*. The results revealed that all tested concentrations (250, 500, and 750 mg/100 ml) exhibited varying degrees of antifungal activity. The strongest antifungal effect was observed against *F. graminearum* at all concentrations, with inhibition indices of 50.64%, 70.07%, and 80.73%, respectively. In contrast, the lowest antifungal activity was recorded against *M. phaseolina*, with inhibition rates of 48.90%, 59.42%, and 63.56%, respectively. These findings suggest that *C. spinosa* extract could serve as an effective natural or biological control agent against fungal pathogens. Further extensive studies are recommended to validate its efficacy under greenhouse and field conditions for the management of soil-borne fungi.

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INTRODUCTION

Soil-borne pathogenic fungi are a major cause of significant yield losses in agriculture across the globe. From ancient times, agriculture has been challenged by the destructive activities of pests and pathogens (Bastakoti et al., 2017). These soil-borne pathogens are responsible for severe plant diseases such as root rots, damping-off, and wilts, which directly hinder plant growth (Janvier et al., 2007; Saba et al., 2022) and reduce the efficiency of water and nutrient uptake (Baligar et al., 2001; Nisa et al., 2022; Azeem et al., 2024). As a result, soil-borne pathogens pose a serious threat to crops grown in both open fields and greenhouse environments.

Among soil-borne pathogens, *Macrophomina phaseolina*, *Alternaria alternata*, *Fusarium graminearum*, and *Rhizoctonia solani* are particularly noteworthy, posing a major threat to global agricultural productivity due to their widespread distribution, broad host range, and ability to survive in soil for extended periods (Iqbal and Mukhtar, 2020a; Bibi et al., 2023; Zeeshan et al, 2023).

These pathogens are responsible for substantial economic losses by reducing crop yield, quality, and marketability (Iqbal and Mukhtar, 2014; Yaseen and Mukhtar, 2024). *M. phaseolina*, the causal agent of charcoal rot, affects over 500 plant species, including soybean, maize, and sunflower, leading to estimated yield losses of up to 50% under drought and heat stress conditions (Iqbal et al., 2014; Iqbal and Mukhtar, 2020b). *A. alternata* causes seedling blights, leaf spots, and fruit rots in numerous horticultural and field crops, affecting both pre-harvest productivity and post-harvest storage (Bibi et al., 2023). *F. graminearum*, a major cause of Fusarium head blight (FHB) in cereals such as wheat and barley, not only decreases grain yield but also contaminates the grain with mycotoxins like deoxynivalenol (DON), posing severe health risks to humans and animals and resulting in trade restrictions (Yaseen et al., 2024). *Rhizoctonia solani* causes root rot, damping-off, and stem canker in a wide range of crops including rice, beans, potatoes, and sugar beet, particularly in intensive farming systems. The costs associated with disease management, reduced productivity, and post-harvest losses due to these pathogens amount to billions of dollars annually on a global scale. In addition, their persistence in soil and resistance to chemical treatments complicate control strategies, making them a critical concern for sustainable crop production and food security (Shahzaman et al., 2015).

Currently, chemical fungicides are widely used to control these diseases. However, their continuous use raises serious concerns due to their harmful effects on the environment and potential risks to human health. Consequently, there is an urgent need to develop eco-friendly and sustainable alternatives for managing soil-borne plant diseases (Lal et al., 2022).

In recent years, natural plant-based extracts, particularly those derived from wild shrubs and medicinal plants, have gained attention as potential alternatives to chemical fungicides. Several plant extracts have demonstrated promising antifungal properties. For example, extracts from *Capparis spinosa* and *Ocimum basilicum* have shown effective antifungal activity (Al-Askar, 2012; Hussain et al., 2024). *C. spinosa*, commonly known as the caper plant, is widely recognized as a rich source of bioactive compounds, including phenolic compounds and flavonoids (Koufan et al., 2022; Eid et al., 2023). These bioactive substances exhibit significant therapeutic potential against various fungal and bacterial pathogens (Alrayes et al., 2018; Shatnawi et al., 2021).

Furthermore, research by Annaz et al. (2022) and Shahrour et al. (2024) has confirmed the antifungal, antibacterial, and even insecticidal properties of *C. spinosa* extracts. Al-Askar (2012) demonstrated that an extract of caper roots significantly inhibited the growth of *R. solani*, *F. oxysporum*, and *Sclerotium rolfsii*, reducing their colony diameters by 5.2 cm, 6.6 cm, and 3.3 cm, respectively, compared to 9.0 cm in the untreated control.

