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Evaluation of the Efficacy of Organic Acids and Some Bacteria in Controlling Wet Blister Disease Caused by the Fungus *Hypomyces perniciosus* on the Edible Mushroom *Agaricus bisporus*

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I. Abstract

This study was conducted at the edible mushroom production farm, Al-Kalsa Agriculture – Tikrit University. It aimed to evaluate the efficacy of organic acids and some bacteria in controlling wet blister disease caused by the fungus *Hypomyces perniciosus* on the edible mushroom *Agaricus bisporus*. Seven bacterial isolates were obtained from the covering layer used in several Iraqi mushroom farms. Isolates A4, A5, and A7 showed the highest activity. The inhibition ratio of the pathogenic fungus in the inhibition zone reached 2.2, 1.8 and 1.5 cm, and these are the most efficient isolates in inhibiting the pathogen. The bacterial isolates A4, A5 and A7 were molecularly identified as *Priestia aryabhattai* strain Aya-4, *Frateuria aurantia* strain Aya-5 and *Pseudomonas fluorescens* strain Aya-6 and were recorded with accession numbers PP320436.1, PP320435.1 and PP320438.1, respectively, at the NCBI site. The results showed an inverse relationship between the growth of the pathogenic fungus *H. perniciosus* and the edible fungus *A. bisporus* with increasing concentrations of the tested organic acids. The results also indicated that the effect of organic acids in inhibiting the growth of *H. perniciosus* was greater than their effect on *A. bisporus*. Propionic, lactic, and acetic acids at a 5% concentration exhibited the highest inhibitory effect compared to the other acids. When studying the effect of three types of bacteria and organic acids on the overall infection severity of two strains of *A. bisporus* under conditions of infection with *H. perniciosus*, the results showed a significant decrease in infection severity for all types of organic acids and isolated bacteria in both the white and brown strains. Citric and propionic acids showed the lowest reduction in infection severity, at 5.9% and 5.98%, respectively, in the brown strain, while *F. aurantia* showed the lowest severity. The infection rate was 3.54% in the white strain of the edible mushroom *A. bisporus*, compared to infection rates of 94.39% and 97.17% in the H. perniciosus treatment of the brown and white edible mushroom strains, respectively. The results also showed a significant increase in fruiting body weights for all organic acids and isolated bacteria in both the white and brown edible mushroom strains.

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Benzoic acid resulted in the highest fruiting body weight of 4.58 kg/20 kg of fermented manure in the brown strain, surpassing the control treatment (not treated with the pathogenic mushroom). Treatments with citric acid and *F. aurantia* yielded the highest fruiting body firmness of 3.85 kg/cm² and 3.74 kg/cm² for the white strain, and 3.42 kg/cm² and 3.34 kg/cm² for the brown strain, respectively. This contrasts with the treatment using only the pathogenic mushroom, which resulted in the lowest fruiting body firmness. The protein content was 0.58 and 0.61 kg/cm² for the white and brown strains, respectively. Treatments with Pr. aryabhattai and *F. aurantia* showed the highest protein content, reaching 27.2% and 27.51% for the white strain, while treatments with benzoic acid and *F. aurantia* yielded the highest protein content, at 27.32% and 27.69% for the brown strain. The study demonstrated the efficiency of some organic acids and local bacterial isolates in reducing wet blister disease caused by the pathogenic fungus *Hypomyces perniciosus* on edible mushroom *Agaricus bisporus*, with the possibility of adopting these treatments as safe and bioavailable alternatives in managing the disease and increasing the productivity and quality of edible mushrooms.

Keywords: Organic acids, *Hypomyces perniciosus*, *Agaricus bisporus*, Biological control

II. Introduction

The white mushroom, *Agaricus bisporus*, is the most widely cultivated edible mushroom species, grown extensively worldwide with a global production exceeding 6 million tons and an estimated annual growth rate of approximately 7% (Kakraliya et al., 2022). It is highly valued by consumers for its economic, nutritional, and medicinal properties (Nasiri et al., 2017; Chechan, 2020). It is rich in vitamins, minerals, proteins, and antioxidants (Nadir et al., 2022). Mushroom cultivation represents a commercially important, microbiologically driven technology for the large-scale recycling of agricultural waste and its transformation into nutritious edibles (Kakraliya et al., 2022). It is a dynamic and rapidly growing industry worldwide (Roysa et al., 2017).

