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Research Article

Morphological and Molecular Identification of Iraqi Desert Truffles and Evaluation of Their Extracts for Antibacterial Activity against Pathogens Associated with Oyster Mushroom (*Pleurotus* spp.)

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ABSTRACT

Desert truffles (*Terfezia* spp.) are valuable hypogeous fungi recognized for their nutritional, medicinal, and antimicrobial properties; however, their potential application in managing bacterial diseases of edible mushrooms remains poorly explored. This study characterized two desert truffle species, *T. nivea* and *T. claveryi*, through morphological, microscopic, and molecular approaches and evaluated their antibacterial potential against oyster mushroom pathogens. Morphological and microscopic observations confirmed the identity of both species, with *T. nivea* characterized by whitish fruiting bodies, clavate asci, and ellipsoid ascospores, whereas *T. claveryi* exhibited brownish peridia, ovoid asci, and globose ascospores. ITS-rDNA amplification produced approximately 650 bp fragments, and sequence analysis confirmed species identity with 99.39% and 99.65% similarity to reference isolates of *T. nivea* and *T. claveryi*, respectively. Ethanolic and aqueous extracts of both truffles exhibited antibacterial activity against *Pseudomonas pleuroti*, *P. agarici*, *P. reactans*, and *P. tolaasii*, with ethanolic extracts generally showing higher inhibitory effects. GC–MS profiling revealed 17 and 9 bioactive compounds in *T. claveryi* and *T. nivea*, respectively, including antimicrobial metabolites such as 9-octadecenamide and ethyl 9,12-octadecadienoate. Application of *T. claveryi* aqueous extract significantly reduced brown blotch disease severity caused by *P. agarici* and enhanced biological efficiency and protein content of oyster mushrooms. The highest biological efficiency (122.78%) and protein content (27.47%) were recorded in *Pleurotus floridanus* treated with the truffle extract. The findings demonstrate that *T. claveryi* possesses promising antimicrobial and growth-promoting potential and could serve as a natural bioactive resource for sustainable management of bacterial diseases in oyster mushroom cultivation.

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Introduction

Truffles (*Terfezia* spp.) are hypogeous edible fungi belonging to the class Ascomycetes and family Terfeziaceae. They develop approximately 5–30 cm beneath the soil surface, where they produce irregularly spherical subterranean fruiting bodies. Truffles are highly valued for their distinctive aroma, exceptional nutritional quality, and diverse medicinal properties. Numerous studies have demonstrated their broad spectrum of biological activities, including antioxidant, antimicrobial, antiviral, antifungal, antiparasitic, hepatoprotective, immunomodulatory, and anticancer effects

(Veeraraghavan et al., 2022). Species of the genus *Terfezia* establish obligate ectomycorrhizal associations with the roots of several host plants, particularly *Helianthemum almeriense* (Cistaceae), upon which their growth and reproductive success depend. This highly specialized symbiotic interaction requires coordinated physiological and molecular communication between fungal hyphae and host roots, making artificial cultivation challenging and natural harvesting labor-intensive. Consequently, truffles are considered rare and economically valuable gourmet fungi (Morte et al., 2025).

Truffles are also recognized for their remarkable nutritional composition. They contain approximately 19.6–27.2% protein, low lipid content, appreciable dietary fiber, and high concentrations of essential minerals, including potassium, phosphorus, iron, copper, zinc, and manganese, together with a complete profile of essential amino acids (Hussain and Al-Ruqaie, 1999). Arid and semi-arid regions, particularly across the Middle East and North Africa, harbor several commercially important desert truffle species, including *Terfezia claveryi*, *T. boudieri*, and *T. nivea*. Beyond their nutritional importance, these species are rich sources of biologically active secondary metabolites, such as polysaccharides, phenolic compounds, flavonoids, terpenoids, and unsaturated fatty acids, which can be extracted using aqueous or organic solvents. Phytochemical investigations have revealed that these metabolites possess considerable antimicrobial activity against a broad range of human and plant pathogenic microorganisms. *In vitro* studies have demonstrated inhibitory effects against bacterial pathogens, including *Staphylococcus aureus* and *Escherichia coli*, with minimum inhibitory concentrations (MICs) ranging from 50 to 100 μg mL^{-1} and growth inhibition of resistant strains approaching 70%, comparable to that of some conventional antibiotics (Khojah et al., 2022; Veeraraghavan et al., 2022). These antimicrobial properties have largely been attributed to their high phenolic and flavonoid contents, which interfere with microbial cell integrity, metabolism, and proliferation, as confirmed through phytochemical characterization, HPLC profiling, and disc diffusion bioassays (Veeraraghavan et al., 2022).

Oyster mushrooms (*Pleurotus* spp.) are among the most extensively cultivated edible mushrooms worldwide because of their simple cultivation requirements, desirable sensory characteristics, and outstanding nutritional and medicinal attributes. They constitute an excellent source of high-quality protein, dietary fiber, B-complex vitamins (B_1, B_2, B_3, B_5, and B_{12}), vitamins C and D, and essential minerals such as potassium, phosphorus, magnesium, and iron, while containing low levels of fat and sodium, making them an attractive alternative to animal-derived protein. Moreover, *Pleurotus* species contain bioactive β-glucans that exhibit immunomodulatory activity by stimulating innate immune responses, while also contributing to cholesterol reduction, cardiovascular protection, and overall human health (Lesa et al., 2022; Wal et al., 2022; Effiong et al., 2024). Despite their commercial importance, oyster mushroom production is frequently constrained by bacterial diseases that reduce both yield and market quality. These infections commonly originate from contaminated cultivation substrates or inadequate environmental management, particularly excessive humidity and unsuitable temperatures, which favor rapid bacterial proliferation. Disease symptoms range from superficial lesions and discoloration to severe tissue maceration and complete fruiting body decay, resulting in substantial economic losses. Among the most destructive bacterial pathogens are *Pseudomonas agarici* and *P. tolaasii*, the causal agents of bacterial blotch disease (Carrasco et al., 2018; Xu et al., 2023; Jung et al., 2026).

Although the nutritional and pharmacological properties of desert truffles have been extensively documented, relatively little is known about their potential as natural antimicrobial agents for controlling pathogens affecting cultivated mushrooms. Therefore, the present study aimed to morphologically and molecularly characterize selected Iraqi *Terfezia* species and to evaluate the antibacterial efficacy of their extracts against bacterial pathogens associated with oyster mushroom (*Pleurotus* spp.) cultivation. This work provides insights into the potential application of desert truffle-derived bioactive compounds as sustainable alternatives to synthetic bactericides for disease management in mushroom production.

Materials and Methods

Bacterial pathogens of oyster mushroom

Four bacterial pathogens associated with oyster mushroom diseases were used in this study: *Pseudomonas pleuroti* strain Iraq-20, *P. reactans* strain Iraq-22, *P. agarici* strain Iraq-21, and *P. tolaasii* strain Iraq-23. These strains were previously identified and deposited in the National Center for Biotechnology Information (NCBI) under accession

numbers PV679803.1, PV679806.1, PV679801.1, and PV679807.1, respectively (Ibrahim and Hassan, 2026).

