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Research Article

Integrated Management of Wet Bubble Disease Caused by *Hypomyces perniciosus* in *Agaricus bisporus* Through Essential Oils and Virulence-Factor Inhibitors

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ABSTRACT

This study was conducted at the Mushroom Production Farm, College of Agriculture, Tikrit University, from November 15, 2023, to April 4, 2024, to identify the causal agent of wet bubble disease in Agaricus bisporus and to assess the effectiveness of selected chemical compounds and essential oils on disease control, yield, and mushroom quality. Morphological and molecular analyses confirmed *Hypomyces perniciosus* as the causal agent, verified by sequencing the ITS region of the 5.8S rRNA gene. The most virulent isolate was deposited in the NCBI database under accession number PP320509.1. Essential oils of thyme, eucalyptus, lemon, coconut, and black seed were evaluated against *H. perniciosus* and *A. bisporus*. Thyme, eucalyptus, and lemon oils exhibited the highest inhibition of pathogen growth (72.94%, 68.23%, and 74.12%, respectively) while causing minimal inhibition of *A. bisporus* (15.79-19.74%). Mineral salts (EDTA, $MgSO_4$, $FeSO_4$, $MnSO_4$, $CuSO_4$, and SDS) were assessed for their effects on chitinase and glucanase activities of *H. perniciosus*. EDTA, $MgSO_4$, and $FeSO_4$ at 0.5 M significantly reduced chitinase activity (0.68-0.89 U/ml) compared with the control (2.63 U/ml), while EDTA at 0.25-0.5 M showed the greatest suppression of glucanase activity (0.36-0.39 U/ml). In cultivation trials, thyme oil resulted in the lowest disease severity (1.62% and 2.83%) in white and brown strains, respectively. EDTA and $FeSO_4$ also markedly reduced disease severity relative to the control. The highest biocontrol efficiency and protein content in fruiting bodies were recorded with thyme oil and selected mineral salts, particularly $FeSO_4$ and $MgSO_4$.

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Introduction

The white button mushroom, *Agaricus bisporus*, is the most widely cultivated edible fungus globally, with annual production exceeding 6 million tons and an estimated annual growth rate of approximately 7% (Kakraliya et al., 2020). It is of considerable importance to consumers owing to its economic value as well as its nutritional and medicinal attributes (Nasiri et al., 2017; Chechan, 2020). *A. bisporus* is a rich source of vitamins, minerals, high-quality proteins, and antioxidants (Nadir et al., 2020). Mushroom cultivation represents a major commercial biotechnology that efficiently utilizes microorganisms to recycle agricultural residues on a large scale, converting them into highly nutritious food products (Kakraliya et al., 2020). Consequently, mushroom production has emerged as a rapidly expanding and dynamic global industry (Royse et al., 2017).

Despite its economic significance, *A. bisporus* is

susceptible to numerous diseases that cause substantial yield losses, primarily due to fungal pathogens, followed by bacterial and viral agents. Among these, wet bubble disease, caused by *Mycogone perniciosa* (= *Hypomyces perniciosus*), is regarded as one of the most destructive diseases of cultivated mushrooms worldwide. The disease manifests in two distinct symptom types: when infection occurs prior to differentiation of the fruiting body into stipe and pileus, severely malformed structures develop that bear little resemblance to normal edible mushrooms. In contrast, infections occurring after differentiation result in thickened and distorted fruiting bodies with deformed gills. Additional symptoms include white mycelial growth on the mushroom surface, tissue softening and rot accompanied by an unpleasant odor, and the exudation of golden-brown droplets. Fan et al. (2012) reported yield losses ranging from 15-30% in China, with severe epidemics leading to complete crop failure. Elevated temperature and humidity further favor disease development and dissemination. Therefore, prompt removal and destruction of infected fruiting bodies are essential to reduce pathogen spread.

The rapid growth and high absorptive capacity of *A. bisporus* make the use of chemical fungicides particularly risky, as pesticide residues can readily accumulate in the edible tissues. Moreover, chemical control strategies pose serious concerns regarding human health, environmental safety, and the potential development of pathogen resistance. Although chemical pesticides have historically been used for mushroom disease management, regulatory restrictions have intensified in recent years. In the European Union, the number of approved synthetic active substances declined from 320 in 2017 to 234 in 2022, and it is projected that up to 50% of the remaining compounds may be withdrawn or lose authorization within the next decade (Marchand, 2023). Consequently, increasing attention has been directed toward environmentally benign alternatives, including plant-derived essential oils (Regnier and Combrinck, 2010) and biological control agents (Navarro et al., 2023; Clarke et al., 2024). In addition, the development and deployment of disease-resistant *Agaricus* strains remain among the most effective and sustainable strategies for long-term disease management (Fu et al., 2016).

Given the nutritional and health-related importance of *A. bisporus* and the severe economic losses caused by wet bubble disease induced by *H. perniciosus*, the present study was undertaken to evaluate safe and natural alternatives, specifically essential oils and selected virulence factor inhibitors, for the integrated management of this economically important disease.

Materials and Methods

Laboratory experiments

Strains of *A. bisporus*

Two Dutch-origin strains of *A. bisporus* were used in this study, namely the A15 strain and the brown strain. Both strains were obtained from the Mushroom Production Farm, College of Agriculture, Tikrit University.

Isolation and identification of the pathogenic fungus

Isolation of *H. perniciosus*

Seven isolates of the pathogenic fungus *H. perniciosus* were collected, including five isolates from Al-Wadaq Farm, Baghdad, and two isolates from the Mushroom Farm, College of Agriculture, Tikrit University. The isolates were initially identified based on morphological characteristics, including colony morphology, mycelial structure, and conidial features. Molecular confirmation of the most virulent isolate was performed through nucleotide sequence analysis of the internal transcribed spacer (ITS) region of the 5.8S rRNA gene, following the protocol described by Hassan et al. (2022).