The objective of the present study is to reduce the reliance on synthetic fungicides and their associated environmental and health hazards by exploring natural alternatives. Specifically, this study aims to evaluate the antifungal activity of alcoholic leaf extracts of *C. spinosa* under *in vitro* conditions against soil-borne pathogenic fungi viz. *M. phaseolina*, *A. alternata*, *F. graminearum*, and *R. solani*.

MAERIALS AND METHODS

Soil samples

A total of 45 soil samples were collected from selected sites in Al Alam in October 2023. Samples were taken at a depth of 5 to 15 cm and stored in plastic bags. They were then promptly transported to the laboratory (AL-Hamandi et al., 2025).

Isolation of soil-borne pathogens

The serial dilution method was used to reduce the concentration of the collected samples. After homogenization, 10 g of each sample was mixed with 100 ml of distilled water. Each sample was serially diluted from 10^1 to 10^8. Subsequently, 1 ml from each dilution was transferred to Petri dishes containing potato dextrose agar (PDA). The dishes were incubated at 27°C for 5 to 8 days. Fungal growth was examined daily, and emerging colonies were transferred to fresh Petri dishes for further incubation at 27°C for one week.

Identification of soil-borne pathogens

Fungal isolates were cultured on PDA, and small portions of the colonies were placed on microscope slides containing 1-3 drops of distilled water. These slides were incubated for eight days at 27°C prior to microscopic examination. Observations were made using an optical microscope at 400× magnification, focusing on key morphological features such as microsclerotia, spore shape, and conidia structure.

Caper plant samples

Caper plants were collected from various locations in the Al Alam region of Salah Al-Din Governorate, Iraq, in

September 2023. The plants were randomly sampled and subsequently identified using molecular techniques (Figure 1).

Figure 1. Caper plant species.

Molecular diagnosis

DNA extraction from caper plant

Genomic DNA was extracted from the caper plant using the Wizard® Genomic DNA Purification Kit (Promega Corporation, USA).

DNA extraction from soil-borne pathogens

DNA from soil-borne pathogens was extracted using the Chelex® 100 rapid extraction method as described by Ding et al. (2007).

PCR amplification

Fungal and plant species were identified using the Polymerase Chain Reaction (PCR) technique with universal primers. The forward primer ITS1-F (TCCGTAGGTGAACCTGCGG) and the reverse primer ITS4-R (TCCTCCGCTTATTGATATGC) were used, following the protocol of White et al. (1990). Each PCR reaction had a final volume of 25 µl, containing 5 µl of DNA template, 4 µl of PreMix, 1 µl of each primer, and 14 µl of distilled water. The PCR conditions were as follows: initial denaturation at 90°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 58°C for 1 min, and extension at 72°C for 50 sec. A final extension was carried out at 72°C for 10 min. The amplified products were analyzed by electrophoresis on a 1.5% agarose gel stained with Safe Rad (Ahmed et al., 2023).

DNA sequencing

The PCR product was used as the template for DNA sequencing. Sequencing was performed using a 3130xl Genetic Analyzer (Applied Biosystems, USA) according to the manufacturer's instructions. The BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) was used for the sequencing reaction. The purified PCR product was prepared accordingly for sequencing.

Preparation of plant extract

Leaves of the caper plant were collected, washed, and shade-dried at 25°C for two weeks. The dried leaves were then chopped into small pieces and further air-dried in an oven at 28°C for five days (Tegelberg et al., 2018; Hama et al., 2024). The dried material was ground into a fine powder using a laboratory mixer and stored in polyethylene bags for further use. Eight grams each of the dried buds, fruits, and leaves of the caper plant were separately immersed in 100 ml of ethanol (8% w/v) and left to stand for 72 h for extraction. The extracts were then filtered using Whatman No. 1 filter paper, following the method described by Mann et al. (2008).

Antifungal activity

The antifungal activity of the caper plant extract was assessed using the growth inhibition assay method described by Shahrour et al. (2024) and Guo et al. (2007). The diameters of the largest and smallest fungal colonies were measured, and the average values were calculated.

The antifungal activity, expressed as the percentage inhibition of mycelial growth (Antifungal Index), was calculated for each treatment in triplicate. Growth inhibition was measured in millimeters at the end of the incubation period, once the fungal mycelium reached the edges of the control plates.

The Antifungal Index (%) was calculated using the following formula:

$$\text{Antifungal Index (\%)} = \frac{100 \times (W - A)}{W}$$

Where:

W = Average diameter of the largest and smallest colonies in the control group.

A = Average diameter of the largest and smallest colonies in the treatment group.

Statistical analysis

The study was conducted *in vitro* using a completely randomized design. Data were analyzed using analysis of variance (ANOVA) in SAS software version 9.1. Treatment means were compared using the least significant difference (LSD) test at a 0.05 significance level (Alaobady and Thalj 2024).