Isolation and identification of truffle fungi

Fruiting bodies of two truffle species exhibiting distinct morphological characteristics (shape, color, and size) were collected from Anbar Governorate, Iraq. Fresh cross-sections were prepared and examined under a light microscope to observe diagnostic morphological features, including ascospores, asci, and the peridium (outer tissue layer), for preliminary species identification.

Molecular identification of truffle fungi

Genomic DNA was extracted from 200–300 mg of fresh truffle fruiting body tissue using the ZR Fungal/Bacterial DNA MiniPrep™ Kit (Zymo Research, USA) according to the manufacturer's instructions. Molecular identification was performed by amplification of the internal transcribed spacer (ITS) region encompassing the 5.8S rRNA gene using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) described by White et al. (1990). Primers were synthesized by Integrated DNA Technologies (IDT), Canada.

PCR amplification was carried out in a final reaction volume of 25 µL containing 1.5 µL genomic DNA template, 5 µL Taq PCR PreMix, 1 µL (10 pmol) of each primer, and nuclease-free water to the final volume. Amplification was performed using an Applied Biosystems GeneAmp PCR System 9700 under the following thermal cycling conditions: initial denaturation at 95°C for 5 min; 37 cycles of denaturation at 95°C for 45 sec, annealing at 58°C for 45 sec, and extension at 72°C for 45 sec; followed by a final extension at 72°C for 7 min.

PCR products were separated on 1.5% agarose gels stained with RedSafe™ nucleic acid stain (Intron Biotechnology, South Korea) and visualized under ultraviolet illumination (302 nm) (Mohammed et al., 2025).

DNA sequencing and phylogenetic identification

PCR-amplified ITS fragments were purified and subjected to bidirectional DNA sequencing using an Applied Biosystems 3730XL DNA Analyzer (Macrogen Inc., South Korea). Approximately 25 µL of purified PCR product together with 10 µL (10 pmol) of each primer were submitted for sequencing. The obtained nucleotide sequences were compared with those available in the NCBI database using the Basic Local Alignment Search Tool (BLAST) to determine species identity based on sequence similarity.

Preparation of aqueous and ethanolic truffle extracts

Aqueous and ethanolic extracts were prepared at a ratio of 1:4 (w/v). Briefly, 100 g of dried truffle powder was mixed separately with 400 mL of distilled water or 96% ethanol following the procedure of Al-Majoul (2025). The mixtures were homogenized using an electric blender at low speed for 5 min and filtered through three layers of sterile cheesecloth to remove coarse particles. The filtrates were centrifuged at 4,000 rpm for 15 min, after which the supernatants were collected and concentrated using a rotary evaporator at 40°C until the solvent was completely removed, yielding approximately 100 mL of concentrated extract. The concentrated extracts were stored until chromatographic analysis and biological evaluation (Tejedor-Calvo et al., 2022).

Antibacterial activity of truffle extracts

The antibacterial activity of aqueous and ethanolic truffle extracts against oyster mushroom pathogenic bacteria was evaluated using the agar well diffusion assay. Briefly, 1 mL of each bacterial suspension was uniformly spread onto nutrient agar plates using a sterile L-shaped glass spreader. Wells (5 mm diameter) were punched aseptically into the agar, and 100 µL of 25% aqueous or ethanolic extract was dispensed into each well. Control wells received 100 µL of distilled water or 96% ethanol corresponding to the respective extraction solvent. Plates were incubated at 30°C for 24 h, after which antibacterial activity was assessed by measuring the diameter of the inhibition zones surrounding each well (Khojah et al., 2022).

GC–MS profiling of bioactive compounds

The chemical constituents of the ethanolic truffle extract were identified using gas chromatography–mass spectrometry (GC–MS). Analysis was performed at the laboratories of the Scientific Research Authority, Ministry of Higher Education and Scientific Research, Republic of Iraq. Individual compounds were identified by comparing their mass spectra with those available in standard spectral libraries.

Evaluation of *T. claveryi* aqueous extract against brown blotch disease under cultivation-room conditions

The aqueous extract of *T. claveryi* was selected for cultivation-room experiments because of its ease of preparation, lower production cost, and comparable antibacterial activity to the ethanolic extract, as demonstrated in preliminary laboratory assays.

Four oyster mushroom species, namely *P. ostreatus*, *P. floridanus*, *P. eryngii* var. *ferulae*, and *P. eryngii* var. *eryngii*, were obtained from the Pilot Project for Edible Mushroom Production, Mushroom Farm, College of Agriculture, Tikrit University. Mushroom cultivation followed the standard protocol described by Hassan and Ibrahim (2023a).

Wheat straw was soaked in water for 10 h and allowed to drain before pasteurization at 70°C for 1 h. After cooling, the substrate was packed into polyethylene bags and inoculated with mushroom spawn at a spawning rate of 2% (w/w). Upon complete substrate colonization and emergence of primordia, a suspension of *P. agarici* (10^8 CFU mL^{-1}) was sprayed uniformly onto the developing primordia until complete wetting was achieved. Fruiting bags were then maintained under standard cultivation conditions until harvest.

For the combined treatment, the aqueous extract of *T. claveryi* was sprayed onto the primordia 24 h after bacterial inoculation to allow sufficient time for bacterial establishment before treatment application.

Disease assessment

Disease severity

Disease severity was assessed using a modified McKinney disease severity index (McKinney, 1923) based on five-point rating scale (Table 1).

Table 1. Disease severity rating scale used for the assessment of disease severity index.

Grade	Disease symptoms
0	Healthy fruiting bodies
1	Bacterial spotting or blotching affecting ≤25% of the fruit body
2	Bacterial spotting or blotching affecting >25–50%
3	Bacterial spotting or blotching affecting >50–75%
4	Bacterial spotting or blotching affecting >75%

Biological efficiency

Biological efficiency (BE) was calculated as an indicator of substrate productivity according to Hassan and Muhammad (2023b):

$$\text{Biological Efficiency (\%)} = \frac{\text{Fresh weight of harvested fruiting bodies (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

Crude protein content

Crude protein content was determined using the semi-micro Kjeldahl method (AOAC, 2002). Protein percentage on a dry weight basis was calculated using the following equation:

Crude protein (%) = Total nitrogen (%) × 6.25

Statistical analysis

The experiment was arranged in a completely randomized design (CRD). Treatments consisted of the control, bacterial inoculation alone, and bacterial inoculation followed by application of the aqueous extract of *T. claveryi* across four oyster mushroom species. Each treatment comprised 10 replicate cultivation bags, resulting in a total of 120 experimental units.

Data were subjected to analysis of variance (ANOVA) using SPSS statistical software. Treatment means were separated using Duncan,s multiple range test at the 5% significance level ($P \leq 0.05$).