Pathogenicity test of *H. perniciosus*

Pathogenicity tests were conducted by inoculating fruiting bodies of *A. bisporus* with a spore suspension of *H. perniciosus*. For each isolate, a freshly grown, actively growing 96-h-old colony was used. Twenty milliliters of sterile distilled water were added to each Petri dish, and the fungal growth was gently scraped using sterile cotton swabs to prepare the inoculum. The concentration of the spore suspension was adjusted to 10^8 CFU ml^{-1} using a hemocytometer.

Healthy fruiting bodies of *A. bisporus* were surface-sterilized with 3% sodium hypochlorite solution for 2 min and subsequently rinsed thoroughly with sterile distilled water. The sterilized fruiting bodies were then immersed in the spore suspension of each fungal isolate. Ten fruiting bodies were used per isolate. Following inoculation, the treated fruiting bodies were incubated at room temperature.

Disease severity was evaluated after incubation using the five-point disease rating scale described by Li et al. (2019a), as follows:

0 = no visible symptoms;

1 = 1-10% disease severity;

2 = 11-25% disease severity;
3 = 26-50% disease severity;
4 = 51-75% disease severity;
5 = >75% disease severity.

Disease symptoms considered during assessment included discoloration, deformation of fruiting bodies, tissue necrosis, and the presence of foul-smelling brown exudates. Disease severity was calculated using McKinney's disease severity index formula (McKinney, 1923).

$$\text{Disease Severity} = \frac{\sum(Number\ of\ infected\ fruiting\ bodies\ at\ degree\ 0\times0+\cdots+Number\ of\ infected\ fruiting\ bodies\ at\ degree\ 4\times4)}{(\text{Highest disease class} \times \text{Total number of fruiting bodies})} \times 100$$

Production of chitinase and glucanase enzymes by the pathogenic fungus *H. perniciosus*

A minimal salt medium for fungal growth was prepared by dissolving 1.73 g K_2HPO_4, 0.68 g KH_2PO_4, 0.68 g $MgSO_4 \cdot 7H_2O$, 4.0 g NaCl, 0.30 g $FeSO_4$, and 0.02 g $CaCl_2 \cdot 2H_2O$ in distilled water, as described by Nwachukwu (2000). To evaluate enzyme production, either 1% (w/v) chitin or 1% (w/v) β-glucan was added separately to the medium as the sole carbon source for the induction of chitinase and glucanase, respectively.

Each medium was inoculated with three agar plugs (0.5 cm diameter) taken from the actively growing margin of a *H. perniciosus* colony and incubated at 25°C in a shaking incubator at 80 rpm for five days. Following incubation, the culture broth was filtered through Whatman filter paper and centrifuged at 5,000 rpm for 10 min. The clear supernatant obtained was used as the crude enzyme extract for subsequent analyses.

Estimation of the inhibitory effects of mineral salts on chitinase and glucanase activity

The inhibitory effects of mineral salts and chemical agents on chitinase and glucanase activities were evaluated using three concentrations (0.50, 0.25, and 0.125 M) of $FeSO_4$, $MgSO_4$, $CuSO_4$, $MnSO_4$, sodium dodecyl sulfate (SDS), and ethylenediaminetetraacetic acid (EDTA).

For each assay, 0.5 ml of the crude enzyme extract was mixed with 0.5 ml of 1% chitin solution and 0.5 ml of the respective salt or chemical solution. Enzyme activities of both chitinase and glucanase were then determined following the method described by Hassan et al. (2022).

Effect of selected essential oils on the growth of the pathogenic fungus *H. perniciosus* and the edible mushroom *A. bisporus*

Potato Dextrose Agar (PDA) medium was amended with thyme, eucalyptus, and lemon essential oils at concentrations of 1, 3, and 5% (v/v). Each essential oil was emulsified using 1% Tween 80 and sterilized by autoclaving at 121°C and 1.5 kg cm^{-2} pressure for 15 min. After sterilization and cooling, chloramphenicol was added to the medium at a concentration of 250 mg L^{-1} to inhibit bacterial contamination. The medium was then poured into sterile Petri dishes and allowed to solidify.

Each plate was inoculated separately with *H. perniciosus* or *A. bisporus* by placing a 0.5 cm diameter mycelial disc taken from the margin of a 5-day-old PDA culture at the center of the plate. All treatments were conducted in triplicate. Control plates containing PDA without essential oils were similarly inoculated. Plates were incubated at 25°C for 7 days.

When the mycelium in the control plates reached the edge of the Petri dishes, radial growth inhibition was assessed by measuring the mean colony diameter along two perpendicular axes using a ruler, following the method of Ibrahim and Hassan (2023).

Mushroom cultivation experiment in growing rooms

Cultivation and production of fruiting bodies

The fungal inoculum of the edible mushroom *A. bisporus* (strains A15 and Brown) and the organic compost were prepared according to the procedures described by Hassan et al. (2022). All stages of mushroom cultivation, from spawning to fruiting body production, were carried out under controlled growing-room conditions. Each experimental unit consisted of a 20 kg compost block inoculated with 2% (w/w) mushroom spawn and packed into nylon bags.

