MJSIR
Mountain Journal of Science and Interdisciplinary Research
PRINT ISSN: 2619-7855
ONLINE ISSN: 2651-7744
January-June 2026 • 86 (1) : 7-19

Characterization and Pathogenicity of *Mycogone perniciosa* Infecting Button Mushroom (*Agaricus bisporus*) and Bioefficacy of Plant Extracts Against the Pathogen

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ARTICLE INFO
Date Received: 08-06-2025
Date Last Revised: 04-19-2026
Date Accepted: 05-21-2026

KEYWORDS

Aleuriospores
Fruiting bodies
Inhibition zone
Phialospores
Soil casing
Sporophore
Wet bubble

Abstract

Wet bubble disease represents a significant challenge in button mushroom cultivation. This study was conducted to verify the identity of the pathogen through morpho-cultural characterization and pathogenicity testing; and to assess the bioefficacy of plant extracts against the pathogen through in vitro agar disk diffusion assay. Infected button mushrooms were collected, isolated, and characterized, which confirmed its identity as *Mycogone perniciosa*. The pathogen produced white to light brown, fluffy mycelium with concentric rings; with hyaline, cylindrical *phialospores* and brown-pigmented, ellipsoid to globose *aleuriospores*. Pathogenicity tests demonstrated that a spore suspension (1.3 x 10^6 spores/mL) inoculated onto *Agaricus bisporus* fruiting bodies caused cap spotting and metallic brown staining within three days, which subsequently developed into white, cottony mycelial growth leading to external and internal decay by the seventh day. Furthermore, results of antifungal activity of eight plant extracts against *M. perniciosa* indicated that a 50 mg/mL concentration of tarragon exhibited the highest inhibitory activity, comparable to that of benomyl, from the third day post-application but diminished by the seventh day. Rosemary also demonstrated an inhibitory effect, but only during the initial 3 days. Other plant extracts, including peppermint, oregano, ginger, lantana, neem, and angel's trumpet, showed only short-term effects.

Introduction

The white button mushroom *(Agaricus bisporus)* is esteemed globally for its nutritional value and economic significance. It serves as an abundant source of nutrients, notably proteins (20-35%) on a dry weight basis, unsaturated fatty acids, minerals, fibers, and vitamins, including B, C, D, and K. Additionally, it is a valuable source of iron, potassium, and phosphorus, and is recognized for its medicinal properties (Prakasam & Singh, 2008). It accounts for approximately 40% of the total global mushroom production (Narendra et al., 2014). Cultivating mushrooms in the Philippines is economically viable due to the availability of substrates and strong market demand (Chang et al., 2014). Nonetheless, the majority of mushrooms available in local markets are imported

from various Asian countries, primarily because domestic production is limited and is continually threatened by pests, such as plant pathogens.

White button mushrooms are cultivated on a specially prepared substrate, a compost produced by fermentation with various thermophilic microorganisms that decompose plant residues and other organic and inorganic materials. These microorganisms break down the compost into readily accessible compounds that serve as the primary source of nutrition for the mushroom mycelium (Kertesz & Thai, 2018). However, it is important to note that harmful fungi also reside within the compost and casing soil. These fungi mainly act as competing molds, adversely affecting spawn, while others infect fruiting bodies at various stages of crop development, producing distinctive disease symptoms that reduce mushroom yield. One of the major mycoparasites of the white button mushroom responsible for causing wet bubble disease is *M. perniciosa* (Magnus) Declar. (Kaur, 2013).

Wet bubble disease was first documented in Paris in 1888 and subsequently caused significant losses in mushroom beds across France (Kaur, 2013). The characteristic symptoms of wet bubble disease include the development of whitish, mold-like mycelial growth on the casing surface and on parts of the fruiting bodies, which eventually spreads to cover the entire cap and results in deformation of the affected mushrooms. A foul-smelling, amber-colored liquid, resembling teardrop-shaped exudate, is exuded and drips from severely infected, tumorous fruiting bodies, representing a typical symptom of the disease (Kouser & Shah, 2013). According to Fletcher and Gaze (2008), the term "wet bubble" is derived from the wet decay phenotype as well as the shape of the affected mushroom tissue.

During the spawn run stage of button mushroom cultivation, their mycelium remains uninfected by *M. perniciosa* due to the presence of hydrophobins that establish a uniform hydrophobic coating on the surface of the mushroom hyphae, thereby preventing pathogen penetration (Kumar et al., 2020). However, after completing the spawn run in the soil casing, fresh air is introduced into the growing environment, and the temperature is reduced to the optimal range for primordia formation. This environment facilitates the activation of *M. perniciosa* conidia and the development of germ tubes that invade the mushroom mycelium. The invading pathogen secretes lytic enzymes such as chitinases, laccases, and cellulases, which degrade host tissues (Kumar et al., 2020). Furthermore, water sprays can influence the dispersal of *M. perniciosa* on the soil casing. Spores may also persist on building surfaces or be transported via crop debris, mites, and flies (Kouser et al., 2015). Additionally, harvesters may facilitate pathogen spread through contact with their hands, tools, and clothing (Pieterse, 2005). *M. perniciosa* can result in yield reductions of approximately 15% to 30%, and in cases of severe infection, losses may reach 50% to 60%, or even cause complete crop failures across entire mushroom farms (Fu et al., 2016).

