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Research Article

Biological Activity of Ethanolic Extract from a New Local Isolate of *Morchella rufobrunnea* against some Fungal Pathogens

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ABSTRACT

Mushrooms are increasingly recognized as valuable sources of bioactive compounds with potential applications in agriculture and medicine. Among them, *Morchella rufobrunnea* has gained attention for its antimicrobial and antifungal properties. However, reports of its occurrence and biological activity remain limited in many regions, including Iraq. The present study aimed to identify *M. rufobrunnea* isolates collected from Iraq using molecular tools and to evaluate their antifungal potential against major phytopathogenic fungi. Molecular identification was performed by sequencing the internal transcribed spacer (ITS) region of DNA. PCR amplification yielded products of approximately 550 bp, and phylogenetic analysis confirmed the specimens as *M. rufobrunnea*. Sequence alignment revealed 99% similarity with reference sequences in the NCBI database, specifically matching accession number OQ808797.1. This represents the first confirmed report of *M. rufobrunnea* in Iraq, expanding its known geographical distribution. The antifungal activity of ethanolic extracts of *M. rufobrunnea* was evaluated against *Rhizoctonia solani*, *Fusarium oxysporum*, *F. graminearum*, *Alternaria alternata*, and *Sclerotium rolfsii*. A concentration-dependent inhibitory effect was observed, with inhibition indices ranging from 24.70% to 55.03%. The highest antifungal activity was recorded at 1000 mg/100 ml, where *F. graminearum* showed maximum susceptibility (55.03%), while *R. solani* exhibited the lowest inhibition (36.93%). Statistical analysis confirmed significant differences among treatments ($LSD_{0-05} = 3.79-6.51$). These findings highlight both the taxonomic significance and the biocontrol potential of *M. rufobrunnea*. Its strong antifungal activity suggests promising applications in sustainable plant disease management.

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Introduction

Morchella rufobrunnea, commonly known as the cinnamon morel, is a relatively recently described species within the *Morchella* genus and is recognized as both a novel culinary delicacy and a valuable medicinal resource. Members of the genus *Morchella* are ecologically important macrofungi that occupy diverse

ecological niches, functioning as saprotrophs, soil-litter decomposers, and root-associated endophytes or mycorrhizal partners. To date, nearly 80 *Morchella* species with considerable morphological and genetic diversity have been documented worldwide, ranging from Arctic to subarctic ecosystems (Khaliq et al., 2025). Since *Morchella* species are among the earliest fungal

species to fructify, usually within two years of a forest fire, these fungi are well-known for their capacity to quickly colonize post-disturbance ecosystems. Foragers and the gourmet food business have long been interested in post-fire morel flushes because of their unique flavor and high market value.

Several closely related morel species, such as *M. snyderi*, *M. populiphila*, *M. esculenta*, and sympatric “snow morels,” are taxonomically and genetically different from *M. rufobrunnea*. These differences are aided by employing morphological and molecular phylogenetic studies (Frąc et al., 2018; Zhang et al., 2021).

Morel mushrooms, especially, *M. rufobrunnea*, are recognized for their excellent cooking attributes. They are also replete with plenty of biologically active secondary metabolites and compounds which possess a broad spectrum of biological activities. The important compounds and metabolites are terpenes and phenolic compounds which have robust free radical scavenging activities, and cytotoxic chemicals whose toxicity might be mitigated through the effect of polysaccharides. As morales have hepatoprotective, antimicrobial, and viricidal properties, they can also be used in pharmaceutical industries (Mustafa, 2021; Tietel and Masaphy, 2022).

Due to deleterious effects of pesticides, searching for natural and environmentally-benign alternatives for the management of plant diseases is imperative and attracting attention of scientists, policymakers and plant protection practitioners. These safer practices are also gaining popularity among the farming community throughout the globe due to serious concerns associated with the use of detrimental pesticides. Many scientific reports have confirmed the antimycotic activities of many morel species. For example, *M. esculenta* extracts have been proved to have inhibitory properties against many phytopathogenic fungi viz., *Colletotrichum*, *Alternaria alternata*, *Bipolaris sorokiniana*, *Neoscytalidium novaehollandiae*, and *N. dimidiatum* (Acay et al., 2022).