White mushrooms are susceptible to numerous diseases that cause significant yield losses. These diseases are primarily caused by biological agents, mainly fungi, followed by bacteria and viruses. One such disease is wet blister, caused by the fungus *Mycogone perniciosa* (*Hypomyces perniciosus*). This is considered one of the most destructive diseases affecting mushroom crops in all countries where *A. bisporus* is produced. The disease presents in two types of symptoms. If the buds (pins) are infected before the fruiting body differentiates into a stem and cap, it produces distorted shapes that bear no resemblance to the edible white mushroom. However, infection after

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differentiation leads to the production of thick fruiting bodies with deformed gills. Symptoms have also been observed in the form of white fungal thread-like growth on the mushroom, leading to rot (emitting a foul odor) and the exudation of golden-brown fluid. Fan et al. (2012) indicated that this disease caused crop losses of 15-30% in China and under severe infestations, infection rates reached 100%.

Organic acids are natural compounds that have proven highly effective in combating fungal diseases that threaten plants, making them a promising alternative to chemical pesticides that are harmful to the environment and human health. Among the natural organic acids with antifungal activity are oxalic acid, salicylic acid, citric acid, ascorbic acid, caffeic acid, and others. These acids have demonstrated their ability to improve plant quality by delaying senescence, controlling diseases, and inhibiting browning. Salicylic acid—one of the most prominent of these acids—enhances plant immunity through multiple mechanisms. It activates internal defense systems by binding to cellular enzymes, allowing for the accumulation of hydrogen peroxide, which acts as a secondary messenger to stimulate acquired systemic resistance against pathogens. (Zhang et al., 2023a; Wang et al., 2019)

Bacteria are among the most promising biological control agents for protecting plants from fungal diseases and have been the subject of numerous studies as a safe and effective alternative to chemical fungicides. Plants are constantly exposed to various pathogens, including fungi, bacteria, viruses, and nematodes, causing significant economic losses in agricultural crops, ranging from 20% to 40% of total global production annually. Scientists have focused their attention on biological control agents as a less toxic and safer alternative to mitigate these diseases. The genera Bacillus and Pseudomonas are at the forefront of bacteria used in this field, as they act through multiple and integrated mechanisms. The traditional mechanisms known to be effective against these bacteria include: production of antimicrobial compounds, competition for sites and nutrients, and induction of systemic resistance in plants (Zhang et al., 2023b; Ayaz et al., 2023). Given the nutritional and health importance of the fungus A. bisporus, and to avoid the losses caused by wet blister disease (*Hypomyces perniciosus*), this study was conducted to evaluate the efficacy of organic acids and certain types of bacteria in controlling this disease.

III. Materials and Methods

A. bisporus Mushroom Strains

Two strains of Dutch origin (strain A15 and Brown) were used, which were prepared at the mushroom production farm of the College of Agriculture, Tikrit University.

Pathogenic fungus *Hypomyces perniciosus*

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The highly pathogenic molecularly identified *Hypomyces perniciosus* isolate Aya-7 (PP320509.1) was used (Al-Jubouri and Hassan, 2025).

Isolation and molecular identification of the bacteria from the casing layer:

Isolation was performed using the dilution method by adding 10 g of casing layer soil taken from three cultures of white edible mushroom *A. bisporus* to 90 ml of sterile distilled water. Dilutions of 10^{-4}, 10^{-5}, and 10^{-6} were used. One ml of these dilutions was then spread in a Petri dish, nutrient broth (NA) was added, and the medium was allowed to solidify. The dishes were incubated for 48 hours. After incubation, the growing isolates were purified and stored for further diagnosis and testing.

Effectiveness of bacteria against the growth of the pathogenic fungus *H. perniciosus* and the edible fungus *A. bisporus*:

The inhibitory efficacy of the bacterial isolates was assessed using a double culture test on PDA medium. A disc of the pathogenic fungus colony was placed in a Petri dish, and three lines were drawn from each bacterial isolate using an inoculation loop 2 cm from the pathogenic fungus. After the incubation period, the inhibition zone (the area between the bacteria and the pathogenic fungus) was measured.

Molecular diagnosis of the bacteria:

Genomic DNA Isolation: A 100 mg smear was taken from a freshly grown (24-hour) pure bacterial colony of each isolate, and genomic DNA was extracted using the ZR Fungal/Bacterial/Yeast DNA Mini Prep™ kit, supplied by ZR USA, according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR): The Maxime PCR PreMix kit (i-Taq) 20 µlrxn (Cat. No. 25025) was used. 16S rRNA was amplified using a primer pair: the universal forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and the universal reverse primer GGTTACCTTGTTACGACTT-3'5' (as described by Miller et al., 2013), supplied by Integrated DNA Technologies, Canada. The reaction mixture (25 µL) consisted of 1.5 µL of DNA, 5 µL of Taq PCR PreMix, forward and reverse primers at a concentration of 10 pmol/mL (1 µL each), and diluted to 25 µL with distilled water. The reaction program consisted of a total of 37 cycles, each containing the following: (a) Initial denaturation at 95°C for 5 minutes – one cycle; (b) Denaturation at 95°C for 45 seconds + plasticization at 58°C for 45 seconds + first elongation at 72°C for 45 seconds – 35 cycles; (c) Second elongation at 72°C for 7 minutes – one cycle. Whole gene amplification was performed using a Thermocycler (Applied Biosystem Gene-amp PCR System 9700). The PCR product was then analyzed by agarose gel electrophoresis (1.5%) and the reaction bands were visualized using UV light at a wavelength of 302 nm after treatment with Intron Korea red stain.