Kouser et al. (2015) described an isolate of *M. perniciosa* exhibiting copious, flocculent mycelium with both even and uneven edges on potato dextrose agar (PDA). The quantity of aerial mycelium ranged from dense to sparse, with colonies transitioning in color from white to pale brown and dark brown following 12-14 days of incubation at a temperature of 24 ± 1°C. The maximum radial growth of 90 mm was observed on compost agar medium after 15 days, followed by PDA with a growth of 75.5 mm. According to Kaur (2013), citing Zhi et al. (1995), microscopic examination reveals that the mycelium of *M. perniciosa* is white, compact, and felt-like. The hyphae are branched, interwoven, septate, hyaline, and approximately 3.5 µm in width. The conidiophores are short, slender, branched, hyaline (200 × 3–5 µm), and possess sub-verticillate to verticillate branches bearing thin-walled, one-celled phialospores measuring 5-10 × 4–5 µm.

Presently, in most European nations, the sole fungicide approved for the control of *M. perniciosa* is Sporgon (prochloraz) (Pyck & Grogan, 2015). Mushrooms can accumulate heavy metals via biosorption (Kulshreshtha et al., 2014), with the amounts accumulated correlating with the concentrations in the substrates. Consequently, increasing awareness among modern consumers of the detrimental effects of chemicals has fostered a "green" consumer profile that demands the elimination of synthetic chemicals in food production and preservation, alongside the extended shelf life of various food products. Recently, interest in natural products has been intensifying due to their availability, reduced side effects, lower toxicity, and superior biodegradability compared to other antimicrobial

agents and preservatives. Plant extracts containing physiologically active biochemicals have significant potential to develop novel agents that offer substantial benefits to humanity. Natural products are garnering increased attention from scientists due to their cost-effectiveness, safety, eco-friendliness, and relevance.

A significant challenge faced by mushroom cultivators is the management of diseases without the use of chemical agents. Currently, numerous researchers are investigating the potential application of biochemicals, such as plant extracts or essential oils derived from various plants exhibiting antimicrobial properties. These extracts, oils, and their constituents have shown potent fungistatic and antibacterial effects against pathogenic fungi and bacteria affecting cultivated mushrooms (Potočnik et al., 2016). According to Dos Santos et al. (2017), essential oils show considerable promise as viable alternatives to traditional fungicides, capable of effectively controlling diseases while being suitable for the organic cultivation of products. Furthermore, essential oils are readily accessible, cost-effective, and offer significant advantages due to their low toxicity to humans and the environment, especially when compared to synthetic compounds. Despite numerous studies emphasizing the antifungal properties of various botanical plants, their potential as environmentally friendly control measures for mushroom diseases remains underexplored. Therefore, further efforts are required to accurately identify mushroom diseases and develop sustainable management strategies.

Generally, the study was conducted to identify potential plants exhibiting fungicidal properties against wet bubble disease of button mushroom, caused by *M. perniciosa*. Specifically, it aimed to characterize *M. perniciosa* by examining its disease symptoms and morpho-cultural features. Additionally, the study assessed the pathogen's infectivity through pathogenicity testing and evaluated the bioefficacy of various plant extracts against *M. perniciosa* via in vitro assays.

Materials and Method

The collection of infected mushroom specimens and the assessment of wet bubble disease incidence were conducted at the Bureau of Plant Industry (BPI) - Button Mushroom Project Growing House, BPI Compound, Guisad, Baguio City. Botanical plant specimens were collected at Loakan Proper, Baguio City, with the exception of the neem, which was gathered at Cuyapo, Nueva Ecija. The collected specimens were subsequently transported to the BPI Serum Laboratory for disinfection and drying procedures. The processes of plant extraction and other laboratory activities were executed at the Department of Plant Pathology Laboratory and the Department of Chemistry Laboratory at Benguet State University (BSU), while the bioassay test was performed at the BSU Crop Protection Laboratory. The entire study was carried out from August 2021 to October 2022.

Collection of Diseased Specimens

Infected fruiting bodies of the white button mushroom were collected and stored in sanitized plastic containers. The specimens were subsequently transported to BSU for the isolation of the causal pathogen.

Isolation of *M. perniciosa* from Infected Fruiting Bodies of Mushrooms

The advancing lesion was disinfected with 10% sodium hypochlorite (Chlorox) for one minute. Cut tissues were rinsed in three washes of sterilized distilled water and then placed equidistantly on a previously plated PDA containing 20% lactic acid to inhibit bacterial growth. Plates were incubated at 25°C in darkness for 7-14 days to facilitate pathogen growth. Fungal growth was monitored, and pure cultures were obtained by sub-culturing the mycelia twice onto fresh PDA plates. The pure culture was subsequently applied in the in vitro evaluation utilizing plant extracts.

Morpho-Cultural Characterization of *M. perniciosa*

The isolates of *M. perniciosa* were characterized based on colony color, texture, and the size of mycelial growth observed on PDA. Morphological features were further examined using light microscopy through the preparation of temporary mounts, and they were described according to specific traits: color, branching pattern, and presence of septa on the conidiophore; the number of spores attached to the conidiophore; as well as the color, shape, and size of phialospores and aleuriospores. A total of 100 spores of

M. perniciosa were measured using an ocular micrometer at 400x magnification. The cultural and morphological characteristics of the fungi were primarily based on the descriptions provided by Li et al. (2019), Kouser et al. (2015), Glamoclija et al. (2008), and Pieterse (2005).