Experimental treatments

The fruiting body production experiment was conducted at the Mushroom Farm, College of Agriculture, University of Tikrit, Iraq. Based on laboratory screening results, the following treatments were applied:

1. *Hypomyces perniciosus* only
2. Control (untreated)
3. *H. perniciosus* + $FeSO_4$
4. *H. perniciosus* + $MgSO_4$
5. *H. perniciosus* + EDTA
6. *H. perniciosus* + thyme oil
7. *H. perniciosus* + eucalyptus oil
8. *H. perniciosus* + lemon oil

Treatments were applied to the casing soil at a rate of 100 kg m^{-2}, with each treatment replicated five times

(five blocks or bags). Each block contained 20 kg of spawned compost. The casing layer was first sprayed with 100 ml of a *H. perniciosus* suspension at a concentration of 1×10^8 CFU ml^{-1}. The treated blocks were then covered with nylon sheets and incubated for three days.

Subsequently, 100 ml of each salt solution (0.5 M) and 100 ml of each essential oil solution (5%) were applied. All treatments were evaluated on both *A. bisporus* strains (A15 and Brown).

Studied traits

Fresh weight

Fresh fruiting bodies were harvested at each flush and weighed using a precision balance. Yield was expressed as grams of fresh fruiting bodies per 20 kg of substrate.

Biological efficiency

Biological efficiency (BE) was calculated using the equation described by Hassan et al. (2022a), as presented below.

$$BE = \frac{\text{Fresh weight of fruiting bodies}}{\text{Dry weight of organic compost}} \times 100$$

Protein content estimation

Protein content was determined using the semi-micro Kjeldahl method by quantifying total nitrogen, and the protein percentage was calculated accordingly (AOAC, 2002).

$$\text{Protein percentage on dry weight basis} = \text{Protein content in dry mushroom powder} \times 6.25$$

Statistical analysis

All experiments were arranged in a Completely Randomized Design (CRD). Data were subjected to analysis of variance (ANOVA) using SPSS statistical software. Treatment means were separated using Duncan's Multiple Range Test and the Least Significant Difference (LSD) test at a 5% level of significance (Al-Rawi and Khalaf Allah, 1980).

Results and Discussion

Morphological diagnosis

Colonies grown on potato dextrose agar (PDA) for seven days at 25°C exhibited coloration ranging from white to off-white (Figure 1A). The mean colony growth rate was 9.96 mm day^{-1}.

In the asexual reproductive stage of *H. perniciosa*, two types of spores were observed:

1. Chlamydospores

These consisted of distinct upper and lower cells. The upper cell was brownish-yellow to brown-gray in color, whereas the lower cell was colorless and transparent. Chlamydospores measured 15.72-20.20 µm in width and 25.61-28.33 µm in length (Figure 1B).

2. Conidia

Conidia were thin-walled, hyaline, and elongated, with dimensions ranging from 9-12 µm in length and 3-7 µm in diameter (Figure 1C).

Microscopic observations indicated that the pathogen produced chlamydospores in considerably greater abundance than conidia (Figure 1D). The identification of *H. perniciosa* based on these morphological characteristics is in agreement with previously published descriptions (Asef and Zare, 2006; Li et al., 2019b; Gu et al., 2021; Karimi et al., 2022).

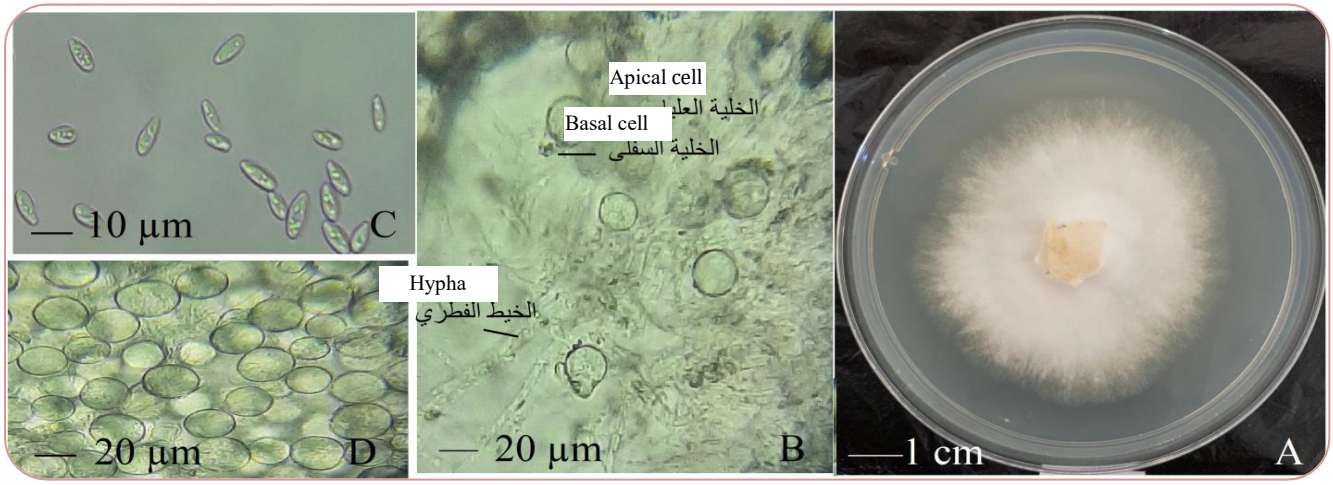

Figure 1. Microscopic and morphological characteristics of *H. perniciosus* colonies: (A) Colony growth on potato dextrose agar (PDA); (B) chlamydospores; (C) conidia; and (D) clusters of chlamydospores.

Pathogenicity of *H. perniciosa* isolates

Figure 2 illustrates the pathogenicity of seven *H. perniciosa* isolates obtained from the casing layers of farms in Al-Wadiq (Baghdad) and from the University of Tikrit (Salahuddin). The results showed that all isolates caused varying levels of infection intensity in the fruiting bodies of *A. bisporus*. Isolate H-7 exhibited the highest infection intensity (92.44%), whereas isolate H-5 showed the lowest (72.92%).