Results and Discussion

Morphological and Microscopic Characteristics of *T. nivea*

The fruiting bodies of *T. nivea* (Figure 1, right) were subglobose to ovoid in shape, with diameters ranging from 7 to 12 cm. The peridium was thick and fleshy, initially whitish with a faint yellowish tinge, gradually turning yellow and ultimately reddish-brown upon maturity. The gleba was firm and compact, varying in color from white to pale yellow or yellowish-pink, and was traversed by numerous sterile, whitish to yellowish veins that formed a characteristic marbled pattern.

The asci were clavate to elongate-ovoid, measuring 65–100 × 40–50 µm, and typically contained 4–6 ascospores, although asci bearing up to eight spores were occasionally observed.

The ascospores were ellipsoid to broadly elliptical, hyaline, smooth-walled, and regular in outline, measuring 12–18 × 10–13 µm (Figure 1, left). These morphological and microscopic characteristics are consistent with the diagnostic features reported for *T. nivea*, thereby confirming the taxonomic identity of the collected specimens.

Morphological and microscopic characteristics of *T. claveryi*

Fruiting bodies

Fruiting bodies were hypogeous, developing entirely below the soil surface or becoming partially emergent at maturity, and measured 5–14 cm in diameter. They were

subglobose, conical, or convex, with irregularly lobed contours. The peridial surface was initially pale brown, gradually turning reddish-brown before fading to whitish at maturity. It was smooth in young specimens but becomes progressively furrowed with age.

Peridium

The peridium was brown, 0.75–2.0 mm thick, with a smooth surface traversed by prominent pinkish veins (Figure 2, right).

Asci and ascospores

The asci were subglobose to ovoid, measuring 60–75 × 70–80 µm, and were irregularly distributed throughout the gleba. Each mature ascus contained eight ascospores. The ascospores were hyaline to pale yellow, globose, measuring 17–20 × 20–25 µm, and were loosely arranged within the ascus (Figure 2, left). This combination of morphological and microscopic features was consistent with the diagnostic characteristics of *T. claveryi*.

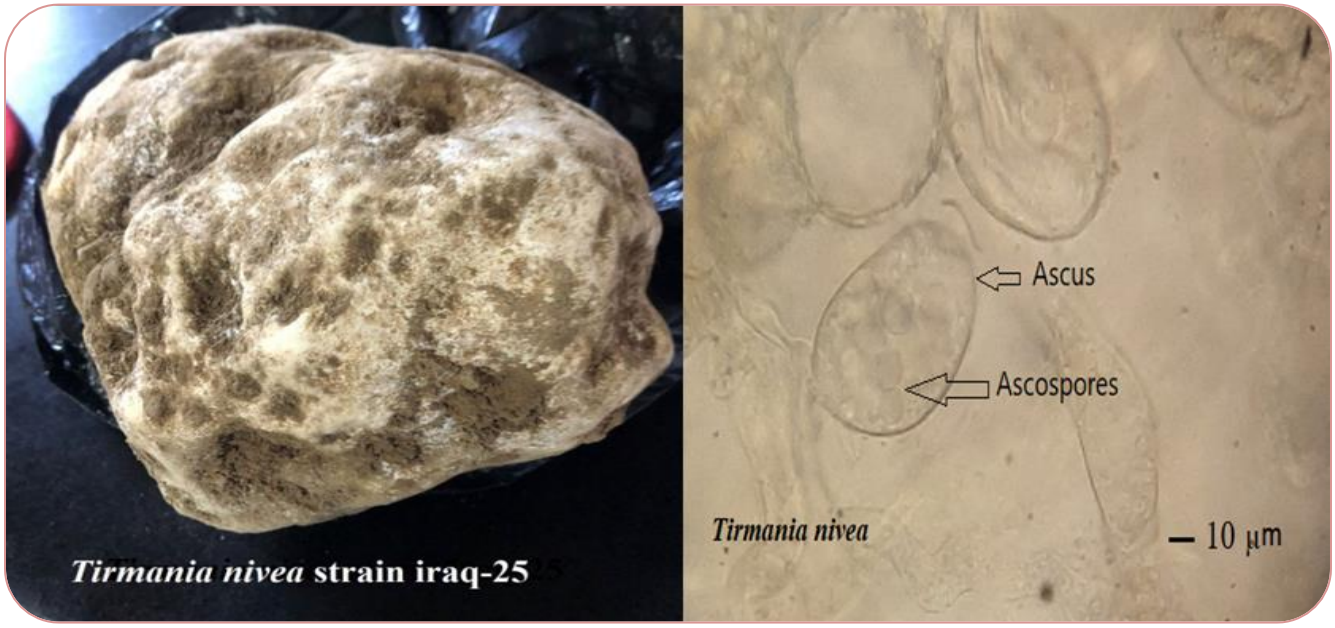


Figure 1. *T. nivea* fruiting body and anatomical features. Right: Mature fruiting body. Left: Anatomical section of the fruiting body.

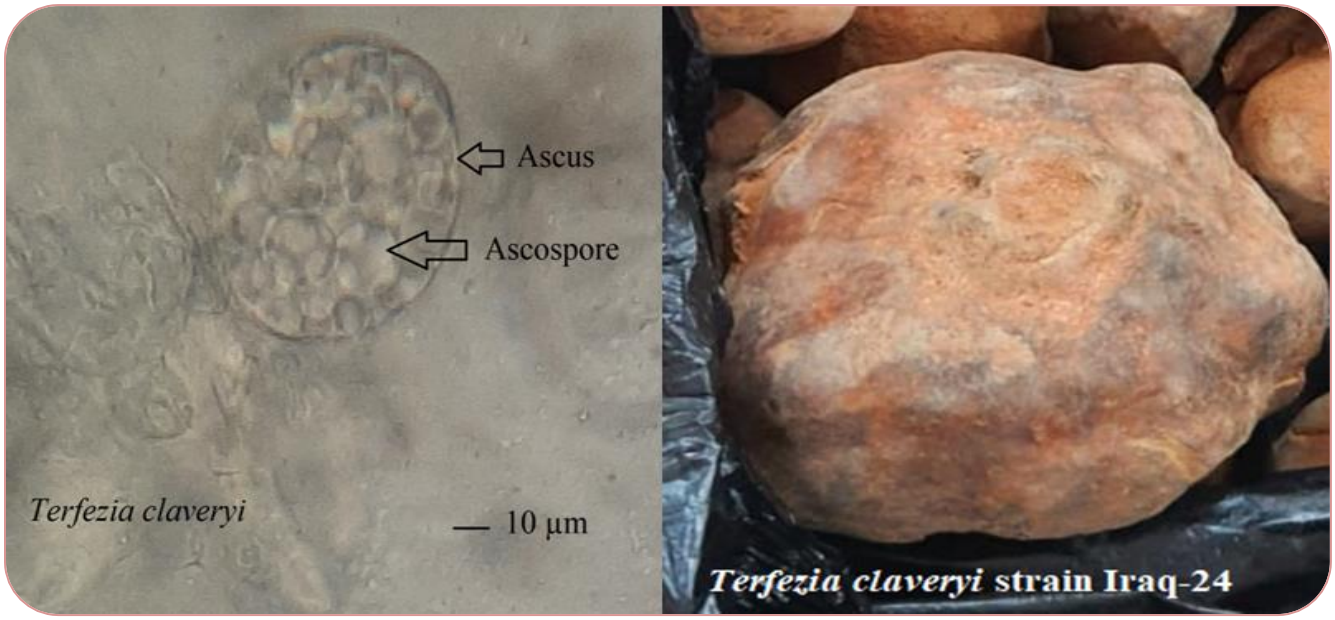


Figure 2. *T. claveryi* truffle. Right: Mature fruiting body; Left: Anatomical section of the fruiting body.