Standardization of the Inoculum

Following Bagsan's (2006) method, fourteen-day-old pure cultures of *M. perniciosa* were scraped and suspended in 10 ml of sterile distilled water. The spore suspensions were pre-standardized by counting spores per milliliter with a haemacytometer. Counts were performed three times, resulting in a fungal suspension concentration of 1.3 x 10^6 spores/mL for the pathogenicity test and 1.24 x 10^6 spores/mL for the bioassay.

Pathogenicity Test of *M. perniciosa* Isolates

To confirm the ability of *M. perniciosa* isolates to induce infection on button mushrooms, a pathogenicity test was conducted. The conidial suspension was standardized using a haemacytometer to achieve a uniform spore concentration. A completely randomized design with two treatments (control and inoculated) and four replicates, each with four samples, was employed in the experiment. Healthy fruiting bodies of button mushrooms were sourced from the cultivation house, scooped along with the substrate, and thereafter placed in disinfected plastic cup containers.

The standardized conidial suspension at 1.3 × 10^6 spores/mL was used as the inoculum, while sterile distilled water served as the control. Both were sprayed at a volume of 1mL per treatment onto the pileus of the button mushroom. The inoculated samples were monitored for 7 days to assess symptom development.

Collection and Air-drying of Botanical Plants

The collected leaves of the plant specimens, along with the rhizome of ginger, were placed in sterile containers. The ginger rhizome was thoroughly washed and later cut into smaller pieces to facilitate faster drying. The specimens were surface sterilized using a 10% sodium hypochlorite (NaCl) solution and rinsed with sterile distilled water. The samples were then air-dried at ambient temperature for a period of three weeks, with the exception of ginger and oregano, which required an extended drying period until the fourth week due to their higher moisture content.

Extraction of Botanical Plants

The procedures established by Balangcod et al. (2012) and Gutierrez et al. (2013) for the preparation of plant extracts via solvent extraction were followed and modified by using 95% ethyl alcohol in place of 95% methanol. The dried specimens were finely chopped and ground using a mortar and pestle. For each specimen, 50g of powdered plant material was soaked in 300mL of 95% ethyl alcohol at ambient temperature for 48 hours. The plant extracts were filtered using Whatman filter paper. The resulting filtrates were transferred to the chemistry laboratory for phytochemical extraction using a rotary evaporator. The extracts were distilled under reduced pressure at 60°C until approximately 20% of the original volume remained. The collected filtrates were then oven-dried at 40°C for 48 hours to further concentrate the extract and eliminate residual solvent, then cooled in a desiccator. The crude extracts were weighed to determine yield and stored at 4°C for 48 hours. Afterward, they were dissolved in acetone to a concentration of 50 mg/mL, which was used in the bioassay.

Dissolving of the Plant Extracts

The crude extracts were prepared according to the modified methods of Rawal and Adhikari (2016) and Bagsan (2006). Each 50mg sample was weighed, placed into a sterile medicinal bottle, and dissolved in 1mL of acetone to obtain a concentration of 50mg/mL.

Bioassay Test

The antifungal activity of plant extracts against *M. perniciosa* was evaluated using the disk diffusion method. The 6 mm paper discs were immersed in the dissolved plant extracts for 30 minutes. Additionally, liquefied lukewarm PDA was poured into sterilized Petri dishes to solidify. Subsequently, a standardized 100 µl suspension of *M. perniciosa* was applied onto the solidified PDA using a calibrated micropipette. The suspension was then spread across the agar surface using an L-shaped rod. Two paper discs were positioned equidistantly on the top surface of each agar plate. The plates were transferred in an incubation chamber maintained at 23°C. The

effects of the biological agents, as indicated by the formation of inhibition zones, were monitored over 7 days. The inhibition zones were measured by determining the clear areas around the paper discs, which represent regions where *M. perniciosa* growth was inhibited by the extracts' antimicrobial activity.

Eleven treatments were prepared and used in the bioassay test: Treatment 1 served as the negative control, was applied with sterile distilled water only; Treatment 2 applied with 50mg of peppermint; Treatment 3 applied with 50mg of oregano; Treatment 4 applied with 50mg of ginger; Treatment 5 applied with 50mg of rosemary; Treatment 6 applied with 50mg of tarragon; Treatment 7 applied with 50mg of lantana; Treatment 8 applied with 50 mg of neem; Treatment 9 applied with 50mg of angel's white trumpet; Treatment 10 applied with Timorex Gold (Bio Fungicide) and Treatment 11 applied with Benomyl Fungicide served as the positive control.

The treatments were replicated four times with four subsamples per replicate and arranged randomly using a Completely Randomized Design (CRD).

Data Gathered

Characteristics of M. perniciosa

The symptoms of the disease on infected mushroom fruiting bodies and the cultural characteristics exhibited by the pathogen on growth media, particularly mycelial growth, colony color, and texture, were observed. Additionally, the physical and morphological features in terms of color, size, and shape of the spores and hyphae were examined, documented, and captured through photomicrography using a compound microscope.

Disease Symptoms Development

The pathogenicity test on the fruiting bodies of the mushrooms was conducted and monitored for 7 days. The development of symptoms based on the characterization of Fletcher and Gaze (2008), and Kouser and Shah (2013) was recorded.

Diameter of Inhibition Zone

The clear zone between the paper disc containing plant extracts and the growth of *M. perniciosa* was measured in millimeters using a ruler. Given the slow growth of the pathogen, the measurement of the inhibition zone commenced on the third day post-incubation, by which time observable growth of the pathogen had occurred.