These findings indicate the presence of pathogenic variability among *H. perniciosa* isolates, resulting in differences in infection severity. Such variability may be attributed to differences in the genetic makeup of the isolates, which are reflected in their physiological behavior during infection of the edible mushroom. Genetic diversity among isolates of pathogenic fungi and its association with differential pathogenicity has been reported in several previous studies (Hassan et al., 2022; Ahmed and Hassan, 2024).

The higher infection intensity observed in certain isolates may be due to the presence of specific virulence factors, such as extracellular digestive enzymes that degrade mushroom fruiting bodies or the production of inhibitory proteins that suppress the growth of *A. bisporus* (Hassan et al., 2022b).

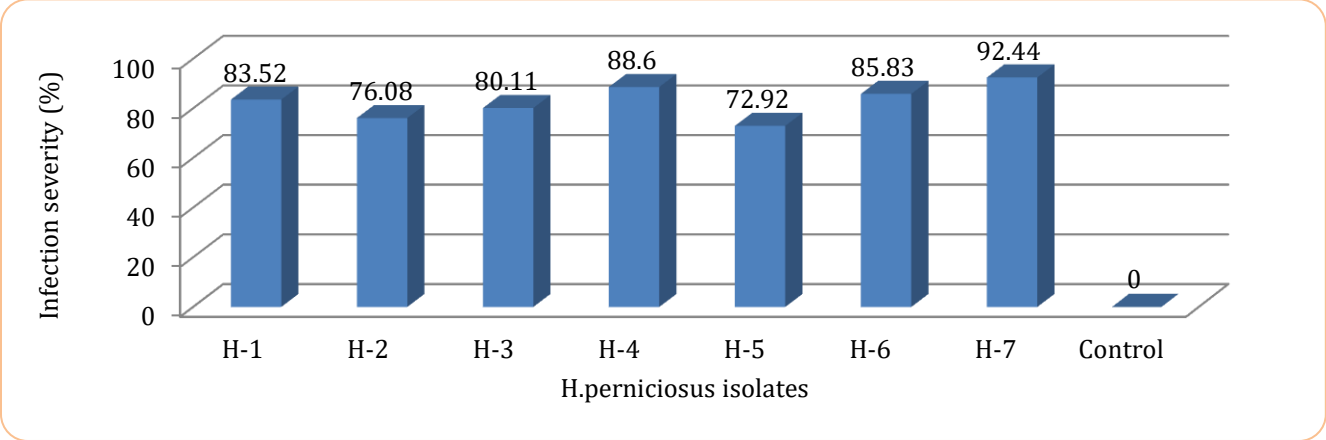

Figure 2. Pathogenicity of *H. perniciosus* isolates on the edible mushroom *A. bisporus*, shown as infection severity (%).

Molecular Identification

The fungal isolates were identified to the species level through nucleotide sequence analysis. The phylogenetic tree (Figure 3) demonstrated a high degree of genetic similarity among several *H. perniciosus* strains isolated from different countries. The highest sequence similarity (99.16%) was observed with *H. perniciosus* strain HP3, originally isolated from China (GenBank accession no. MK584650.1).

The *H. perniciosus* isolate Aya-7 obtained in the present study was deposited in the NCBI GenBank database under accession number PP320509.1. This represents the first molecular-level record of this fungal species from Iraq.

Analysis of the 5.8S rRNA gene nucleotide sequence showed complete concordance between *H. perniciosus* isolate Aya-7 and globally reported isolates. Minor nucleotide variations reported among isolates may arise due to environmental influences, intensive use of chemical pesticides, or soil and air contamination, which can induce genetic mutations and result in sequence divergence (Hassan et al., 2022a).

Effect of selected mineral salts on the inhibition of chitinase enzyme extracted from the pathogenic fungus *H. perniciosus*

The results presented in Figure 4 illustrate the effects of selected mineral salts on chitinase activity. The data revealed significant differences among the treatments. EDTA, $MgSO_4$, and $FeSO_4$, applied at concentrations of 0.25 M and 0.5 M, exhibited the strongest inhibitory effects on chitinase activity. Specifically, chitinase activities were reduced to 0.72 and 0.68 units mL^{-1} for EDTA, 0.94 and 0.89 units ml^{-1} for $MgSO_4$, and 0.89 and 0.82 units ml^{-1} for $FeSO_4$ at 0.25 M and 0.5 M, respectively, compared with the control, which showed an activity of 2.63 units ml^{-1}. In contrast, $MnSO_4$ enhanced chitinase activity at both tested concentrations. Chitinase activities of 2.78 and 2.86 units ml^{-1} were recorded at 0.25 M and 0.5 M $MnSO_4$, respectively, exceeding the activity observed in the control treatment.

Figure 3. Phylogenetic tree of multiple *H. perniciosus* strains isolated from different countries.