Keeping the above-mentioned in view, the current research was done to test the mycotoxic activities of ethanol extracts of mycelia of *M. rufobrunnea* against some selected phytopathogenic fungal species which have been recently recognized in the Al-Alam region of Salah al-Din Governorate, Iraq. The outcomes of these investigations will be helpful in designing control strategies which will base on biological, nonhazardous

and sustainable plant disease management approaches and help in curtailing reliance on artificially synthetic detrimental chemical fungicides.

Materials and Methods

Collection of pathogenic fungal isolates

The phytopathogenic fungi that live in soil viz., *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria alternata*, and *F. graminearum* (Al-Jbory et al., 2023) were taken from previously identified cultures that were kept in the Plant Pathology Laboratory at the College of Agriculture, Tikrit University, Iraq. Before being used in the study, these fungal phytopathogens were further cultivated on Potato Dextrose Agar (PDA) at 4°C.

Identification of *M. rufobrunnea*

M. rufobrunnea fruiting bodies that had just formed were obtained from multiple sites in the Al-Alam area, which is located in the Salah Al-Din Governorate of Iraq. These fruiting bodies were identified on the basis of morphological characteristics of the stipe, pileus, and spore morphology under a light microscope. Molecular identification was subsequently performed, marking the first report of *M. rufobrunnea* in Iraq. The confirmed isolate was deposited in the NCBI GenBank database with an assigned accession number.

Molecular identification

DNA extraction

Genomic DNA was extracted from fresh mycelia of *M. rufobrunnea* following the method described by Dong and Zhao (2006) with minor modifications. Approximately 100 µl of Tris-EDTA (TE) buffer was added to crushed hyphae. The mixture was supplemented with 200 µl of Chelex-100 resin, heated at 95°C for 10 min, and then centrifuged. The supernatant containing genomic DNA was collected and stored at -20°C until use.

PCR amplification

The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using the universal primers ITS1 (forward: 5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (reverse: 5'-TCCTCCGCTTATTGATATGC-3'). PCR reactions were performed in a total volume of 20 µl containing 10 µl of Master Mix, 5 µl of template DNA, 1 µl of each primer (10 pmol/µl), and nuclease-free water to make up the final volume. The thermal cycling conditions are provided in Table 1. Amplified products were analyzed by agarose gel electrophoresis and visualized under UV light.

Table 1. PCR amplification protocol for the ITS region.

Step	Temperature (°C)	Cycles	Time (min)
Initial Denaturation	1	94	4
Denaturation	33	94	1
Annealing		58	1
Extension		72	1
Final extension	1	72	10

Preparation of mushroom ethanolic extract

The ethanolic extract of *M. rufobrunnea* was prepared following the method of Mahdi et al. (2024) with modifications. Fresh mushrooms were air-dried at room temperature, ground into a fine powder using a milling machine, and stored in airtight containers. A total of 61 g of powdered sample was placed into 500 ml of 95% ethanol in a conical flask, covered, and shaken intermittently every 30 min for 6 h. The mixture was then left to stand for 48 h at room temperature.

The extract was filtered through Whatman No. 1 filter paper, and the residue was re-extracted under the same conditions to ensure maximum yield. Both the filtrates were mixed thoroughly and concentrated by using a rotary evaporator maintained under reduced pressure at a maximum temperature of 40°C until complete dryness (Nwofor et al., 2019). The crude extracts were placed in bottles, tightly closed and stored at a temperature of 4°C until these were used in subsequent study.

Preparation of *M. rufobrunnea* extracts

In order to make the stock solution of *M. rufobrunnea* extract (80 mg/ml), 400 mg of crude extract was mixed with 20 ml of 5% dimethyl sulfoxide (DMSO) in distilled water. This stock solution was further used to make subsequent concentrations of 250, 500, 750, and 1000 mg/100 ml by adding requisite amount of distilled water (Moazeni and Khademolhoseini, 2016).