Other Observation on Disease Incidence of M. perniciosa

An assessment of wet bubble disease incidence on white button mushrooms was conducted to determine the status of the disease at the BPI Button Mushroom Project Growing House. The number of fruiting bodies infected with *M. perniciosa* per square meter was counted and recorded. Data was collected over 6 weeks, from September 15 to October 20, 2022. The prevailing temperature and relative humidity inside the growing house were recorded. The disease incidence percentage was calculated using the formula from Kouser et al. (2013), modified to count infected fruiting bodies per square meter (m^2) rather than per tray/bag.

$$\text{Disease incidence} = \frac{\text{Number of infected fruiting bodies per square meter}}{\text{Total number of fruiting bodies per square meter}} \times 100$$

Data Analysis

A pooled Analysis of Variance (ANOVA) test was conducted on the bio-efficacy test results, comparing the inhibition zones of the experimental variable (extracts) with those of the control variable (positive and negative controls). The Tukey Honest Significant Difference (HSD) was employed as a post hoc test to identify statistically significant differences among treatments at $\alpha = 0.05$.

Results and Discussion

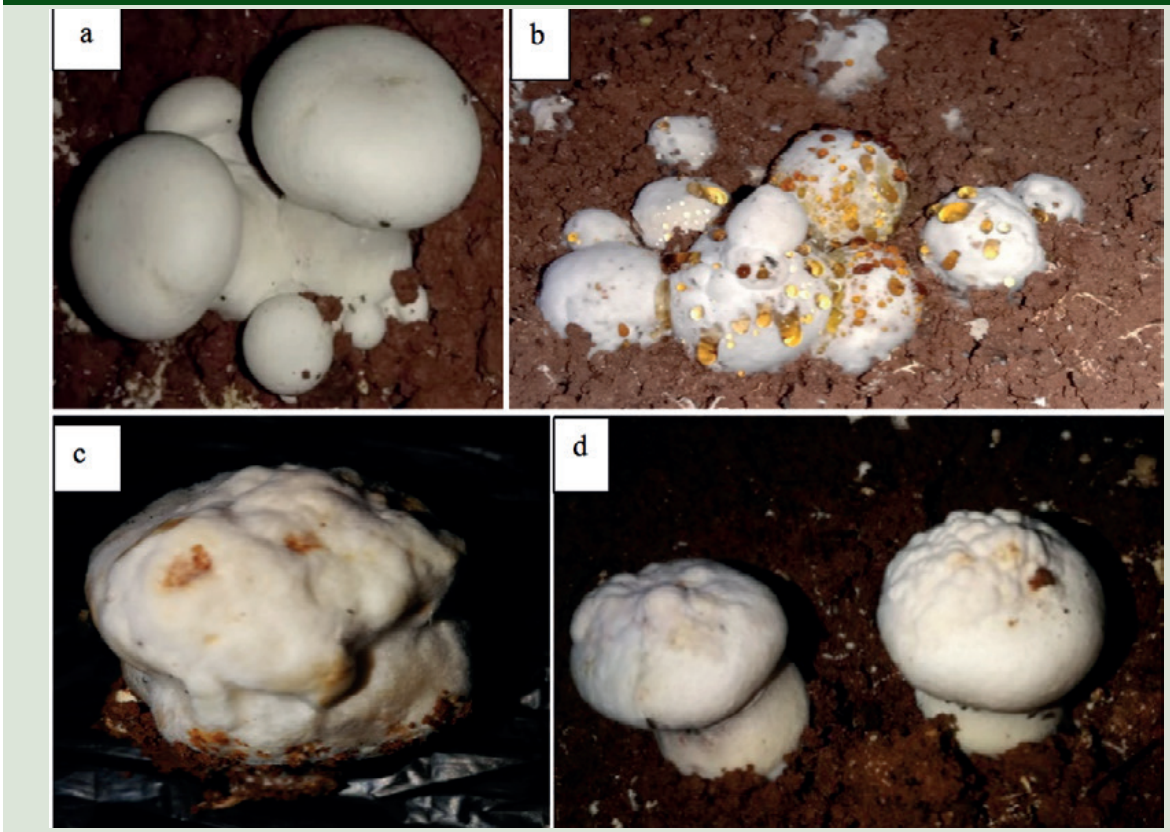
Characteristics of *M. perniciosa*

M. perniciosa caused malformation of the fruiting body of the white button mushroom. A white fluffy mycelial growth of the pathogen developed on the fruiting body, appearing as tumorous fungal masses (Figure 1).

M. perniciosa was initially white and fluffy but turned brown as it ages. Small amber to dark brown drops of liquid were observed on the

Figure 1

Symptoms of M. perniciosa on Fruiting Bodies of Button Mushroom: a) Healthy Button Mushroom; b) Infected Primordia and Distorted Pinheads with Brown Liquid Exudate; c) Malformed Mushroom with Swollen Stipe and Deformed Pileus; d) Tumoroid Masses on the Pileus. Photos were Taken at the BPI Button Mushroom Project Growing House, BPI Compound, Guisad, Baguio City



surface of distorted, undifferentiated mushroom tissue. This observation conforms with the description of Fletcher and Gaze (2008) and Kouser and Shah (2013) on wet bubble disease symptoms.