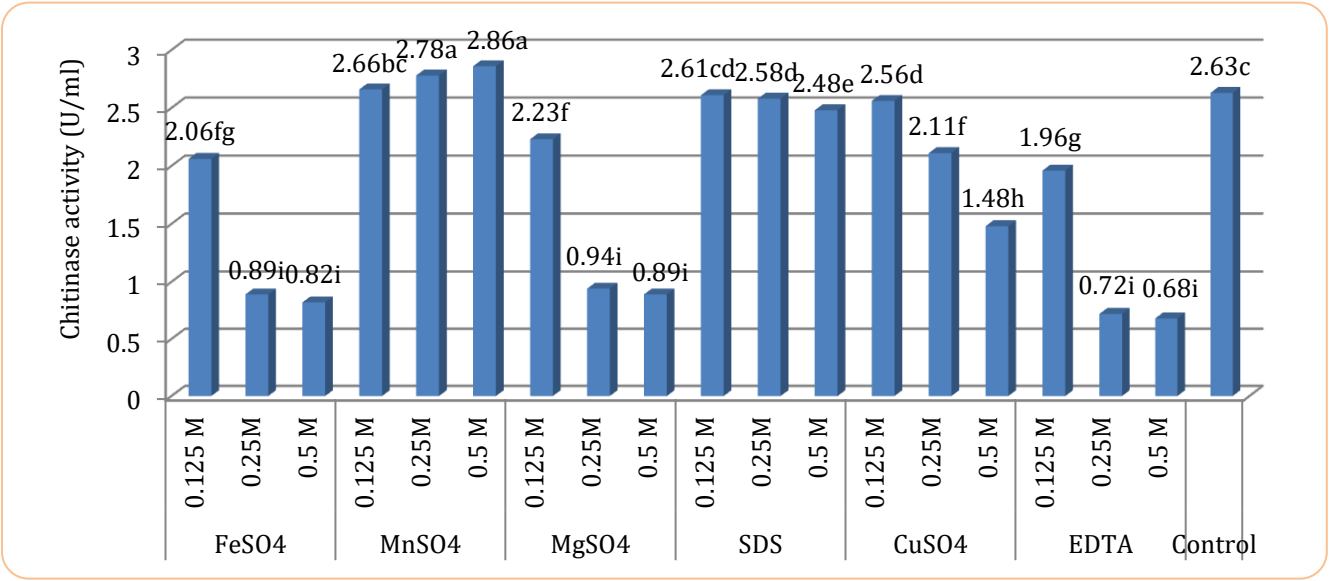

Figure 4. Effect of selected mineral salts on the inhibition of chitinase extracted from the pathogen *H. perniciosus.*

Effect of selected mineral salts on the inhibition of β-glucanase extracted from the pathogenic fungus *H. perniciosus*

The results presented in Figure 5 demonstrated variation in the inhibitory effects of different mineral salts on the activity of β-glucanase extracted from *H. perniciosus*. Treatment with EDTA at concentrations of 0.25 M and 0.5 M resulted in the greatest inhibition, with the lowest enzymatic activities of 0.39 and 0.36 U ml^{-1}, respectively. This was followed by $MgSO_4$ and $FeSO_4$ at the same concentrations, which also markedly reduced enzyme activity. In contrast, the control exhibited a substantially higher β-glucanase activity of 2.3 U ml^{-1}. At lower concentrations, $MnSO_4$, SDS, and $CuSO_4$ did not cause significant inhibition of β-glucanase activity.

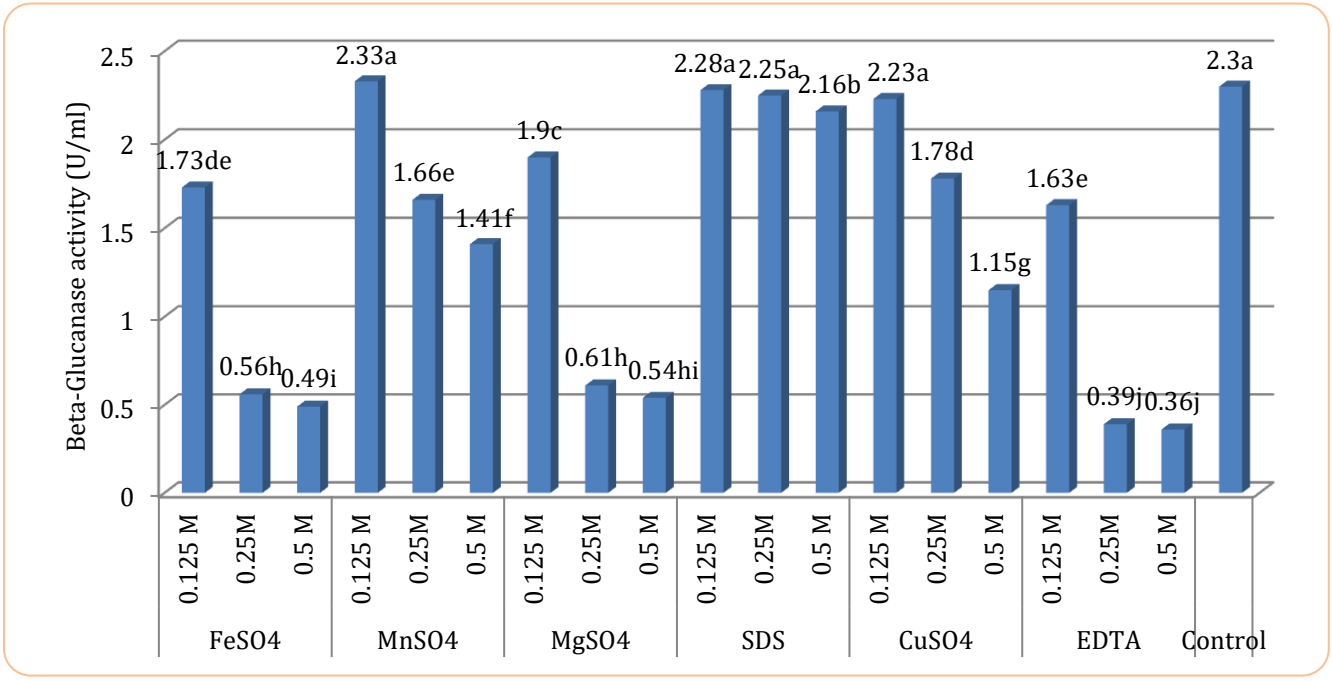

Figure 5. Effect of selected mineral salts on the inhibition of β-glucanase Extracted from the pathogen *H. perniciosus.*

Chitinase is a key enzyme produced by pathogenic fungi and plays a critical role in host invasion by degrading chitin in the cell wall of edible mushrooms. It cleaves glycosidic bonds within chitin polymers, yielding N-acetylglucosamine units and thereby facilitating fungal penetration and tissue colonization (Yang et al., 2018). β-Glucanase, in contrast, hydrolyzes β-glucans, the second most abundant structural component of the fungal hyphal cell wall, releasing glucose-rich mono-, di-, and oligosaccharides that are essential for fungal growth and nutrition. Consequently, inhibition of these two enzymes disrupts cell wall degradation, limits pathogen penetration, and protects edible mushroom tissues.