Assessment of fungicidal activity of *M. rufobrunnea*

The mycelial growth inhibition method was employed to evaluate the fungicidal efficacy of *M. rufobrunnea* ethanolic extracts (Shahrour et al., 2024). After carefully combining the *M. rufobrunnea* extracts with PDA at the appropriate concentrations, the mixture was transferred into sterile petri dishes and let to set. After that, 5 mm discs of each pathogen's actively growing mycelia were added to the center of these extract-modified petri plates. The controls were PDA petri plates that had not been supplemented with *M. rufobrunnea* extracts. Three replications of each

treatment were made, and the petri plates were incubated for five to seven days at $25 \pm 2^\circ\text{C}$. Colony diameters of each pathogenic fungus on treated and control media plates were measured, and the Antifungal Index (AFI) was calculated (Al-Jbory et al., 2023).

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

Where:

W represents average colony diameter (mm) in the control and

A shows average colony diameter (mm) in the treatment

The results of AFI were shown as % inhibition of growth of pathogenic fungi with all the concentrations.

Statistical analysis

The Completely Randomized Design (CRD) was used in the experiment. Each treatment was replicated three times. All the data regarding inhibition of mycelial growth were subjected to one-way analysis of variance (ANOVA) using statistical software SAS.v.9.1. The Least Significant Difference (LSD) test was used to compare the treatment means at a probability level of 5%.

Results and Discussion

Molecular identification of *M. rufobrunnea*

M. rufobrunnea samples used in this study were collected from Iraq and were identified on the basis of internal transcribed spacer (ITS) region of DNA. The PCR products obtained had an approximate size of 550 base pairs (Figure 1). The phylogenetic tree constructed from the ITS sequences (Figure 2) confirmed the identification, representing the first recorded occurrence of *M. rufobrunnea* in Iraq. Sequence analysis revealed 99% similarity to reference sequences in the NCBI database, with the closest match corresponding to accession number OQ808797.1.

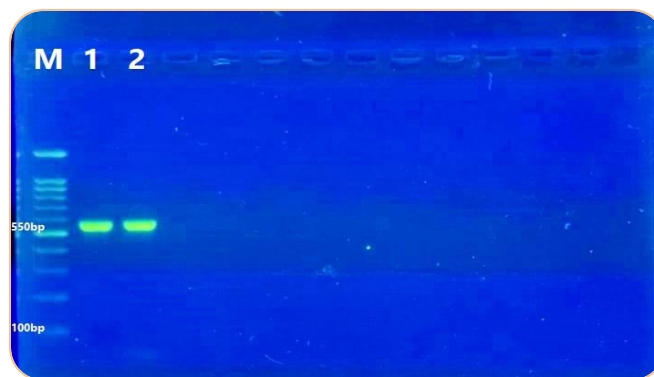


Figure 1. PCR product of *M. rufobrunnea* showing a 550 bp band on agarose gel electrophoresis.

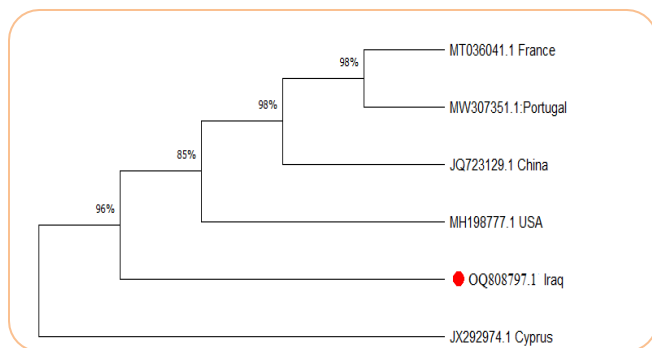


Figure 2. Maximum likelihood phylogenetic tree of *M. rufobrunnea* based on ITS sequences.

Antifungal activity

The antifungal activity of *M. rufobrunnea* ethanolic extract increased progressively with higher concentrations against all tested phytopathogenic fungi (Table 2). At the lowest concentration (250 mg/100 ml), inhibition indices ranged from 24.70% (*R. solani*) to 30.40% (*F. oxysporum*).

A similar trend was observed at 500 mg/100 ml, where inhibition ranged between 29.33% (*R. solani*) and 31.03% (*F. graminearum*).

Further increases in concentration to 750 mg/100 ml resulted in greater antifungal effects, with inhibition indices varying from 32.57% (*R. solani*) to 38.63% (*F. graminearum*). The maximum antifungal activity was consistently recorded at the highest concentration (1000 mg/100 ml), with *F. graminearum* exhibiting the highest inhibition (55.03%), followed by *F. oxysporum* (46.43%), *A. alternata* (45.20%), *S. rolfii* (37.67%), and *R. solani* (36.93%).