The pure culture of *M. perniciosa* was observed and described based on its cultural and morphological characteristics as summarized in Table 1.

Figure 2 shows the colony growth of *M. perniciosa* on PDA after 7 days of incubation. It produced white to light brown, copious flocculent mycelium having villous or concentric circles on PDA, which conforms to the description of Kouser et al. (2015); Glamoclija et al. (2008) and Pieterse (2005).

It was also observed that it has slow growth with a colony size of 40 mm after 7 days of incubation at 23°C. Szumigaj-Tarnowska et al. (2015) cited that some pathogenic strains of *M. perniciosa* were slow growing on PDA, highly pigmented, and produced numerous aleuriospores.

The structures of the pathogen were observed and characterized using a compound microscope, as shown in Figure 3. It was observed that the phialospores of *M. perniciosa* were hyaline, cylindrical in shape, borne singly, thin-walled, and with an average measurement of 7.6 x 2.4µm. The size of the measured phialospores is almost close to the measurement done by Li et al. (2019) with a size of 6.7-25.8 x 2.5-7.8 µm. It was also observed that the chlamydospores or aleuriospores of *M. perniciosa* were two-celled with thick-walled upper cells, warty, ellipsoidal to globose in shape, hyaline to brown, and had an average measurement of 13.5 x 7.5µm, which is within the range of measurement done by Li et al. (2019), having a size of 6.5-25.2 x 2.5-7.8µm.

Table 1

Morpho-Cultural Characteristics of M. perniciosa After 5 days of Incubation

Characteristics	*Mycogone perniciosa*		
A. Cultural			
Color of the colony	white to light brown		
Colony texture	copious flocculent mycelium having villous or concentric circles		
Colony size	40mm on the 7th day		
B. Morphological			
	Phialospore	Aleuriospore	Conidiophore
Color	hyaline	hyaline to brown pigmented	hyaline
Shape	cylindrical	ellipsoid to globose	
Size	mean:7.6 x 2.4 µm	mean: 13.5 x 7.5 µm	

Figure 2

M. perniciosa on Potato Dextrose Agar After 7 days of Incubation. a) Surface and b) Reverse

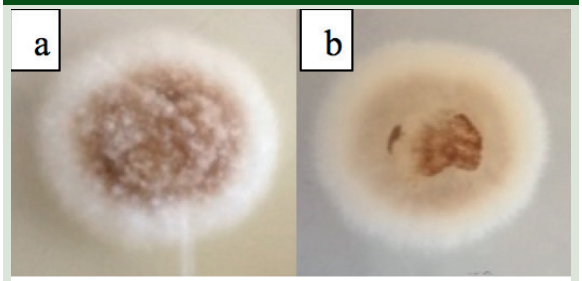
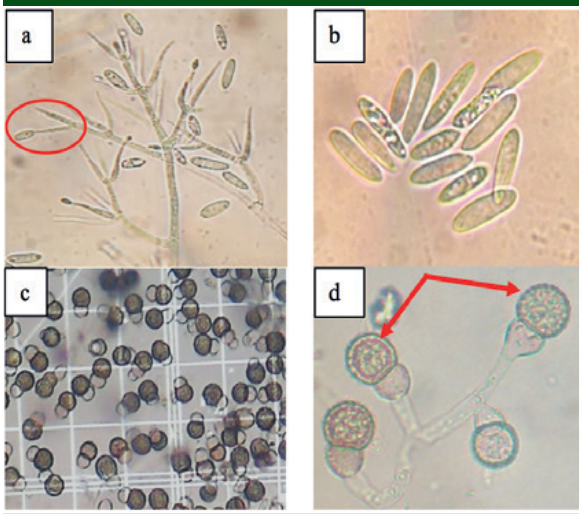


Figure 3

Morphological Structures of M. perniciosa a) Phialospores Attached to the Conidiophore (100x); b) Phialospores (400x); c) Aleuriospores (100x) and d) Aleuriospores aAttached to the Conidiophore (400x)



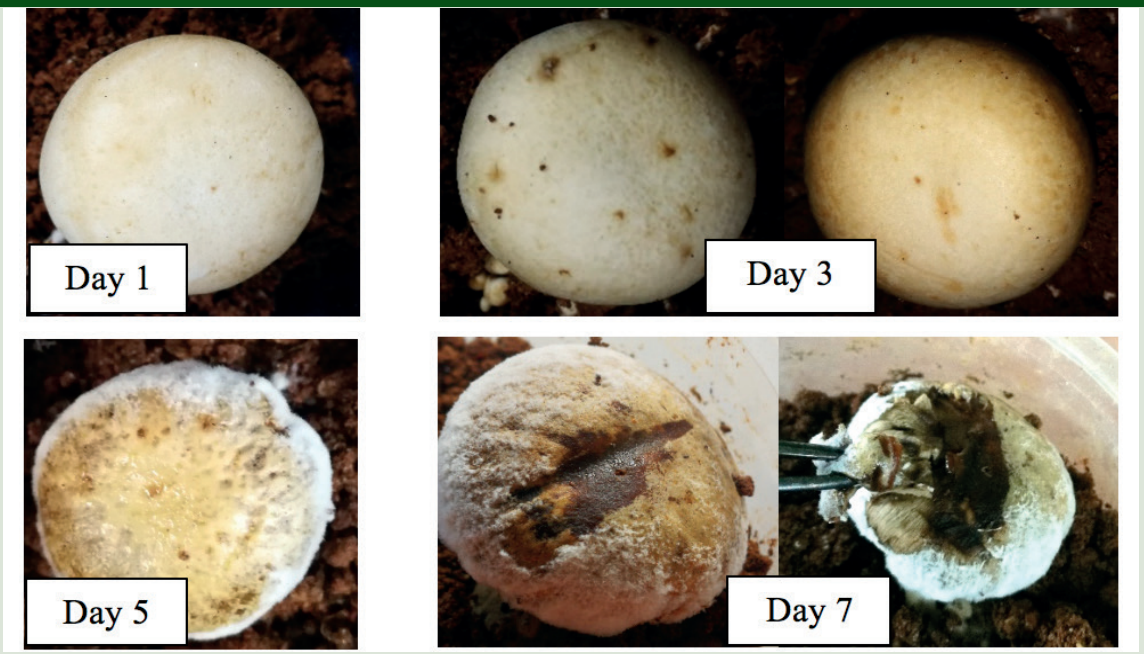
The discrepancies between the actual measurements of phialospores and aleuriospores and the description provided by Li et al. (2019) may be attributed to the genetic variability of the pathogen influenced by environmental factors. Single spores are attached to the septated, hyaline, upright, and verticillate conidiophore.