Mineral elements play diverse roles in regulating enzymatic activity; some function as activators, whereas others act as inhibitors. These elements may bind to the active site of enzymes, blocking substrate access and suppressing catalytic activity, or induce conformational changes that reduce enzyme efficiency. In addition, certain metal ions can interfere with enzyme-mediated chemical reactions, further diminishing enzymatic performance (Cheba et al., 2016).

Effect of selected essential oils on the growth of the pathogenic fungus *H. perniciosus* and the edible mushroom *A. bisporus*

The results presented in Figure 6 revealed statistically significant differences among treatments. Growth inhibition of both fungi increased progressively with increasing concentrations of essential oils. Thyme, eucalyptus, and lemon oils at a concentration of 5% exhibited the strongest inhibitory effects against the pathogenic fungus compared with the edible mushroom. The radial growth of *H. perniciosus* declined from 8.5 cm in the control to 2.3, 2.7, and 2.2 cm, respectively. In contrast,

the growth of *A. bisporus* decreased modestly, from 7.6 cm in the control to 6.4, 6.2, and 6.1 cm, respectively.

In percentage terms, growth inhibition caused by thyme, eucalyptus, and lemon oils was markedly higher in the pathogenic fungus than in the edible mushroom. The inhibition rates for *H. perniciosus* were 72.94%, 68.23%, and 74.12%, respectively, whereas growth reduction in *A. bisporus* was limited to 15.79%, 18.42%, and 19.74%, respectively.

Mushroom production experiments in growing rooms

The results presented in Table 1 demonstrate the effects of selected chemical agents and essential oils on the total infection severity (%) of two *A. bisporus* strains following inoculation with the pathogenic fungus *H. perniciosus*. All treatments resulted in a significant reduction in infection severity compared with the control. The combined treatment of *H. perniciosus* and thyme essential oil produced the lowest infection severity, recording values of 1.62% in the white strain and 2.83% in the brown strain.

Among the mineral salt treatments, EDTA and $FeSO_4$ markedly reduced infection severity to 5.66% in the white strain and 3.26% in the brown strain, respectively. In contrast, the untreated control exhibited very high infection severity, reaching 97.17% in the white strain and 94.39% in the brown strain.

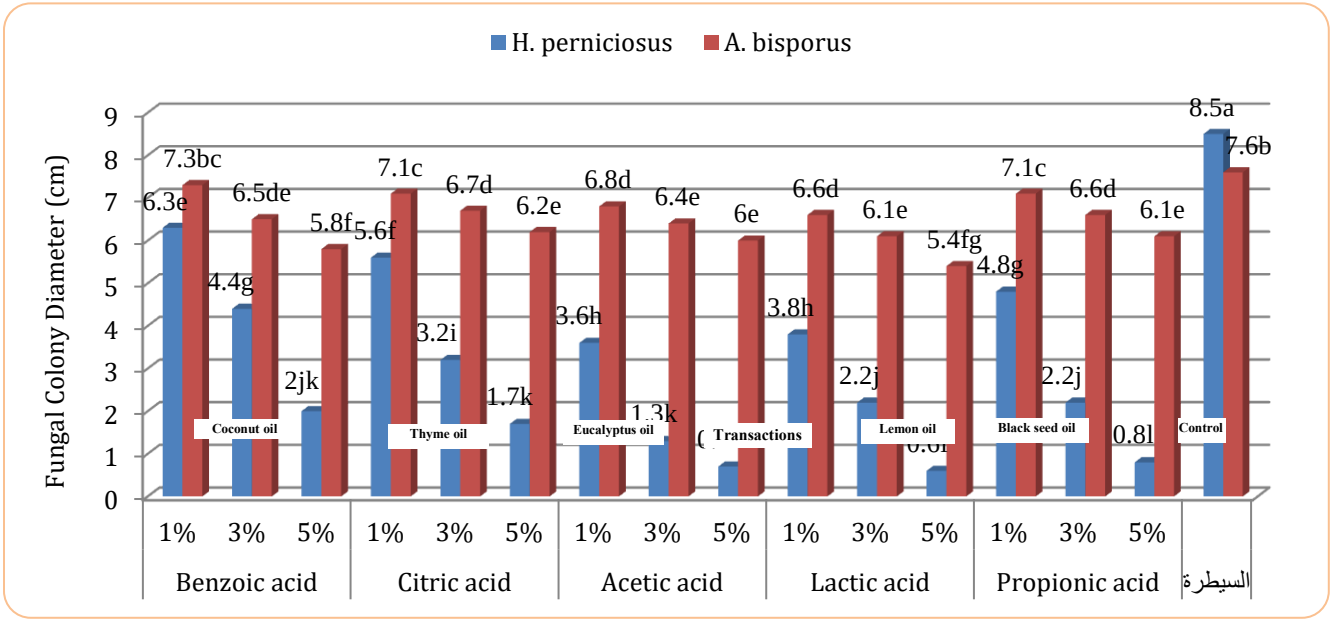

Figure 6. Effects of selected essential oils on the growth of the pathogenic fungus *H. perniciosus* and the edible mushroom *A. bisporus.*

Table 1. Effect of selected chemical agents and essential oils on the total infection severity (%) of two *A. bisporus* strains inoculated with *H. perniciosus*.