Nucleotide Sequencing Analysis

The nucleotide sequences of the amplified gene were determined by polymerase chain reaction (PCR) after obtaining the 16S rRNA gene amplification product. This was done by sending 25 µl of the PCR product and 10 µl of each primer to the Korean company Biotechnology Lab (the apparatus used was the Applied Biosystem 3730XL DNA Sequencer). The results were compared using a web-based computer program (the Basic Local Alignment

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Search Tool (BLAST)) with the National Center for Biotechnology Information (NCBI) database. This database was used to match the nucleotide sequences of the 16S rRNA gene of the bacterial isolates included in the search and to determine their type based on the match in the aforementioned database.

Effect of certain organic acids on the growth of the pathogenic fungus *H. perniciosus* and the edible fungus *A. bisporus*

PDA medium was enriched with three concentrations (1%, 3%, and 5%) of organic acids, including citric acid, acetic acid, lactic acid, propionic acid, and benzoic acid. The medium was then sterilized in an autoclave at 121°C and a pressure of 1.5 kg/cm² for 15 minutes. After sterilization, the flasks were allowed to cool. Just before solidification, the antibiotic chloramphenicol was added at a rate of 250 mg/L to inhibit bacterial growth. The resulting medium was distributed onto Petri dishes and allowed to solidify. After solidification, the dishes were inoculated with isolates of the pathogenic fungus *H. perniciosus* and the edible fungus *A. bisporus* separately by placing a disc of each fungus, 0.5 cm in diameter, at the edges of the fungal colony (grown on the PDA medium and aged five years). (days) with three replicates for each fungal isolate. PDA media without any testing materials were inoculated with the same fungi as a control treatment. All plates were incubated at 25°C for 7 days. When the mycelium reached the edges of the plates in the control plates, inhibition of radial growth was calculated by measuring the average diameter of two perpendicular colonies using a ruler (Ibrahim & Hassan, 2023).

Cultivation and Production of Fungal Fruiting Bodies:

Fungal inoculum for the edible mushroom *A. bisporus* strains A15 and brown, compost, and all stages of mushroom cultivation up to fruiting boll production were carried out according to (Mohammad et al., 2020).

Experimental Treatments: The treatments below were conducted in an experiment for the production of fungal fruiting bodies at the mushroom farm, College of Agriculture, Tikrit University, Iraq. The experiment included the following treatments: *H. perniciosus only; H. perniciosus* + citric acid; *H. perniciosus* + acetic acid; *H. perniciosus* + lactic acid; *H. perniciosus* + propionic acid; *H. perniciosus* + benzoic acid; *H. perniciosus* + Pr. aryabhattai; *H. perniciosus* + *F. aurantia; H. perniciosus* + *Ps. Fluorescens* and Control (Untreated)

Treatments were performed on the covering layer. Each treatment was sprayed with 100 ml of a suspension of the pathogenic fungus at a concentration of 10^8 CFU/ml, then covered with nylon and left for three days of incubation. Afterward, 100 ml at a 5% concentration of each organic acid, as well as 100 ml at a concentration of 10^8 CFU/ml for each of the bacteria that promote the growth of the edible fungus and inhibit the pathogenic fungus, were used, based on the results of laboratory experiments. These treatments were applied to two edible fungus strains of *A. bisporus*: strain A.15 and the brown strain.

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Studied Characteristics

Infection Severity

Infection severity was measured according to the 5-point pathological index developed by Li et al. (2019):

From 0 to 5, where: 0: No symptoms; 1: 1-10%; 2: 11-25%; 3: 26-50%; 4: 51-75%; 5: More than 75%. This was based on symptoms including discoloration, fruit body malformation, necrosis, and foul-smelling brown exudate.

The severity of the infection was measured using the McKinney equation (1923) as follows:

Infection severity = (Number of fruit bodies in infected disease grade × 4 + … + 0 × 0 number of fruit bodies in infected disease grade) / (Total number of fruit bodies × highest disease grade) × 100

Fresh weight: The fruit bodies were weighed for each harvest using a sensitive scale, and the weights were calculated based on (g soft fruit body/20 kg medium).

Fruit body hardness: Hardness was estimated (kg/cm²) using a hardness meter (China), and 5 readings were taken for each replicate in each treatment.