Statistical analysis indicated that differences in antifungal activity among treatments were significant ($LSD_{0.05} = 3.79-6.51$), confirming a clear dose-dependent effect of the *M. rufobrunnea* extract. Overall, *F. graminearum* was the most susceptible species to the extract, while *R. solani* was the least sensitive across all concentrations tested.

Table 2. Antifungal efficacy of *M. rufobrunnea* extract at different concentrations (mg/100 ml).

Treatments	Concentration (mg/100 ml)				
	Antifungal index (%)				
	0	250	500	750	1000
<i>A. alternata</i>	0.0	25.13	29.53	33.63	45.20
<i>S. rolfii</i>	0.0	26.23	30.46	33.40	37.67
<i>F. graminearum</i>	0.0	28.93	31.03	38.63	55.03
<i>F. oxysporum</i>	0.0	30.40	30.50	35.30	46.43
<i>R. solani</i>	0.0	24.70	29.33	32.57	36.93
LSD (0.05)	0.0	3.79	6.15	5.46	6.51

There were five replications for each treatment.

The fungicidal capability of *M. rufobrunnea* might be due to its diversity in chemical makeup which can comprise of various compounds like alkaloids, carotenoids, chitosan, flavonoids, polyphenols, steroids, and tocopherols (Tietel and Masaphy, 2018; Acay et al., 2022). Members of Basidiomycetes, like *Morchella*, have been reported to produce a large number of secondary metabolites having strong antimicrobial and antimycotic activities (Suay et al., 2000). There are many reports that chitosan, particularly, possessed strong mycotoxic effectiveness. There are also several documented studies which have proved the efficacy of nanoparticles of chitosan in controlling numerous phytopathogenic fungi (Kashyap et al., 2015). The biologically synthesized chitosan-based nanoparticles have also been found to inhibit the growth of many plant pathogens (Acay et al.,

2022). Low molecular weight chitosan formulations have proved substantial fungicidal effectiveness for the management of *A. alternata*, *Rhizopus oryzae*, and *Aspergillus niger* (Ziani et al., 2009; Sharp, 2013). Chitosan preparations of low molecular weights have shown considerable antifungal efficacy to control pathogenic fungi such as *A. alternata*, *Rhizopus oryzae*, and *Aspergillus niger* affecting many vegetable crops (Ziani et al., 2009; Sharp, 2013).

The antimycotic efficiency of *Morchella* species has also been related to high levels of flavonoids, oxalic acid, succinic acid, sterols, and numerous organic acids (Ullah et al., 2022). Specifically, high contents of flavonoids are crucial for estimating fungistatic and fungicidal activities of *Morchella* species (Fatima et al., 2025). It is well documented that the fruiting bodies of *M. rufobrunnea*

contain sugars 1.1%, flavonoids 0.42%, organic acids 0.85%, sterols 0.13%, and fatty acids and triglycerides 0.18% (Dissanayake et al., 2021). Furthermore, the biological activity and efficiency of *Morchella* mushrooms are owing to their high quantities of ascorbic acid, phenolic compounds, tocopherols, and vitamin D (Tietel and Masaphy, 2018).

M. rufobrunnea contain a large number of naturally occurring biological control agents which have successfully controlled many pathogenic fungi of agricultural crops of great economic significance. These natural biologically active chemicals can prove substitutes to hazardous fungicides and can be helpful in mitigating environmental pollution. Therefore, development of *Morchella* based nanoparticles and biopesticides can offer a feasible tactic for sustainable management of plant pathogens.

Conclusion

It is concluded from the present investigation that ethanolic extracts from *M. rufobrunnea* possess substantial antifungal activities against the test pathogenic fungi of agricultural crops of economic significance. These ethanolic formulations from *Morchella* contain several biologically active plant-derived compounds and can be helpful in formulating and manufacturing biopesticides reducing dependency on deleterious pesticides. Nonetheless, additional comprehensive research is needed to assess its practical utility in agricultural management systems, particularly, in order to explore and exploit the development of *Morchella*-based nano-formulations as antifungal biocontrol agents.

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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