Treatments	*A. bisporus* fungus Strain		Mean of Treatments
	White Strain	Brown Strain	
H. perniciosus only	97.17	94.39	95.78
H. perniciosus + $FeSO_4$	6.78	3.26	5.02
H. perniciosus + $MgSO_4$	6.99	4.02	15.5
H. perniciosus + EDTA	5.66	5.42	5.54
H. perniciosus + Thyme Oil	1.62	2.83	32.2
H. perniciosus + Lemon Oil	4.97	5.34	65.1
H. perniciosus + Eucalyptus Oil	5.9	6.31	16.1
Control (untreated)	0	0	0
Mean of Strains	11.21	10.56	

LSD 0.05: Treatments = 1.23; Strains = 1.02; Interaction = 1.47.

Production indicators

Total fresh weight of *A.bisporus* strains

The results presented in Table 2 demonstrate the effects of selected chemical agents and essential oils on the total fresh weight (kg per 20 kg compost) of fruiting bodies of two *A. bisporus* strains under infection with the pathogenic fungus *H. perniciosus*. All treatments resulted in a significant increase in productivity compared with the *H. perniciosus*-only control.

The combined treatment of *H. perniciosus* and thyme oil produced the highest fresh weight in both the white and brown strains, reaching 6.18 and 5.66 kg per 20 kg compost, respectively. Among the chemical agents, $FeSO_4$ and $MgSO_4$ showed the highest productivity in the brown strain, yielding 5.48 and 5.15 kg per 20 kg compost, respectively, with no significant difference between them. In contrast, the *H. perniciosus*-only treatment recorded markedly lower fresh weights of 0.40 and 0.83 kg per 20 kg compost for the white and brown strains, respectively.

Biological efficiency (%)

Table 3 summarizes the effects of selected chemical agents and essential oils on the biological efficiency (%) of two *A. bisporus* strains under infection with *H. perniciosus*. Significant differences were observed among the treatments. The combined treatment of *H. perniciosus* and thyme oil resulted in the highest biological efficiency, reaching 103.07% in the white strain and 94.40% in the brown strain.

Among the chemical agents, $FeSO_4$ and $MgSO_4$ produced the highest biological efficiency in the brown strain, with values of 91.27% and 85.70%, respectively, and no significant difference between these two treatments. In contrast, the *H. perniciosus*-only treatment resulted in markedly reduced biological efficiency, recording 6.70% and 13.77% in the white and brown strains, respectively.

Table 2. Effect of Selected chemical agents and essential oils on the total fresh weight (kg/20 kg compost) of fruiting bodies of two *A. bisporus* Strains Infected with *H. perniciosus*.

Treatments	*A. bisporus* fungus Strain		Mean of Treatments
	White Strain	Brown Strain	
H. perniciosus only	0.4	0.83	0.62
H. perniciosus + $FeSO_4$	3.95	5.48	4.72
H. perniciosus + $MgSO_4$	3.86	5.15	4.51
H. perniciosus + EDTA	4.44	4.54	4.49
H. perniciosus + Thyme Oil	6.18	5.66	5.92
H. perniciosus + Lemon Oil	4.73	4.57	4.65
H. perniciosus + Eucalyptus Oil	4.33	4.16	4.25
Control (untreated)	4.44	4.42	4.43
Mean of Strains	4.10	4.34	

LSD 0.05: Treatments = 0.25; Strains = 0.26; Interaction = 0.34.

Table 3. Effect of selected chemical agents and essential oils on the biological efficiency (%) of two *A. bisporus* strains infected with *H. perniciosus*.

Treatments	*A. bisporus* fungus Strain		Mean of Treatments
	White Strain	Brown Strain	
H. perniciosus only	6.7	13.77	10.24
H. perniciosus + $FeSO_4$	65.87	91.27	78.57
H. perniciosus + $MgSO_4$	64.33	85.8	75.07
H. perniciosus + EDTA	73.93	75.67	74.8
H. perniciosus + Thyme Oil	103.07	94.4	98.74
H. perniciosus + Lemon Oil	78.9	76.23	77.57
H. perniciosus + Eucalyptus Oil	72.2	69.25	70.73
Control (untreated)	73.93	73.73	73.83
Mean of Strains	68.39	72.27	

LSD 0.05: Treatments = 3.16; Strains = 3.32; Interaction = 3.78.

Protein content (%)

The results presented in Table 4 demonstrate clear treatment-dependent variation in the protein content of *A. bisporus*. The combined treatment of *H. perniciosus* with thyme oil produced the highest mean protein content in both white and brown strains, reaching 28.34% and 28.40%, respectively. Among the chemical treatments, $FeSO_4$, $MgSO_4$, and EDTA resulted in comparatively high protein contents in the brown strain (28.22%, 27.89%, and 27.28%, respectively), with no statistically significant differences among them. In contrast, the treatment with *H. perniciosus* alone recorded substantially lower protein contents, amounting to 7.11% in the white strain and 7.82% in the brown strain.

Table 4. Effect of selected chemical agents and essential oils on the protein content (%) of two *A. bisporus* strains infected with *H. perniciosus.*

Treatments	*A. bisporus* fungus Strain		Mean of Treatments
	White Strain	Brown Strain	
H. perniciosus only	7.11	7.82	7.47
H. perniciosus + $FeSO_4$	25.42	28.22	26.82
H. perniciosus + $MgSO_4$	25.33	27.89	26.61
H. perniciosus + EDTA	25.91	27.28	26.6
H. perniciosus + Thyme Oil	28.34	28.4	28.37
H. perniciosus + Lemon Oil	26.89	27.31	27.1
H. perniciosus + Eucalyptus Oil	25.8	26.9	26.35
Control (untreated)	24.31	25.16	24.74
Mean of Strains	24.72	25.97	

LSD 0.05: Treatments = 1.12; Strains = 1.06; Interaction = 1.33.