RESULTS AND DISCUSSION

Microscopic identification

Soil analysis revealed the presence of fungi originating from different soil types. The classification of these fungi is primarily based on the morphology of their spores and hyphae. The identification of fungal genera was

accomplished through detailed microscopic examination. Morphological analysis indicated that the spores and hyphae belonged to four distinct fungal species. The results showed that 15 samples (33.3%) were identified as *Fusarium* spp., 14 samples (31%) as *Rhizoctonia* sp., 12 samples (26.6%) as *Alternaria* spp., and 4 samples (8.8%) as *M. phaseolina*, as illustrated in Figure 2.

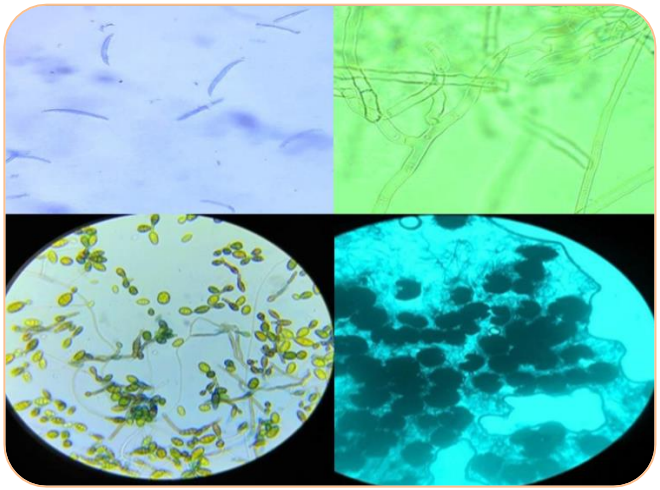

Figure 2. Fungi species A: *Fusarium* spp.; B: *Rhizoctonia* sp.; C: *Alternaria* spp.; and D: *M. phaseolina*.

Molecular diagnosis of fungi and plant

To confirm the morphological identification of the five studied fungal and plant isolates (F1, F2, F3, F4, and P1), molecular diagnosis was performed. The internal transcribed spacer (ITS) gene of the isolates was amplified (Figure 3). The amplified and purified DNA fragments of the fungal and plant isolates were sequenced in the forward direction. The sequences of the amplified ITS gene indicated that the fragments obtained from the five isolates were approximately 550 bp in length. These sequences were submitted to GenBank (NCBI) (Table 1) and showed 100% similarity with *R. solani*, *M. phaseolina*, *F. graminearum*, *A. alternata*, and *C. spinosa*.

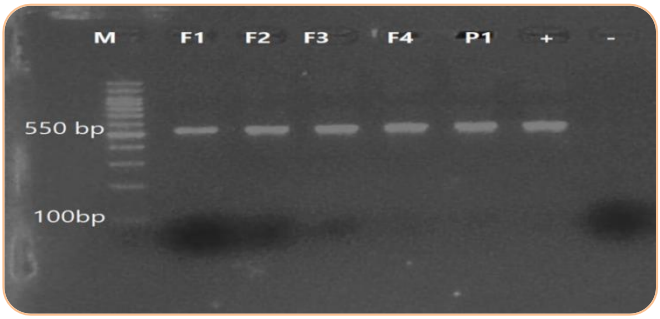

Figure 3. PCR amplification of five fungal and plant isolates using ITS1/ITS4 primers.
Lanes: M = DNA ladder; F1 = *R. solani*; F2 = *M. phaseolina*; F3 = *F. graminearum*; F4 = *A. alternata*; P1 = *C. spinosa*; + = positive control; – = negative control.

Antifungal activity

The results presented in Table 2 and Figure 4 indicate that ethanolic extracts of the caper plant exhibited strong antifungal activity against soil-borne fungi at various concentrations (250, 500, and 750 mg/100 ml). The concentration of 750 mg/100 ml was found to be the most effective against all tested fungi, *R. solani*, *M. phaseolina*, *F. graminearum*, and *A. alternate*, *in vitro*. Among these, *F. graminearum* was the most sensitive, with an antifungal index of 80.73%, followed by *R. solani* with 78%.

Table 1. Identification of soil-borne pathogens and Capparis spinosa based on phenotypic characteristics and ITS gene sequencing.

Isolates	Phenotypic identification	Diagnosis by ITS gene	NCBI Acc.No
F1	*R. solani,*	*R. solani,*	PQ097591.1
F2	*M. phaseolina,*	*M. phaseolina,*	PQ097588. 1
F3	*F. graminearum*	*F. graminearum*	PQ097589.1
F4	*A. alternata.*	*A. alternata.*	PQ097590.1
P1	*C. spinosa*	*C. spinosa*	PQ196142.1

Table 2. Antifungal activity of caper plant extract at different concentrations (mg/100 mL).