Protein Content Estimation: The protein content was measured using the semi-micro kjeldal method for nitrogen estimation, and the nitrogen content was determined using the following equation: Protein content based on dry weight = Protein content in dry mushroom powder × 6.25 (AOAC, 2002).

Statistical Analysis

The experiments in this study were conducted using a completely randomized design (CRD). Variance was analyzed using SPSS software, and means were compared using Duncan's multiple range test and the least significant difference (LSD) test at a probability level of 0.05 (Al-Rawi & Khalafallah, 1980).

IV. Results and Discussion:

Efficacy of Isolated Bacteria in Inhibiting Pathogenic Fungi

The results shown in Figure (1) indicate the effect of bacteria isolated from the covering layer used in several Iraqi mushroom cultures on the growth of the pathogenic fungus *H. perniciosus* in dual cultures. Isolates A4, A5, and A7 recorded the highest inhibition of the pathogenic fungus, with inhibition zones of 2.2, 1.8, and 1.5 cm, respectively. These are the most efficient isolates in inhibiting the pathogen but showed no effect on inhibiting the edible fungus. While isolates A2, A3, and A6 inhibited the pathogenic fungus, they also showed an effect on inhibiting the edible

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fungus. Despite the variation in the bacteria's ability to inhibit the pathogenic fungus *H. perniciosus*, bacterial isolate A1 showed no effect on the growth of either pathogenic fungus. One of the reasons for the varying inhibition of the growth of pathogenic fungi by bacteria may be attributed to the fact that they are different types, and each type has a genetic makeup, and its genetic difference is reflected in some vital factors such as the activity of enzymes that break down the tissues of pathogenic fungi, such as protease, chitinase, and glucanase enzymes. Such enzymes have the main role in attacking the tissues of the pathogen (Xu et al., 2023).

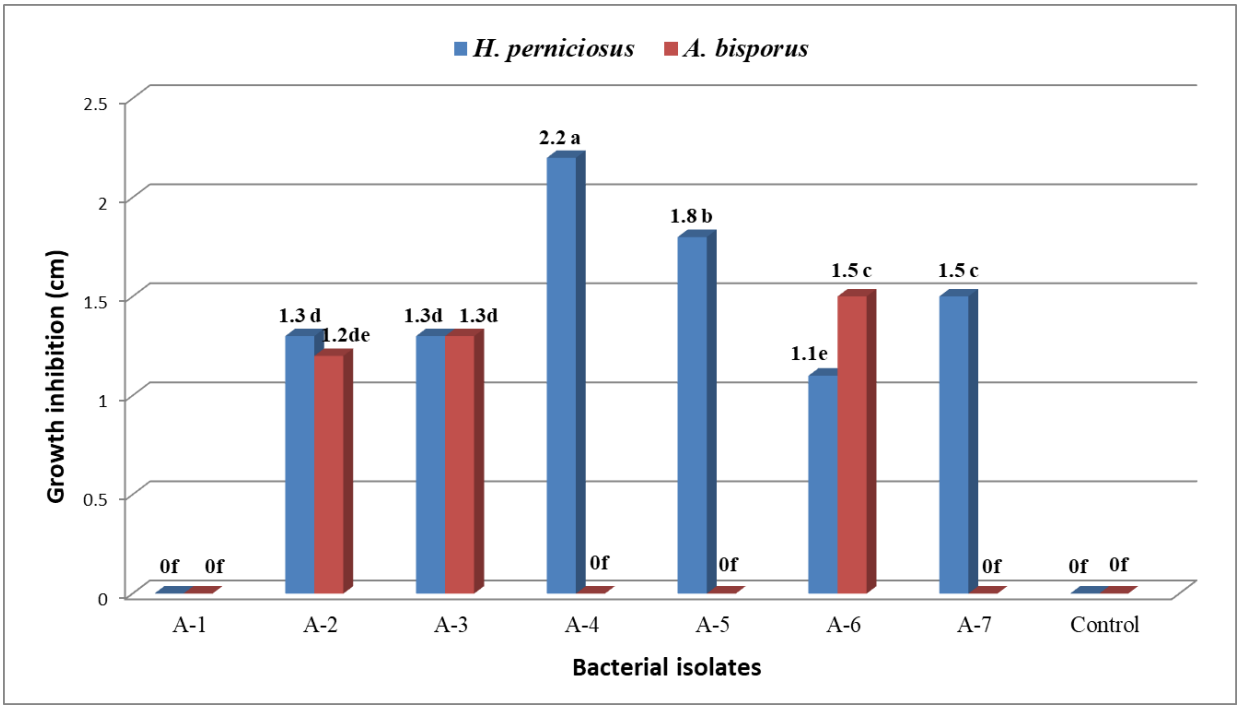

Figure (1) Effect of bacteria isolated from the capping layer on the growth of the pathogenic fungus *H. perniciosus* and the edible fungus *A. bisporus*.