The fruiting bodies of truffle fungi exhibited considerable morphological variation, ranging from nearly spherical to irregularly ovoid forms, representing one of their most distinctive taxonomic characteristics. Such variation is primarily attributed to the hypogeous growth habit of truffles, where the developing ascocarps are subjected to soil compaction, mechanical pressure, and interactions with surrounding plant roots, all of which influence the final morphology of the fruiting body (Veeraraghavan et al., 2022). Fruiting body coloration also differed between the identified species. *T. nivea* was characterized by a white to light-gray peridium with white gleba, whereas *T. claveryi* exhibited a brown to dark-brown peridium enclosing a cream to light-brown gleba (Morte et al., 2025). The morphological identification of *T. claveryi* in the present study was consistent with previous descriptions reported by Mahmoud (2025) and Mohamed-Benkada et al. (2024). Likewise, the diagnostic characteristics of *T. nivea* agreed with those described by Moreno et al. (2000), Bouzadi et al. (2017), and Emhmed et al. (2023).

Molecular identification

PCR amplification of the internal transcribed spacer (ITS) region using ITS-specific primers successfully amplified DNA fragments from both truffle species (Figure 3). The presence of a single amplicon of approximately 650 bp confirmed successful amplification of the fungal ITS region, supporting the taxonomic identification of the isolates as true fungi.

Sequence analysis of the ITS region further confirmed the identity of the two truffle species (Table 2). The isolate *T. nivea* strain Iraq-25 exhibited 99.39% nucleotide sequence similarity with *T. nivea* isolate A1, whereas *T. claveryi* strain Iraq-24 showed 99.65% similarity with *T. claveryi* voucher MA:FU 24880, originally reported from Italy and Hungary, respectively. The ITS sequences generated in this study were deposited in the National Center for Biotechnology Information GenBank database under accession numbers PV679782.1 (*T. nivea* strain Iraq-25) and PV679778.1 (*T. claveryi* strain Iraq-24), providing molecular confirmation of species identity.

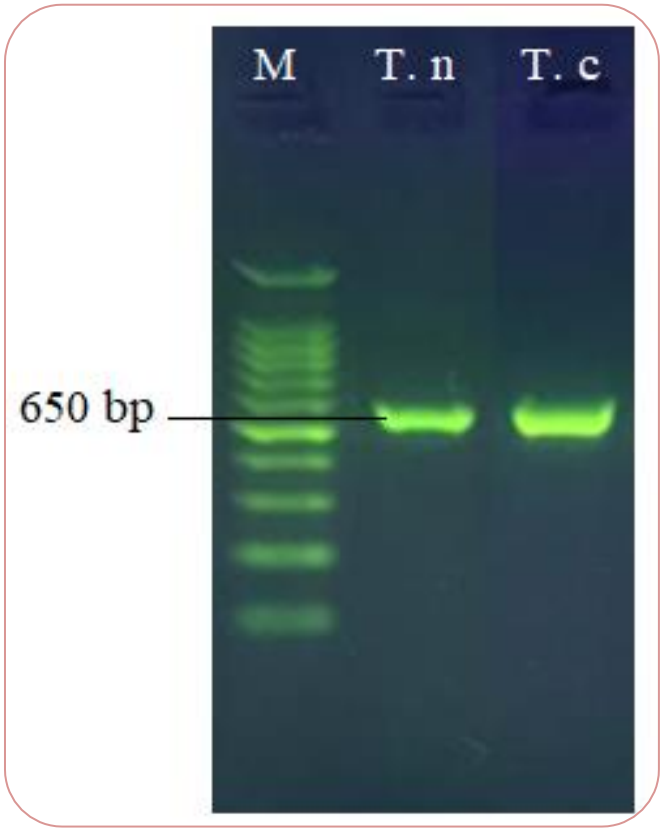


Figure 3. PCR amplification of the internal transcribed spacer (ITS) region from *T. nivea* and *T. claveryi*. Amplified products were separated by electrophoresis on a 1.5% agarose gel in 1× TBE buffer at 5 V cm^{-1} for 1.5 h. M, DNA molecular weight marker; T.n, *T. nivea*; T.c, *T. claveryi*. The expected ITS amplicon size was approximately 650 bp.

Table 2. Molecular identification of Iraqi desert truffles based on ITS-rDNA sequence similarity with reference sequences in the NCBI GenBank database.

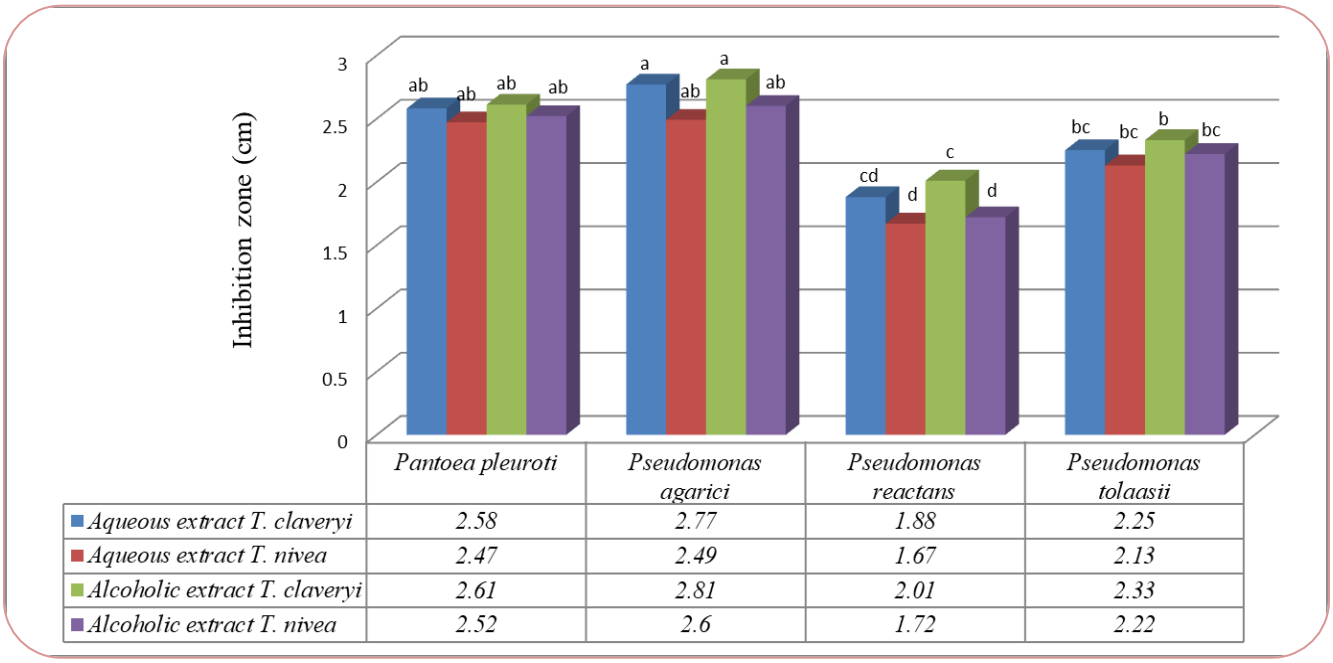
The fungus species with the highest similarity	Accession number	Country	(%) Similarity	Species and strain of fungus recorded in this study	Accession number of fungi recorded in this study
Tirmania nivea isolate A1	LT718218.1	Italy	99.39	*Tirmania nivea* strain iraq-25	PV679782.1
Terfezia claveryi voucher MA:FU 24880	HQ698077.1	Hungary	99.65	*Terfezia claveryi* strain Iraq-24	PV679778.1