Discussion

Essential oils comprise a wide range of bioactive compounds with well-documented antifungal properties. Thyme oil contains the phenolic compounds thymol and carvacrol, which exert antifungal activity primarily by disrupting fungal cell membranes through the solubilization of lipids and phospholipids. This disruption leads to leakage of intracellular constituents, disturbance of ionic homeostasis (K^+ and H^+), and inhibition of mitochondrial enzymes, ultimately resulting in fungal cell death (Hyldgaard et al., 2012). Lemon oil is rich in limonene, β-pinene, and γ-terpinene, which compromise plasma membrane integrity, increase membrane permeability, and cause leakage of cellular components. In addition, these compounds inhibit cellular respiration by interfering with mitochondrial function (Fisher and Phillips, 2008). Eucalyptus oil contains 1,8-cineole (eucalyptol), α-pinene, and limonene, which disrupt fungal membrane structure, impair membrane-associated proteins, and inhibit hyphal growth. These effects are often accompanied by suppression of the fungal antioxidant system, leading to the accumulation of reactive oxygen species (ROS) and subsequent oxidative damage (Tyagi and Malik, 2011). Collectively, the antifungal activity of essential oils extends beyond mycelial growth inhibition to include suppression of pathogenic spore germination (Glamočlija et al., 2009).

The results of the present study demonstrated that essential oils exerted a stronger inhibitory effect on the pathogenic fungus *H. perniciosus* than on the cultivated mushroom *A. bisporus*. This differential sensitivity may be explained by taxonomic and structural differences between the two fungi. *H. perniciosus* belongs to the phylum Ascomycota, whereas *A. bisporus* is a basidiomycete. Basidiomycetes generally possess cell walls enriched with chitin and β-glucans, which reduce membrane permeability and limit the penetration of essential oil components. Furthermore, basidiomycete membranes typically contain higher levels of ergosterol, conferring greater membrane stability against compounds that disrupt lipid bilayers. Additional tolerance mechanisms in basidiomycetes include well-developed detoxification systems involving cytochrome P450 enzymes, laccases, and peroxidases, which can metabolize terpenes and phenolic compounds into less toxic derivatives. Efflux mechanisms also contribute to

tolerance, as ABC and MFS transporter families actively expel hydrophobic compounds, including essential oil constituents, thereby reducing intracellular accumulation (Isikhuemhen et al., 2009; Taheri et al., 2023).

Moreover, treatments targeting the pathogenic fungus were applied to the casing layer, where *H. perniciosus* actively proliferates, resulting in direct exposure to essential oils and mineral salts. In contrast, *A. bisporus* primarily colonizes the compost layer, which likely reduced its exposure to these treatments and minimized adverse effects on its growth.

The observed reduction in yield and protein content of *A. bisporus* under pathogen-only treatment can be attributed to the aggressive colonization of fruiting bodies by *H. perniciosus* and the secretion of hydrolytic enzymes, such as chitinases, glucanases, and proteases. These enzymes degrade fungal cell walls and proteins, enabling the pathogen to utilize decomposition products as nutrient sources (Saadi and Hassan, 2023). In contrast, treatments involving essential oils and mineral salts restored fruiting body yield to levels comparable to the pathogen-free control. This improvement is likely due to the suppression of the pathogenic fungus through direct antifungal effects of essential oils and indirect inhibition of pathogen virulence by mineral salts. These mechanisms may also account for the increased protein content observed in fruiting bodies under these treatments.

The antifungal efficacy of essential oils against pathogenic fungi associated with cultivated mushrooms is consistent with previous findings reported for *Pleurotus* spp. (Hassan and Ibrahim, 2022), while the role of mineral salts in suppressing pathogen virulence agrees with the observations of Al-Darraji and Hassan (2022).

Mineral salts inhibit chitinase and glucanase activity, enzymes that function as key virulence factors enabling pathogenic fungi to invade *A. bisporus* tissues. Since the fruiting bodies of the cultivated mushroom are fully developed and chitin and glucan biosynthesis is largely complete at maturity, these inhibitors exert little or no detrimental effect on *A. bisporus*. In contrast, inhibition of these enzymes effectively suppresses the growth and pathogenic activity of *H. perniciosus*. These findings are consistent with Ahmed and Hassan (2024), who reported that inhibition of virulence-related enzymes in *Lecanicillium fungicola*, the causal agent of dry bubble disease in *A. bisporus*, using zinc sulfate significantly reduced disease incidence.

Conclusions

The findings of this study confirm that *H. perniciosus* is the primary causal agent of wet bubble disease in *A. bisporus*, as verified through both morphological and molecular identification. Essential oils, particularly thyme oil, exhibited strong antifungal activity against the pathogen while exerting minimal adverse effects on the cultivated mushroom. In addition, selected mineral salts, including EDTA, $FeSO_4$, and $MgSO_4$, effectively reduced the activity of virulence-associated enzymes and alleviated disease severity. *In vivo* application under commercial mushroom cultivation conditions demonstrated that thyme oil, in combination with iron and magnesium salts, provided the most effective disease suppression, improved biological efficiency and yield, and enhanced protein content in the fruiting bodies. Overall, these results highlight the potential of essential oils and mineral salts as environmentally friendly and effective alternatives for managing wet bubble disease and improving the productivity and quality of cultivated mushrooms.

Authors' Contributions

AMMA designed the study, prepared the materials, collected and analyzed the data, and assisted with disease scoring. AAKH supervised the study. AMMA wrote the manuscript, and all authors reviewed and approved the final version.

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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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