Molecular identification of bacterial isolates:

The molecular identification of bacterial isolates A4, A5, and A7, which showed the highest inhibition of the pathogenic fungus without any effect on the growth of the edible fungus A. bisporus, was based on the nucleotide sequence of the 16S rRNA gene. Figure (2) shows the bands generated by electrophoresis of the PCR product using a specific primer for amplifying the 16S rRNA gene. The electrophoresis results show bands of 1250 base pairs, indicating the accuracy of the PCR test.

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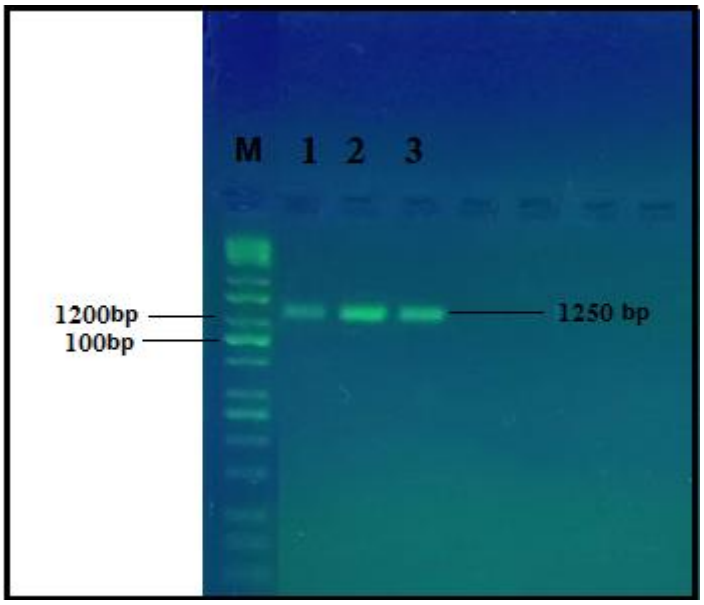

Figure (2) Electrophoresis of the PCR product of the identified bacterial samples. The polymerase chain reaction (PCR) product was transferred to a 1.5% agarose gel at 5 V/cm² using 1x TBE solution for 1 hour and 30 minutes. M: DNA indicator (100).

Table (1) shows the percentages of similarity and conformity of the bacterial isolates to globally registered bacterial strains and their international numbers in the GeneBank database. Bacterial isolates A4, A5, and A7 were identified as *Priestia aryabhattai* strain Aya-4, *Frateuria aurantia* strain Aya-5, and *Pseudomonas fluorescens* strain Aya-6, and were registered under accession numbers PP320436.1, PP320435.1, and PP320438.1, respectively. They showed 99.35%, 99.42%, and 98.78% concordance with global strains registered in the NCBI database, isolated from China, the United States, and Italy, respectively.

Table 1. Molecular identification of the bacterial isolates was based on the percentage of 16S rRNA gene sequence concordance with bacterial strains registered in the global gene bank at the NCBI database.

Highest matching bacterial strains	Accession number	country	Similarity %	Species and strain of bacteria recorded in this study	The accession number for the bacteria recorded in this study
Priestia aryabhattai strain HIB-027	ON698027.1	China	99.35	*Priestia aryabhattai* strain Aya-4	PP320436.1
Frateuria aurantia strain STP853.3	MT827108.1	USA	99.42	*Frateuria aurantia* strain	PP320435.1

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				Aya-5	
Pseudomonas fluorescens strain PF85	MF838663.1	Italia	98.78	*Pseudomonas fluorescens* strain Aya-6	PP320438.1

Effect of Some Organic Acids on the Growth of the Pathogenic Fungus *H. perniciosus* and the Edible Fungus *A. bisporus*:

The results in the figure 2. show an inverse relationship between the growth of the pathogenic fungus *H. perniciosus* and the edible fungus *A. bisporus* with increasing concentrations of the tested organic acids. The results also indicate that the effect of organic acids in inhibiting the growth of the pathogenic fungus *H. perniciosus* is greater than their effect on the edible fungus *A. bisporus*. Propionic, lactic, and acetic acids at a concentration of 5% exhibited the highest inhibitory effect compared to the other acids. The most important reason for the effect of organic acids in inhibiting fungal pathogens is likely due to their binding to cellular enzymes, which allows for the accumulation of hydrogen peroxide. This peroxide has harmful effects on the pathogenic fungus, in addition to causing hyphae distortion and increasing cell membrane permeability.