***In vitro* antibacterial activity of aqueous and ethanolic extracts of desert truffles**

The *in vitro* antibacterial assay revealed significant variation in the inhibitory activity of aqueous and ethanolic extracts of *T. claveryi* and *T. nivea* against bacterial pathogens associated with oyster mushrooms. As shown in Figure 4, the ethanolic extract of *T. claveryi* produced the largest inhibition zone against *P. pleuroti* (2.61 cm), although its activity did not differ significantly from that of the ethanolic extract of *T. nivea* or the aqueous extracts of both truffle species. Likewise, no significant differences were observed between aqueous and ethanolic extracts of either species against *P. agarici*, despite the ethanolic extract of *T. claveryi* exhibiting the greatest inhibitory effect. Against *P. reactans*, the ethanolic extract of *T. claveryi* demonstrated the strongest antibacterial activity, producing an inhibition zone of 2.01 cm, whereas all extracts displayed statistically comparable activity against *P. tolaasii*.

Overall, ethanolic extracts consistently exhibited greater antibacterial activity than aqueous extracts, although these differences were not statistically significant. The relatively higher efficacy of ethanolic extracts is likely attributable to the superior extraction efficiency of ethanol for lipophilic and moderately polar bioactive metabolites, including phenolics, flavonoids, terpenoids, and other antimicrobial constituents. These findings are consistent with those of Gouzi et al. (2011), who reported pronounced bacteriostatic activity of aqueous extracts of *T. claveryi* against *S. aureus* (86.48% inhibition) and *P. aeruginosa* (71.11% inhibition) at concentrations ranging from 4% to 11% using the agar diffusion method.

The observed differences in antibacterial efficacy among extracts are most likely attributable to qualitative and quantitative variations in their bioactive metabolite profiles, which were subsequently confirmed by GC–MS analysis. In addition, intrinsic differences among the bacterial species, including genetic diversity, cell envelope composition, physiological adaptability, and antimicrobial resistance mechanisms, may have influenced their susceptibility to truffle extracts. The comparatively lower inhibition observed against *P. reactans* may be associated with its production of extracellular lipodepsipeptides, particularly the white line-inducing principle (WLIP), which contributes to bacterial defense and survival (Lo Cantore et al., 2006). Furthermore, the presence of multidrug efflux pumps, enzymatic detoxification systems, and adaptive stress responses may reduce bacterial susceptibility to truffle-derived phenolic and terpenoid compounds. Differences in membrane permeability, limited interaction between antimicrobial metabolites and cellular targets, and inducible resistance mechanisms may also contribute to the variability in antibacterial activity observed among the tested pathogens (Lopes et al., 2022).



	Pantoea pleuroti	*Pseudomonas agarici*	*Pseudomonas reactans*	*Pseudomonas tolaasii*
Aqueous extract T. claveryi	2.58	2.77	1.88	2.25
Aqueous extract T. nivea	2.47	2.49	1.67	2.13
Alcoholic extract T. claveryi	2.61	2.81	2.01	2.33
Alcoholic extract T. nivea	2.52	2.6	1.72	2.22

Figure 4. Effects of aqueous and ethanolic extracts of desert truffles on the inhibition of oyster mushroom-associated pathogenic bacteria.

Bioactive compounds in desert truffles

GC–MS analysis of the ethanolic extract of *T. claveryi* (Figure 5; Table 3) identified 17 chemical constituents, although four chromatographic peaks (7, 8, 11, and 14) could not be assigned to known compounds. In contrast, GC–MS profiling of the *T. nivea* extract (Figure 6; Table 4) revealed nine distinct bioactive constituents. Among the identified metabolites, 9-octadecenamide and ethyl 9,12-octadecadienoate (linoleic acid ethyl ester) are recognized for their broad-spectrum antibacterial properties. These compounds exert antimicrobial activity by disrupting bacterial membrane integrity and interfering with essential metabolic processes, thereby contributing to the inhibitory effects observed against the tested bacterial pathogens in the present study (Izzati et al., 2026).

Ethyl 9,12-octadecadienoate exhibits a multifaceted mode of antibacterial action. It inhibits the bacterial enoyl-acyl carrier protein reductase (FabI), a key enzyme involved in fatty acid biosynthesis (Zheng et al., 2005). Furthermore, it suppresses biofilm formation in Gram-positive bacteria, particularly *S. aureus*, even at sub-minimum inhibitory concentration (sub-MIC) levels (Dilika et al., 2000). In addition, this compound interferes with quorum-sensing signaling in *P. aeruginosa* by reducing intracellular cyclic di-GMP concentrations, thereby impairing bacterial communication, biofilm maturation, and virulence (Kim et al., 2020; Kobayashi et al., 2020). Collectively, these mechanisms provide a plausible biochemical basis for the pronounced antibacterial activity of the desert truffle extracts observed in the present study.

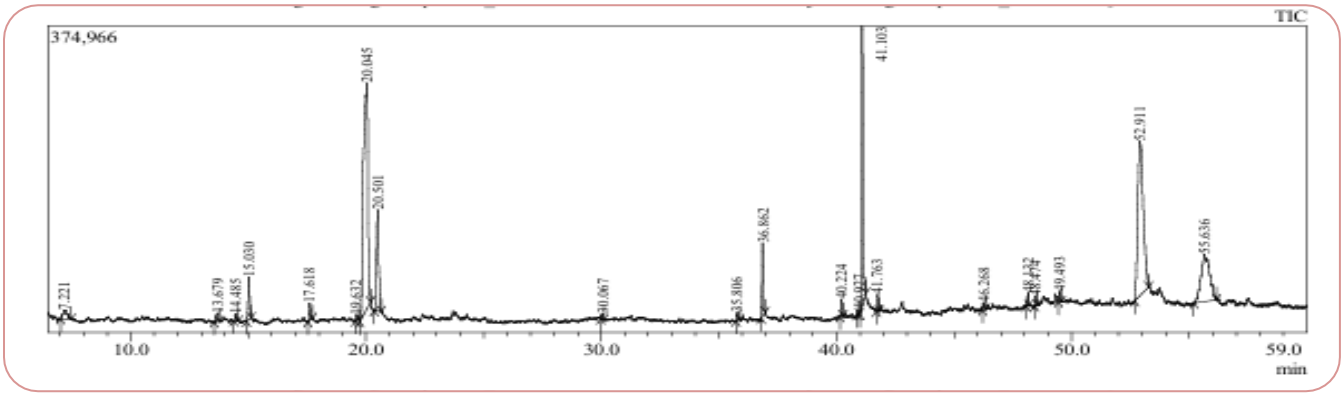


Figure 5. GC–MS chromatogram of bioactive compounds identified in the *T. claveryi* truffle extract.