Fungal strains	Concentration (mg/100 ml)			
	Antifungal index (%)			
	0	250	500	750
R. solani,	0.0	50.28	69.81	78.00
M. phaseolina	0.0	48.90	59.42	63.56
F. graminearum	0.0	50.64	70.07	80.73
A. alternata	0.0	47.25	67.20	73.80
LSD (0.05)	0.0	3.5	2.5	2.9

Each treatment was replicated three times.

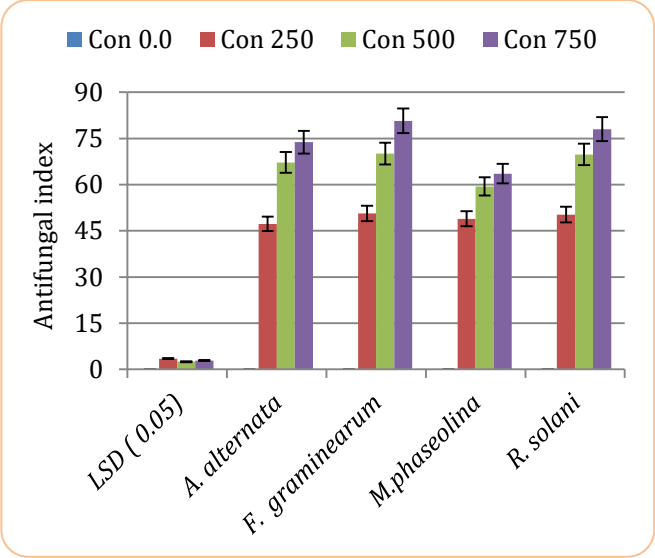

Figure 4: Antifungal activity of caper plant extract at different concentrations (mg/100 ml).

These findings are supported by previous studies such as Gaind et al. (1969), who demonstrated that various extracts from *Capparis* species exhibited biological activity against a wide range of pathogens. Compared to the control treatment (0.0 mg/100 ml), the least antifungal effect was observed against *M. phaseolina* at the same concentration, with an antifungal index of 63.56%. This result is consistent with findings reported by Al-Askar (2012), Sherif et al. (2013), Abu-Shama (2019), and Shahrour et al. (2024). Notably, the aqueous extract from the aerial parts of the plant has also demonstrated antifungal properties. Therefore, *C. spinosa* could be considered a valuable natural source of antifungal agents (Ali-Shtayeh and Abu Ghdeib, 1999).

The antifungal activity of caper plants can be attributed to their rich phytochemical composition (Rathee et al., 2010). These include alkaloids, flavonoids, polyphenols, steroids, saponins, tannins, glycosides, triterpenes, and phlobatannins, which are present in ethanolic extracts from caper flower buds, fruits, and leaves (Abu-Shama, 2019). Moreover, the presence of total phenolic compounds, rutin, tocopherols, carotenoids, and vitamin C may significantly contribute to the antimicrobial effects of the plant (Mahboubi et al., 2012), and the flavonoid content is also a crucial quality indicator for determining antifungal activity (Fatima et al., 2025).

Previous chemical analyses have also identified alkaloids, lipids, indoles, glucosinolates, polyphenols, and flavonoids in caper extracts (Tlili et al., 2010). The antifungal activity of these extracts is likely related to this complex chemical composition (Mustafa, 2011). Additional constituents reported in *C. spinosa* include β-sitosterol, vanillic acid, p-hydroxybenzoic acid, protocatechuic acid, daucosterol, uracil, butanedioic acid, and uridine (Yu et al., 2006). Biological control agents have also been shown to be effective in reducing disease severity when used against plant pathogens (Al-Jbory et al., 2025).

Developing antifungal agents based on these compounds offers a promising approach for controlling pathogenic fungi. These natural products are not only effective but also represent an environmentally friendly alternative to synthetic fungicides for managing plant diseases.

CONCLUSION

Based on the findings discussed in this study, it can be concluded that ethanolic leaf extracts of the caper plant are effective in combating soil-borne pathogens. This efficacy is likely due to the abundance of bioactive compounds present in the extract. The results suggest that caper extract could play an important role in the natural management of plant pathogens. Therefore, further in-depth research, especially involving caper-based nanoparticles, is necessary to evaluate its full potential in plant disease control.

AUTHORS' CONTRIBUTIONS

WKA designed the study and prepared the materials, collected and analyzed the data; ATM helped in disease scoring. WKA and AAA supervised the studies; WKA wrote the manuscript; All the authors proofread and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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