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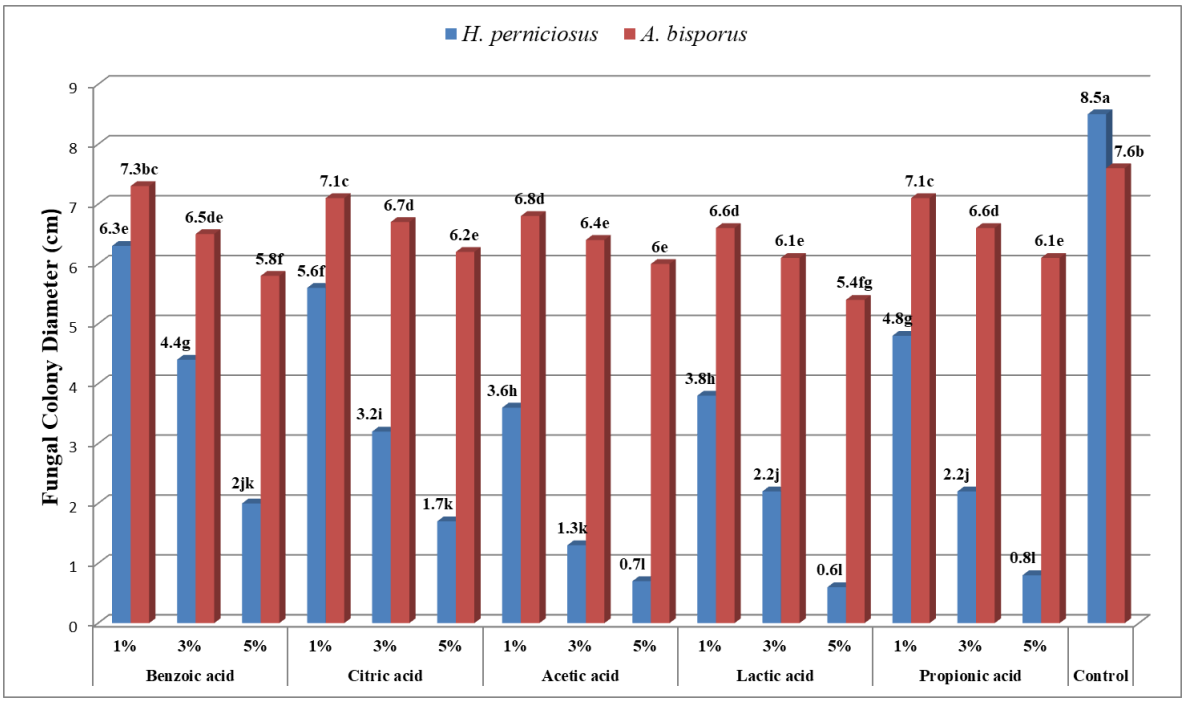

Figure (2) shows the effect of some types of organic acids on the growth of the pathogenic fungus *H. perniciosus* and the edible mushroom *A. bisporus*.

The effect of some biotic factors and organic acids on infection parameters, yield, and quality of two strains of the edible mushroom *A. bisporus* under conditions of infection with the pathogenic fungus *H. perniciosus*.

Infection severity

Table (2) shows the effect of three types of bacteria and organic acids on the overall infection severity of two strains of the edible mushroom *A. bisporus* under conditions of infection with the pathogenic fungus *H. perniciosus*. The results showed a significant decrease in infection severity for all types of organic acids and isolated bacteria in reducing infection severity for both the white and brown edible mushroom strains. Citric acid and propionic acid showed the lowest decrease in infection severity, at 5.9% and 5.98% in the brown strain, respectively, while the bacterium *F. aurantia* showed the lowest infection severity at 3.54% in the white strain. The white strain of the edible mushroom *A. bisporus* was compared to the brown and white strains of the pathogenic fungus *H. perniciosus*, which exhibited infection rates of 94.39% and 97.17%, respectively. It is observed that infection severity decreased significantly in the organic acid and bacterial treatments. This may be attributed to the role of organic acids in inhibiting pathogen enzymes and disrupting cell membranes, while the bacterial infection reduction is attributed to the production of antimicrobial compounds, competition for sites and nutrients, and the induction of systemic plant

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resistance. Furthermore, novel mechanisms such as interference with the quorum-sensing system of pathogenic fungi and the remodeling of microbial communities in the root zone may also play a role (Ayaz et al., 2023).

Table (2) shows the effect of some biotic factors and organic acids on the overall infection severity (%) of two strains of the edible mushroom *A. bisporus* with the pathogenic fungus *H. perniciosus*.