Table 3. Bioactive compounds identified in the fruiting bodies of *T. claveryi*.

Peak #	Compound	Area %	R. Time	Peak #	Compound	Area %	R. Time
1	3-Pentanol, 3-ethyl-	1.17	7.221	12	9,12-Octadecadienoic acid, methyl	0.57	40.224
2	trans-2,3-Epoxynonane	0.42	13.679	13	Hexatriacontane	0.14	40.927
3	Isosorbide Dinitrate	0.28	14.485	14	Unknown	12.35	41.103
4	n-Amyl isovalerate	2.02	15.030	15	9,12-Octadecadienoic acid, ethyles	0.62	41.763
5	3-Ethyl-3-heptanol	0.76	17.618	16	9-Octadecenamide, (Z)-	0.30	46.268
6	Bis[(2Z)-Hex-2-en-l-yioxy] (dimetl)	0.23	19.632	17	Hexanoic acid, 2-Dimethylaminoethyl	0.44	48.132
7	Unknown	30.96	20.045	18	Cyclohexanol, 4-[(trimethylsilyl)ox	0.35	48.474
8	Unknown	7.08	20.501	19	Phenol, 2,4-bis(1-methyl-l-phenyle	0.38	49.493
9	Hexadecane	0.25	30.067	20	7,22-Ergostadienol	25.89	52.911
10	7,9-Di-tert-butyl-l-oxaspiro (4,5)de	0.45	35.806	21	Ergosterol	12.50	55.636
11	Unknown	2.85	36.862				

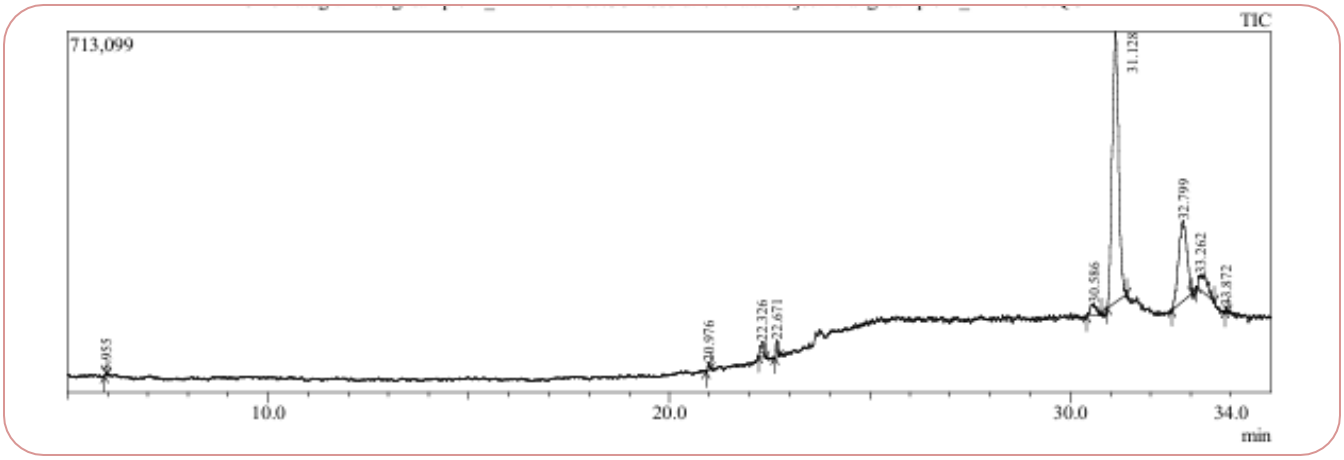


Figure 6. GC–MS chromatogram of bioactive compounds identified in the *T. nivea* truffle extract.

Table 4. Bioactive compounds identified in the fruiting bodies of *T. nivea*.

Peak #	Name	R. Time
1	Propanedioic, dihydroxy-	5.955
2	Ethyl tridecanoate	20.976
3	Acetic acid, (2,4-dichlorophenoxy)	22.326
4	Linoleic acid ethyl ester	22.671
5	Tri(2-Ethylhexyl) trimellitate	30.586
6	Ergosta-5,22-dien-3-ol,(3.beta.,22)	31.128
7	Ergosterol	32.799
8	2-tert-Butyl -4,6-bis(3,5-di-tert-butyl)	33.262
9	N-Methyl-l-adamantaneacetamide	33.872

Park and Kim (2020) reported that the major antibacterial compounds identified in fungal extracts, whose bioactivities have been extensively documented in the scientific literature, include ergosterol, which exhibits antibacterial activity, particularly after esterification; octadecadienoic acid (linoleic acid) and its derivatives (methyl and ethyl esters), which are unsaturated fatty acids with well-established antimicrobial properties; and phenol, 2,4-bis(1-methylphenyl), a phenolic compound known to suppress bacterial proliferation and biofilm formation. Other bioactive constituents include ergostadienol, a sterol structurally related to ergosterol, as well as hexatriacontane, 3-pentanol, 3-ethyl-3-heptanol, cyclohexanol derivatives, and epoxide compounds such as epoxynonane, which may contribute to antimicrobial activity through direct or indirect mechanisms, including inhibition of bacterial growth (Pu et al., 2010). The failure to detect four compounds in the *T. claveryi* extract may be due to the presence of previously uncharacterized metabolites, compounds occurring below the detection threshold, or the absence of corresponding entries in the available GC–MS spectral databases.

Effect of aqueous extract of *T. claveryi* on brown spot disease caused by *P. agarici*

Infection severity

As illustrated in Figure 7, the aqueous extract of *T. claveryi* significantly reduced the severity of brown spot disease caused by *P. agarici* across all tested oyster mushroom species. The application of the fungal extract resulted in a marked suppression of disease development, with the minimum infection severity values recorded as 12.60% and 13.71% in *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae*, respectively. These findings demonstrate the potential of *T. claveryi* aqueous extract as an effective natural antimicrobial agent for reducing bacterial disease severity in cultivated oyster mushrooms.