Ability of the Pathogen to Cause an Infection Through Pathogenicity Test

The disease progression following the inoculation of healthy mushroom fruiting bodies with *M. perniciosa* are illustrated in Figure 4. Symptoms of the disease commenced on the third day of observation, manifesting as spots on the cap surface and as metallic brown staining of the caps. By the fifth day, the development of white, fluffy, or cottony mycelial growth of the pathogen is evident, leading to a water-soaked appearance of the external parts of the fruiting body and internal tissue necrosis, as indicated by rotting, observed on the seventh day post-inoculation. These findings are consistent with the descriptions provided by Du et al. (2021), Kouser and Shah (2013), and Fletcher and Gaze (2008) in their respective pathogenicity tests.

Figure 4

Wet Bubble Disease Symptoms on Mature Fruiting Bodies of Button Mushrooms After 7 days of Inoculation



According to Kaur (2013), the infection of *M. perniciosa* during the early stage of mushroom development results in the formation of undifferentiated masses of mushrooms, while spotting symptoms develop on infected mature fruiting bodies. The moderate to high virulence levels of the yellow to brown *M. perniciosa* isolates is attributed to a large amount of conidia production, and the production of secondary metabolites (e.g., pigments and mycotoxins) which play an important role in pathogenesis (Li et al., 2019).

Bioefficacy of the Different Plant Extracts Against *M. perniciosa* Through *In Vitro* Conditions

The inhibitory effects of the plant extracts against *M. perniciosa* determined by inhibition zones were first observed and recorded on the third day after treatment application using a bioassay test by the disk diffusion method. Statistical analysis revealed highly significant differences among treatments in the inhibition zones, as determined by Tukey HSD mean comparisons at each observation day (Table 2).

On the third day, all plant extracts exhibited inhibitory effects against *M. perniciosa*. Tarragon showed the largest inhibition zone at 8.55mm, followed by the benomyl fungicide (positive control) with 6.62mm, then rosemary at 6.54mm. There was no significant difference between benomyl and rosemary. Oregano's inhibition zone of 3.62mm was not significantly different from ginger (2.91mm), lantana (2.60mm), peppermint (2.44mm), and angel's trumpet (2.36mm). Likewise, ginger, lantana, peppermint, and angel's trumpet did not differ significantly from neem, which had the smallest zone at 1.83 mm. Timorex Gold, a biofungicide, did not produce any inhibition zone until the seventh day of observation.

On the 4th day, tarragon continues to exhibit the largest inhibition zone at 7.85mm, followed by benomyl fungicide at 5.86mm. Subsequently, rosemary measures 3.95mm, and oregano measures 1.98mm, with these differences not being statistically significant. Ginger, lantana, angel's trumpet, peppermint, and neem, with inhibition zones of 1.54 mm, 1.19mm, 1.15mm, 0.94mm, and 0.79mm respectively, showed narrow zones of inhibition that are not significantly different from each other. This suggests that their inhibitory effects on the pathogen are minimal.

On the 5th day, tarragon had the largest inhibition zone (6.09mm), comparable to the

Table 2

Morpho-Cultural Characteristics of M. perniciosa After 5 days of Incubation

Treatments	Inhibition Zone (mm)				
	Day 3	Day 4	Day 5	Day 6	Day 7
T1 – Sterile Distilled Water (Negative control)	0.00^{e}	0.00^{e}	0.00^{c}	0.00^{c}	0.00^{b}
T2 – Peppermint	2.44^{cd}	0.94^{de}	0.00^{c}	0.00^{c}	0.00^{b}
T3 – Oregano	3.62^{c}	1.98^{d}	1.22^{bc}	0.00^{c}	0.00^{b}
T4 – Ginger	2.91^{cd}	1.54^{de}	0.68^{c}	0.00^{c}	0.00^{b}
T5 – Rosemary	6.54^{b}	3.95^{c}	2.88^{b}	2.35^{b}	1.41^{b}
T6 – Tarragon	8.55^{a}	7.85^{a}	6.09^{a}	5.05^{a}	4.53^{a}
T7 – Lantana	2.60^{cd}	1.19^{de}	0.53^{c}	0.00^{c}	0.00^{b}
T8 – Neem	1.83^{d}	0.79^{de}	0.35^{c}	0.00^{c}	0.00^{b}
T9 – Angel's trumpet	2.36^{cd}	1.15^{de}	0.54^{c}	0.00^{c}	0.00^{b}
T10 – Timorex Gold (Biofungicide)	0.00^{e}	0.00^{e}	0.00^{c}	0.00^{c}	0.00^{b}
T11 – Benomyl fungicide (Positive control)	6.62^{b}	5.86^{b}	5.71^{a}	5.30^{a}	4.93^{a}