Treatments	*A. bisporus* strain		Average of treatment
	White strain	Brown strain	
Pathogenic fungus *H. perniciosus* only	97.17	94.39	95.78
Pathogenic fungus *H. perniciosus* + citric acid	8.16	5.9	7.03
Pathogenic fungus *H. perniciosus* + acetic acid	8.31	7.15	7.73
Pathogenic fungus *H. perniciosus* + lactic acid	6.57	6.17	6.37
Pathogenic fungus H. *perniciosus* + propionic acid	6.27	5.98	6.13
Pathogenic fungus *H. perniciosus* + benzoic acid	7.05	5.33	6.19
Pathogenic fungus *H. perniciosus* + P. aryabhattai	4.27	5.46	4.87
Pathogenic fungus *H. perniciosus* + *F. aurantia*	3.54	4.48	4.01
Pathogenic fungus *H. perniciosus* + Ps. *fluorescens*	6.16	6.96	6.56
Control (no treatment)	0	0	0
Average of Strain	14.75	14.18	
LSD $_{0.05}$	Treatment; 1.23 , Strains; 1.02 , Interaction; 1.47		

Productivity (Total Fresh Weight of *A. bisporus* Strains)

Table (3) shows the effect of three types of bacteria and organic acids on the total fresh weight (kg/20 kg fermented manure) of the fruiting bodies of two *A. bisporus* strains under conditions of infection with the pathogenic fungus *H. perniciosus*. The results showed a significant increase in fruiting body weight for all types of organic acids and isolated bacteria, and for both the white and brown edible mushroom strains. Benzoic acid showed the highest fruiting body weight, reaching 4.58 kg/20 kg fermented manure in the brown strain, exceeding the control treatment (not treated with the pathogenic fungus), which reached 3.99 kg/20 kg fermented manure. Meanwhile, the bacterium *F. aurantia* recorded the highest fresh weight, reaching 5.35 kg/20 kg fermented manure in the white strain of *A. bisporus*, exceeding the control treatment. The non-pathogenic fungus treatment yielded 3.82 kg/20 kg of compost, while the treatment with the pathogenic fungus *H. perniciosus* alone recorded the lowest yields of 0.83 and 0.4 kg/20 kg of compost for the brown and white edible mushroom strains, respectively.

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Table (3) Effect of some biotic factors and organic acids on the total fresh weight (kg/20 kg of compost) of the fruiting bodies of two *A. bisporus* strains under conditions of infection with the pathogenic fungus *H. perniciosus*

Treatments	*A. bisporus* strain		Average of treatment
	White strain	Brown strain	
Pathogenic fungus *H. perniciosus* only	0.4	0.83	0.62
Pathogenic fungus *H. perniciosus* + citric acid	3.36	4.33	3.85
Pathogenic fungus *H. perniciosus* + acetic acid	3.29	3.79	3.54
Pathogenic fungus *H. perniciosus* + lactic acid	4.04	4.22	4.13
Pathogenic fungus *H. perniciosus* + propionic acid	4.17	4.3	4.24
Pathogenic fungus *H. perniciosus* + benzoic acid	3.84	4.58	4.21
Pathogenic fungus *H. perniciosus* + *P. aryabhattai*	5.04	4.52	4.78
Pathogenic fungus *H. perniciosus* + *F. aurantia*	5.35	4.95	5.15
Pathogenic fungus *H. perniciosus* + *Ps. fluorescens*	4.22	3.88	4.05
Control (no treatment)	4.44	4.42	4.43
Average of Strain	3.82	3.99	
LSD $_{0.05}$	Treatment; 0.25 , Strains; 0.26 , Interaction; 0.34		

Fruiting Body Firmness

Table (4) shows the effect of some biotic factors and organic acids on the fruiting body firmness (kg/cm²) of two edible mushroom strains under conditions of infection with the pathogenic fungus **H. perniciosus*. The treatment with citric acid and **F. aurantia** resulted in the highest fruiting body firmness, reaching 3.85 and 3.74 kg/cm² respectively for the white strain and 3.42 and 3.34 kg/cm² respectively for the brown strain. This is compared to the treatment with the pathogenic fungus alone, which resulted in the lowest fruiting body firmness, at 0.58 and 0.61 kg/cm² for the white and brown strains, respectively.

Table (4) Effect of some biotic factors and organic acids on the fruiting body firmness (kg/cm²) of two edible mushroom strains under conditions of infection with the pathogenic fungus *H. perniciosus*

Treatments	A. bisporus strain		Average of treatment
	White strain	Brown strain	
Pathogenic fungus *H. perniciosus* only	0.58	0.61	0.59
Pathogenic fungus *H. perniciosus* + citric acid	3.85	3.42	3.64
Pathogenic fungus *H. perniciosus* + acetic acid	2.28	2.48	2.38
Pathogenic fungus *H. perniciosus* + lactic acid	2.43	2.61	2.52
Pathogenic fungus *H. perniciosus* + propionic acid	2.56	2.69	2.63
Pathogenic fungus *H. perniciosus* + benzoic acid	2.23	2.97	2.6
Pathogenic fungus *H. perniciosus* + P. aryabhattai	3.43	2.91	3.17
Pathogenic fungus *H. perniciosus* + *F. aurantia*	3.74	3.34	3.54
Pathogenic fungus *H. perniciosus* + *Ps. fluorescens*	2.61	2.27	2.44
Control (no treatment)	2.23	2.41	2.32
Average of Strain	2.59	2.57	
LSD $_{0.05}$	Treatment; 0.12 , Strains; 0.13 , Interaction; 0.17		