Biological efficiency

The results presented in Figure 8 demonstrate that the aqueous extract of *T. claveryi* significantly improved the biological efficiency of all tested oyster mushroom cultivars under brown blotch disease pressure caused by *P. agarici*. Among the evaluated cultivars, *P. floridanus* exhibited the highest biological efficiency, reaching 122.78% under pathogen-infected conditions following treatment with the *T. claveryi* extract. These findings indicate that the extract not only suppressed disease development but also enhanced mushroom productivity by promoting physiological performance and fruiting body development.

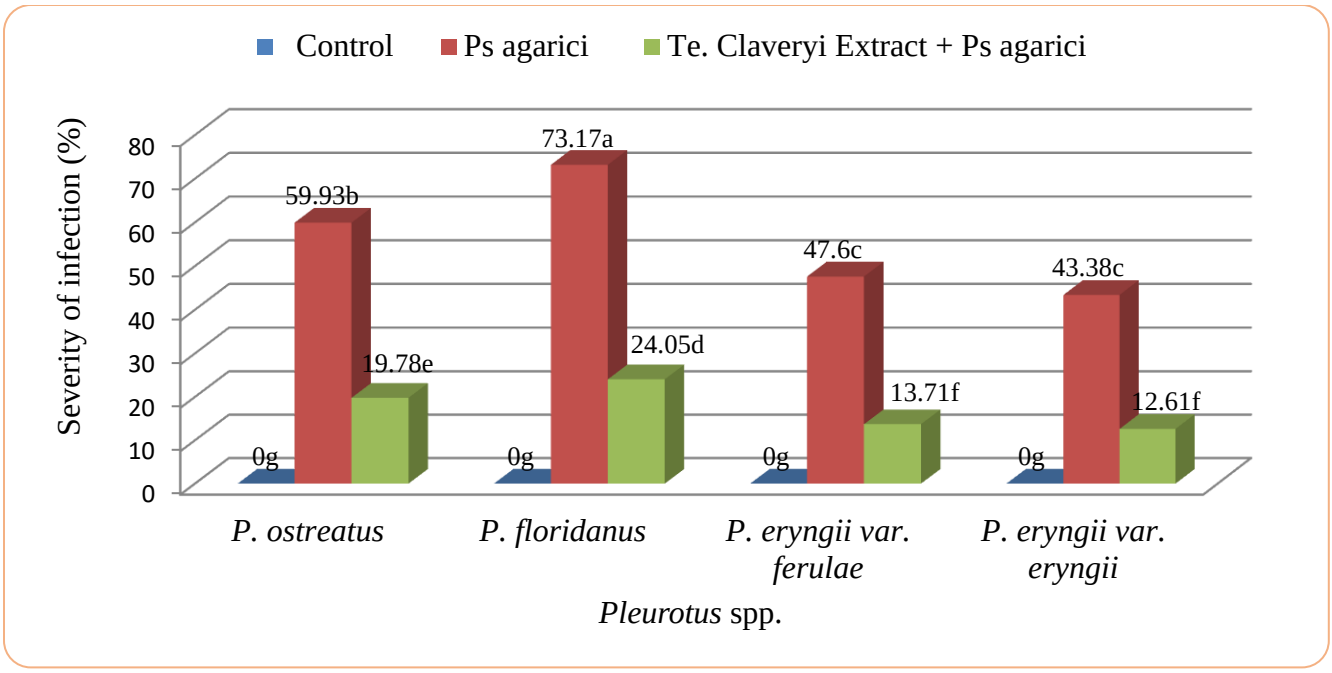


Figure 7. Effect of aqueous extract of *T. claveryi* on the severity of brown blotch disease caused by *P. agarici*.

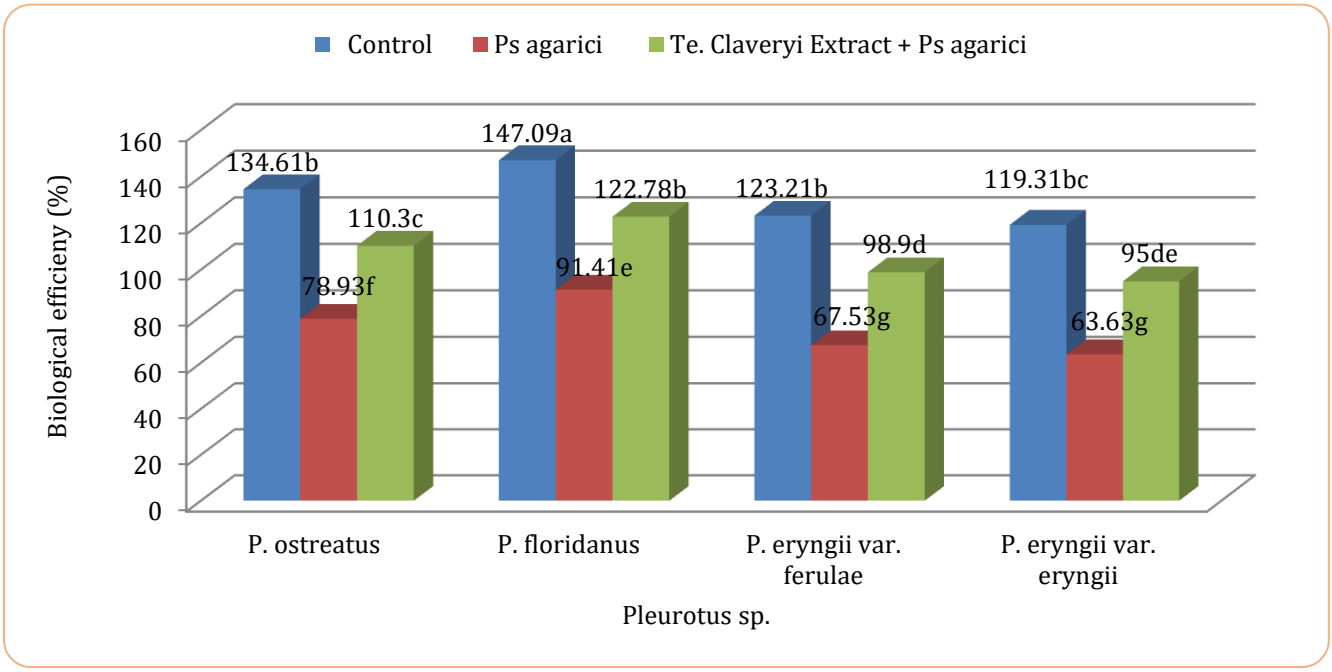


Figure 8. Effect of aqueous extract of *T. claveryi* on the biological efficiency of oyster mushroom species under brown blotch disease conditions caused by *P. agarici*.

Protein content

The results illustrated in Figure 9 revealed that application of the aqueous extract of *T. claveryi* significantly increased the protein content of all tested oyster mushroom species challenged with *P. agarici*. The highest protein accumulation was recorded in the fruiting bodies of *P. floridanus*, reaching 27.47% under brown blotch disease conditions. All mushroom cultivars treated with the *T. claveryi* extract exhibited significantly higher protein contents compared with the pathogen-only treatment, demonstrating the protective and growth-promoting potential of the extract.