**Means with the same letters are not significantly different according to Tukey HSD test at α = 0.05*

benomyl fungicide (positive control; 5.71mm). Secondly, rosemary exhibited an inhibition zone of 2.88mm, which was not significantly different from oregano's 1.22mm. Peppermint no longer demonstrated any inhibitory effect on the pathogen, while ginger (0.68mm), angel's trumpet (0.54mm), lantana (0.53mm), and neem (0.35mm) displayed very narrow inhibition zones, which were not significantly different from peppermint and the negative control (sterile distilled water).

On the 6th and 7th days, only tarragon with inhibition zones measuring 5.05mm and 4.53mm demonstrated an inhibitory effect comparable to that of the benomyl fungicide (positive control), which exhibited inhibition zones of 5.30mm and 4.93mm, respectively. Rosemary also maintained its inhibitory effect, with inhibition zones of 2.35mm on the sixth day and 1.41mm on the seventh day of observation.

The antifungal activity of the plant extracts used in the experiment is attributed to their phenolic compounds, which have been widely described in various scientific literature. Tarragon extracts have phenolic compounds such as flavonoids (Ekiert et al., 2021); 1-Methoxy-4-(2-propenyl) benzene, also known as methyl chavicol (Obolskiy et al., 2011), and sabinene (Badea & Delian, 2014). Estragole is a phenylpropanoid, and sabinene is a terpene that is highly lipophilic by nature and has a low molecular weight, thereby capable of disrupting the cell membrane, causing cell death, or inhibiting the sporulation and germination of food spoilage fungi (Nazzaro et al., 2017). Likewise, according to Kheiria et al. (2021), rosemary has high phenolic compounds such as phenolic acids and flavonoids. The major polyphenolic components of rosemary are carnosic acid, carnosol, rosmarinic acid, and hesperidin (Nieto et al., 2018). It was also reported by Tanović et al. (2009), as cited by Regnier and Combrink (2010), that oregano oil was characterized by high levels of thymol and carvacrol, which were effective inhibitors of *M. perniciosa*.

The active component of Timorex Gold biofungicide is tea tree oil, primarily made up of aromatic terpene hydrocarbons that are highly volatile and poorly water-soluble. According to Regnier and Combrinck (2010), who tested essential oils for controlling wet bubble disease in *Agaricus bisporus* both in vitro and in vivo, tea tree oil was ineffective. Similarly, Potočnik et al. (2010) observed that at a 1% concentration, tea tree oil does not combat cobweb disease *(Cladobotryum dendroides)* in edible mushrooms.

Tiwari et al. (2011) identified that the key factors affecting extract quality include the plant part selected, the solvent used, and the extraction

method. The impact of plant phytochemicals depends on the plant material's nature, processing degree, moisture level, and particle size. Differences in extraction techniques influence the amount and secondary metabolite profile, depending on factors such as extraction type, duration, temperature, solvent properties, concentration, and polarity.

Other Observation

At the BPI Button Mushroom Project Growing House, the fruiting bodies emerged 30 days after planting (inoculating the spawn). During the first week, no disease incidence (0%) was observed at 23-24°C and 91% relative humidity. By the second week, disease incidence increased to 20% when temperatures were around 24°C and relative humidity was 90.95%. By the third week, the disease incidence decreased to 17%, with room conditions at 21-24°C and 90.67% relative humidity (Figure 5).

Wet bubble disease was most common during the 4th week of observation, with a 44% infection rate at 23-25°C and 91% relative humidity, likely due to increased yield and more infected fruiting bodies. The incidence declined in the 5th week to 9% at 20-24°C and 90.52% humidity. By the 6th week, it further decreased to 5% at 23-24°C and 90.91% humidity. Kouser and Shah (2013) noted a large number of *M. perniciosa* spores on the mycelial mat of white button mushrooms at 95% humidity, while several studies, including Siwulski et al. (2011), Glamoclija et al. (2008), and Pieterse (2005), identified 25°C as the optimal temperature for the pathogen's growth and development.