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Protein Percentage

Table (5) shows the effect of some biotic factors and organic acids on the protein percentage (%) of two edible mushroom strains under conditions of infection with the pathogenic fungus *H. perniciosus*. The results show that the treatment with *Pr. aryabhattai* and *F. aurantia* gave the highest protein percentage, reaching 27.2% and 27.51%, respectively, for the white strain, while the treatment with benzoic acid and *F. aurantia* gave the highest protein percentage, reaching 27.32% and 27.69%, respectively, for the brown strain.

Table (5) Effect of some biotic factors and organic acids on the protein percentage (%) of two edible mushroom strains under conditions of infection with the pathogenic fungus *H. perniciosus*

Treatments	*A. bisporus* strain		Average of treatment
	White strain	Brown strain	
Pathogenic fungus *H. perniciosus* only	7.11	7.82	7.47
Pathogenic fungus *H. perniciosus* + citric acid	24.83	27.07	25.95
Pathogenic fungus *H. perniciosus* + acetic acid	24.76	26.53	25.65
Pathogenic fungus *H. perniciosus* + lactic acid	25.51	26.96	26.24
Pathogenic fungus *H. perniciosus* + propionic acid	25.64	27.04	26.34
Pathogenic fungus *H. perniciosus* + benzoic acid	25.31	27.32	26.32
Pathogenic fungus *H. perniciosus* + *P. aryabhattai*	27.2	27.26	27.23
Pathogenic fungus *H. perniciosus* + *F. aurantia*	27.51	27.69	27.6
Pathogenic fungus *H. perniciosus* + Ps. *fluorescens*	25.69	26.62	26.16
Control (no treatment)	24.31	25.16	24.74
Average of Strain	23.79	24.95	
LSD $_{0.05}$	Treatment; 1.12 , Strains; 1.06 , Interaction; 1.33		

The results proved the role of organic acids and bacteria in increasing the productivity of edible mushrooms from fruiting bodies, and at the same time increasing the firmness of the fruiting bodies and increasing their protein content. This may be attributed to reducing the damage to the fruiting bodies of edible mushrooms through the inhibition of pathogenic fungi by organic acids and bacteria. At the same time, the metabolic products of bacteria may have a role in increasing the firmness of the fruits and their protein content. This may be attributed to the secretion by bacteria of some compounds such as the cyclic tetrapeptide called Arthropeptide {cyclo-(L-Pro-L-Leu-L-cHyp-L-Tyr)}, which has antifungal activity, especially against ascomycetes (including pathogenic fungi), as it works to disrupt fungal cell membranes, tear their walls, and inhibit the germination of pathogenic fungal spores, thus preventing their growth (Gomez et al., 2023). In addition to the interaction between bacteria and edible fungi, which promotes vegetative and reproductive growth of the fungi, enhances the substrate with nutrients, and improves fungal productivity (Frey-Klett et al., 2011). While the growth enhancement and weight gain by bacteria can be attributed to their concentration in fruiting bodies, they effectively reduce their enzymes, especially peptidase and lipase enzymes, thus preventing the breakdown of fruiting bodies (Oh and Lim, 2021). The bacteria associated with edible fungi benefit from nutrients such as sugar and amino acids in the edible fungi instead of hydrolyzing the

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fungal cell wall, which promotes the development of a mutually beneficial habitat between the fungi and bacteria (Bánfi et al., 2021). The bacteria promote the growth of the edible fungi *A. bisporus*, especially in the early stages of growth, which increases the biomass, speed, and size of the fruiting body as a result of the bacteria secreting volatile organic compounds. In addition, they play a role in reducing the colonization period of the edible fungi's hyphae in the culture medium. The bacteria that promote the growth of edible fungi also inhibit pathogens and break down their walls, so the edible fungi can also benefit from the decomposing materials (Orban et al., 2023).

Conclusion

The study demonstrated the efficacy of certain organic acids and local bacterial isolates in reducing wet blister disease caused by the pathogenic fungus *Hypomyces perniciosus* in the edible mushroom *Agaricus bisporus*. These treatments contributed to reducing the severity of infection and improving the yield and quality of the fruiting bodies. Furthermore, some molecularly identified bacterial isolates and organic acids proved superior in inhibiting pathogen growth compared to their effect on the edible mushroom. The results confirm the potential of these treatments as safe and effective bioavailable alternatives for disease management and increasing the yield and quality of edible mushrooms.

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