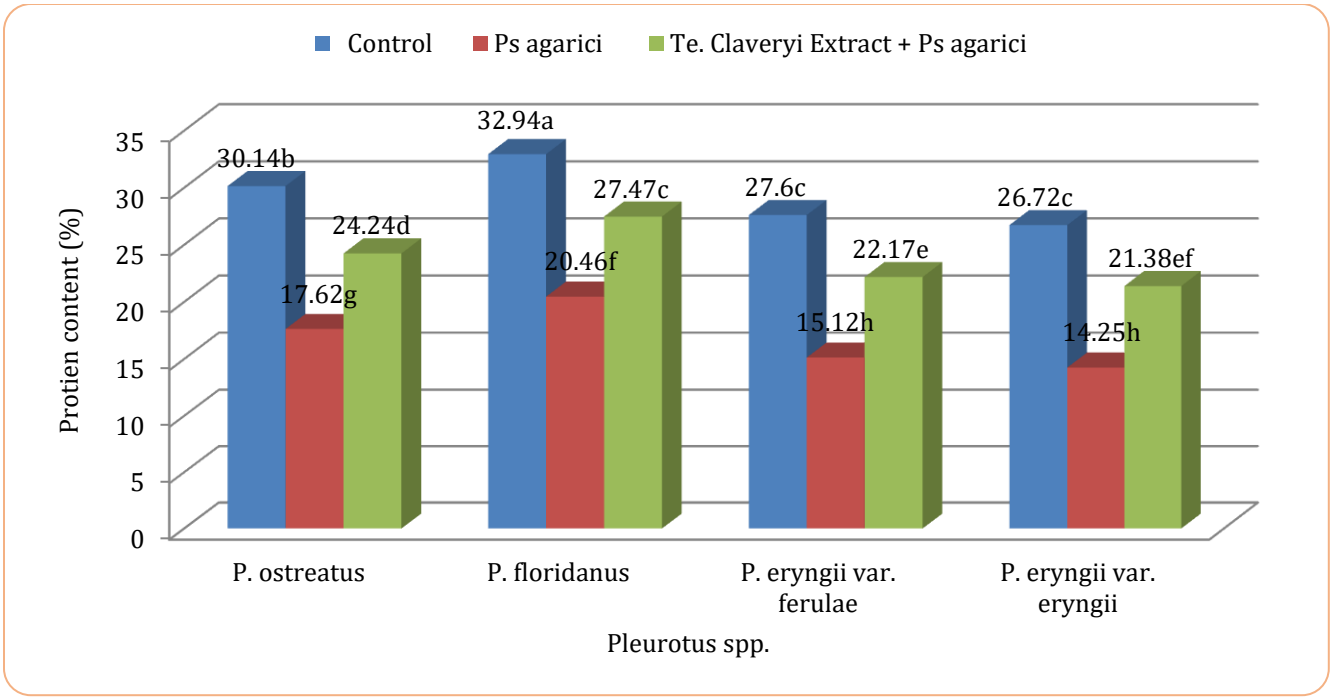


Figure 9. Effect of aqueous extract of *T. claveryi* on protein content of oyster mushroom cultivars affected by brown blotch disease caused by *P. agarici*.

The highest disease severity, lowest biological efficiency, and reduced protein content were observed in *P. agarici*-only treatments. These detrimental effects may be attributed to the pathogenic mechanisms of *P. agarici*, particularly the secretion of extracellular hydrolytic enzymes, including proteases, which degrade host tissues and impair fruiting body development. The pathogen-derived proteases contribute to cellular disintegration and membrane disruption, resulting in the release of phenolic compounds from vacuoles. In the presence of oxygen, these phenolics undergo oxidation mediated by polyphenol oxidase, leading to the formation of dark-brown melanin pigments responsible for the characteristic discoloration and browning symptoms associated with bacterial brown blotch disease (Murata et al., 1995).

The aqueous extract of *T. claveryi* significantly alleviated disease severity, which may be associated with its diverse bioactive constituents, including ergosterol, unsaturated fatty acids, and phenolic compounds identified through GC–MS analysis, as well as antimicrobial proteins and enzymes (Ibrahim and Hassan, 2026). Phenolic compounds represent the predominant bioactive components and play a central role in antimicrobial activity through membrane disruption, oxidative stress modulation, and inhibition of microbial growth (Khojah et al., 2022). In addition, ethyl tridecanoate exhibits multifaceted antibacterial activity by targeting essential cellular processes, including inhibition of DNA gyrase-mediated replication and transcription, while simultaneously compromising membrane integrity, leading to cytoplasmic leakage and bacterial cell death (Hazra et al., 2020). Collectively, these antimicrobial mechanisms contribute to reduced pathogen colonization, decreased disease severity, and subsequent improvements in mushroom yield and nutritional quality.

Significant variation in susceptibility among oyster mushroom cultivars was observed, which may be attributed to genetic differences affecting physiological responses, biochemical defense mechanisms, and hyphal wall composition. Such inherent variability can influence pathogen recognition, colonization efficiency, and resistance expression. In particular, *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae* exhibited comparatively greater resistance to *P. agarici*, possibly due to their stronger hyphal architecture, compact fruiting body structure, and enhanced metabolic efficiency, which collectively contribute to improved defense against bacterial invasion (Zhang et al., 2022).

Conclusions

Morphological and molecular characterization confirmed the identity of Iraqi desert truffles as *T. nivea* and *T. claveryi*, both of which contained bioactive metabolites exhibiting significant antibacterial activity. Extracts obtained from both aqueous and ethanolic fractions demonstrated considerable inhibitory effects against major bacterial pathogens associated with oyster mushrooms, with *T. claveryi* consistently exhibiting superior antimicrobial potential. Notably, the aqueous extract of *T. claveryi* effectively reduced brown blotch severity caused by *P. agarici* while simultaneously enhancing biological efficiency and protein accumulation across oyster mushroom cultivars. These findings highlight the potential application of *T. claveryi* extract as a natural biostimulant and environmentally sustainable biocontrol agent for improving disease management, productivity, and nutritional quality in commercial mushroom cultivation systems.

Author Contributions

AAH and MTI conceptualized and designed the study. MTI conducted the field investigations and carried out the experimental work. AAH provided overall supervision, ensuring methodological rigor, analytical consistency, and continuous refinement of the research throughout all stages of the project. AAH, MTI, and WKA prepared the initial draft of the manuscript, including the organization of the content, compilation of the results, and integration of the preliminary interpretations. All authors subsequently critically reviewed and revised the manuscript, contributed to its intellectual refinement, verified the accuracy of the data and analyses, and approved the final version for submission.

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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

Policy Addressed

This study supports policies promoting indigenous fungal biodiversity conservation, molecular authentication of native Terfezia species, and development of truffle-derived biopesticides for integrated disease management. Adoption of these sustainable bio-based antimicrobials can reduce synthetic bactericide use, improve oyster mushroom productivity and quality, and strengthen environmentally responsible agricultural production systems.

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