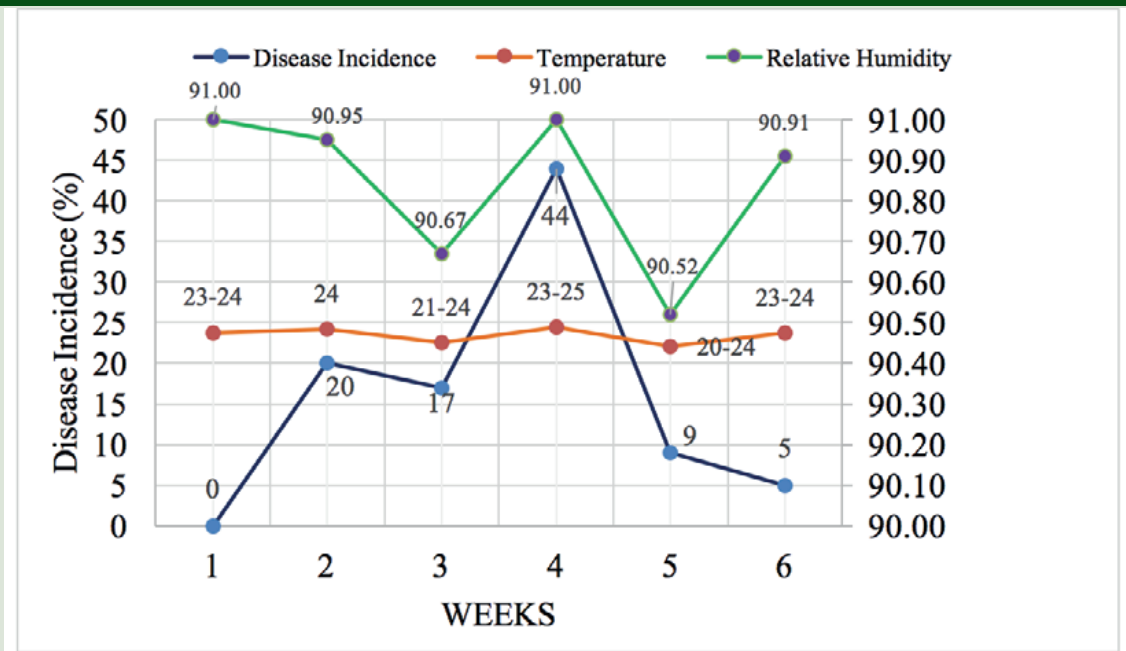
Wet bubble outbreaks are typically linked to contamination of the casing, water supply, or other dust and debris (Pyck & Grogan, 2015). The infection caused by *M. perniciosa* depends on several factors, including temperature, relative humidity in growing rooms, and the pathogenicity of the isolates (Szumigaj-Tarnowska et al., 2015).

Conclusions

M. perniciosa was characterized by symptoms and morpho-cultural characteristics. The observations matched those of the pathogen described in earlier research. Additionally, pathogenicity testing confirmed its ability to infect white button mushrooms, as it caused

Figure 5

Disease Incidence of M. perniciosa on Prevailing Room Temperature and Relative Humidity at the Bureau of Plant Industry Button Mushroom Project Growing House



characteristic brown spots that progressed to fluffy or cottony mycelial growth, leading to internal and external decay within 7 days of inoculation.

The results of the bioassay test indicated that extracts of tarragon at a concentration of 50mg/mL exhibited the highest inhibitory effect against *M. perniciosa* on the third day of observation, followed by rosemary. These extracts demonstrate potential for use as antifungal agents to manage wet bubble disease in button mushrooms. While peppermint, oregano, ginger, rosemary, lantana, neem, and angel's trumpet showed phytotoxic effects, these effects were limited to a short duration, suggesting that the pathogen could easily develop resistance. Therefore, application should be considered within a brief time frame. The bio-efficacy of plant extracts against pathogens varies depending on the concentration of active components such as phenolic compounds and terpenes successfully extracted. Tarragon showed the greatest inhibitory effect on the third day; however, its efficacy gradually diminished to nearly 50% by the seventh day. This indicates that reapplication should occur at five-day intervals, taking into account that, according to the pathogenicity test, the pathogen began to infect the fruiting bodies three days after inoculation.

Recommendation

Tarragon extract at 50 mg/mL exhibits the most significant inhibitory effect against *M. perniciosa* on button mushrooms, followed by rosemary. Considering that the pathogen begins to infect after three days of inoculation, it is therefore recommended to apply tarragon extract at five-day intervals, as its efficacy gradually diminishes to approximately 50% after seven days. Similarly, rosemary extract should be re-applied within three days, as its efficacy markedly decreases to approximately 60% on the fourth day. Although both tarragon and rosemary demonstrate considerable effects against the pathogen, further validation of their antifungal activity must be conducted on button mushroom beds. Additionally, plant extracts with minimal antifungal efficacy such as peppermint, oregano, ginger, lantana, neem, and angel's trumpet could be combined, or the concentration and application intervals of each extract could be adjusted to enhance effectiveness against the pathogen. Further research should be conducted to evaluate the effects of botanical plants on *M. perniciosa* using alternative extraction procedures and solvent types, particularly aqueous solutions at varying concentrations. Furthermore, future research should incorporate continuous monitoring of agroclimatic conditions throughout the cropping cycle. In this study, these parameters were recorded solely during disease incidence assessments within the growing environment, and not during pathogenicity testing, which limited the ability to correlate pathogen growth with host response.

It is also suggested that the isolated *M. perniciosa* will be subjected to further study on its genetic variability on button mushrooms at a wider scale on existing farms in the Cordillera to further understand the nature of its infectivity and distribution for a successful application of management strategies.

Acknowledgment

The researcher would like to acknowledge the Department of Agriculture – Bureau of Plant Industry Button Mushroom Project, headed by Ms. Mary Ann Guerrerro for allowing the collection of mushroom specimens and gathering of necessary data for the conduct of this study; to Dr. Judith G. Lawilao for mentoring; to Prof. Asuncion L. Nagpala and Prof. Bonie S. Ligat for sharing their invaluable technical expertise and moral support and to Han B. Bonoy for his assistance in the data analysis.

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