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Research Article

Efficacy of botanical extracts for controlling wet bubble disease caused by *Mycogone perniciosa* in white button mushrooms

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ABSTRACT: Wet bubble disease, caused by *Mycogone perniciosa*, is a major fungal problem in *Agaricus bisporus* (button mushroom). It leads to serious yield losses, particularly in India. Chemical fungicides offer limited control and raise environmental concerns. This study investigated eight locally available plants as potential biocontrol agents. Aqueous and ethanol extracts were prepared and evaluated *in vitro* using the poisoned food technique at concentrations of 1%, 2%, and 3%. Their effects on the mycelial growth of *M. perniciosa* and *A. bisporus* were assessed. At 3% concentration, *Curcuma longa* completely inhibited *M. perniciosa.* Ethanol extracts of *Mentha longifolia, Juglans regia*, and *Urtica dioica* also displayed strong activity, while *Azadirachta indica* ethanol extract significantly reduced pathogen growth. *In vivo* trials confirmed these observations. Mushroom beds treated with selected botanicals showed reduced disease incidence and improved fruit body quality compared to untreated controls. The findings demonstrate that plant-based extracts, particularly ethanol formulations, hold promise as eco-friendly alternatives to synthetic fungicides for managing wet bubble disease. They can be integrated into sustainable mushroom production systems to reduce chemical dependence and promote safer cultivation practices.

KEYWORDS: *Agaricus bisporus*, biocontrol, mushroom, *Mycogone perniciosa*, wet bubble disease

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INTRODUCTION

Cultivated globally, mushrooms are recognised for their numerous nutritional and therapeutic properties. Throughout history, mushrooms have been regarded as a culinary marvel and used as a base for fine dining meals due to their distinct flavour. Although over 2,000 mushroom species exist in the wild, only approximately 25 are regularly used as food, with a few cultivated for economic purposes. Because of their appealing taste, texture, and aroma, medical qualities, and economic relevance, mushrooms are regarded as a delicacy with great nutritional and functional value. They are also recognised as nutraceutical foods. The line separating edible from medicinal mushrooms is not always clear-cut, though, as many commonly consumed edible species have therapeutic qualities and some that are used medically are also edible. *A. bisporus* is the most widely grown mushroom globally, followed *by Lentinus edodes, Pleurotus* spp.*,* and *Flammulina velutipes* (Gea *et al*., 2021).

Agaricus bisporus (Lange.) Imbach, commonly referred to as European or white button mushrooms, is one of the most commercially grown mushrooms, widely cultivated around the globe. India's agricultural sector yields nearly 600 million tonnes of byproducts annually, which serve as a viable substrate for mushroom cultivation. Among cultivated mushrooms, the white button mushroom (*A. bisporus*) is the most popular worldwide, contributing approximately 31% to global output and 85% to India's total mushroom production (Sharma, 2017). In the 1990s, white button mushroom farming became commercially viable in India, and a number of export-focused, high-tech farms were established in cooperation with foreign technology companies. Still, small farms produce a sizable portion of the world's mushrooms. Mushroom infections are the main obstacles preventing this crop in the valley from developing quickly enough to become a cottage business. Several bacterial, viral, and fungal diseases infest the crop, causing it to fail entirely or partially, or at least degrade the quality of the produce.

In Jammu and Kashmir, button mushrooms are predominantly cultivated in residential rooms. In limited cases, dedicated structures are used; however, the lack of environmental regulation and compost pasteurization in these facilities creates favourable conditions for the emergence and spread of pathogens and competitive molds (Amin *et al*., 2021). The three types of fungal diseases—dry bubble, wet

bubble, and cobweb—that cause crop losses in mushroom farms differ in severity (Arora, 2003). The most damaging of them is Wet Bubble Disease (*M. perniciosa*), which results in soft rot or decomposition of the entire fruiting body. If the infection is not well contained promptly, the entire crop is badly infected.

The illness is identified by the appearance of yellowish, moldy mycelium growth on some fruit bodies. According to Fletcher *et al.* (1975), they generated enormous, uneven, confluent lumps of *A. bisporus* without any indication of organogenesis or differentiation. Often, these tumorous entities appeared as teardrops, flowing with amber liquid. *M. periniciosa* is a frequent contaminant that severely reduces button mushroom output in the Kashmir valley. Although some workers have recommended using fungicides to combat this disease, fungitoxicants are rarely used by mushroom producers to cure this devastating illness. This option was frequently deemed non-economic by them. The goal of the current study was to use botanicals to treat this critical condition in an environmentally responsible and commercially feasible manner.

MATERIALS AND METHODS

A study was conducted during 2008-2009 at the Mushroom Research and Training Centre, Division of Plant Pathology, SKUAST-Kashmir, Shalimar, Srinagar, to evaluate the effectiveness of botanicals in managing wet bubble disease in *A. bisporus* (Lange) Imbach.

In vitro assessment

To assess their antifungal potential, eight readily accessible botanicals (Table 1) were subjected to *in vitro* testing via the poisoned food technique. Their aqueous and ethanol extracts were tested against *A. bisporus* and *M. perniciosa* at 1%, 2%, and 3% concentrations (Figures 1 and 2).

Formulation of plant extracts

Aqueous extract

The medium-sized leaves and tender shoots of each plant, except turmeric, whose rhizomes were utilised, were employed to create the aqueous extracts (Figure 3) (Singh *et al.*, 2022). The used botanicals were carefully cleaned under the shade and then rewashed with tap water. Turmeric rhizomes and walnut hulls were ground in an electric grinder after being dried until they were brittle. One litre of sterilised distilled water was added to the plant material to dilute it, followed by filtration through four layers of muslin cloth. The resulting filtrate was further purified under aseptic conditions using Whatman filter paper No. 41, and subsequently passed through a bacteria-proof Sietz filter. This final filtrate was considered the 100% aqueous extract. To prepare test

Table 1. Botanicals used *in vitro against both A. bisporus and M. perniciosa*

Scientific name	Vernacular name	Plant Part utilised
Curcuma longa	Turmeric	Rhizome
Matricaria sp.	Shale-lout	Whole plant (leaves and tender shoots)
Artemisia annua	'Tethwan'	Foliage
Mentha longifolia	'Pudina'	Whole plant
Lavandula officinalis	Lavender	Whole plant
Juglans regia	Walnut	Hull
Urtica dioica	Soi	Whole plant
Azadirachta indica	Neem	Leaves



Figure 1. Locally available botanicals were used in the study.

concentrations of 1%, 2%, and 3%, the appropriate volume of this extract was mixed with 50 millilitres of sterilised distilled water and then aseptically incorporated into 50 millilitres of double-strength molten PDA medium in a 250-millilitre Erlenmeyer flask, thereby achieving the desired double strength of each concentration. After that, the modified



Figure 2. Evaluation of dried botanicals.

PDA was aseptically transferred into sterile petri plates. *A. bisporus* (14-day-old culture) and *M. perniciosa* (7-day-old culture) each had a 5 mm diameter disc that was aseptically inserted into the centre of the petri plates.

Each treatment was replicated three times. The percentage mycelial growth inhibition of the test fungus was used to evaluate the relative efficacy of the test botanicals' aqueous extract. The per cent inhibition of mycelial growth was determined by the formula (Vincent, 1974):

$$\text{Mycelial Growth Inhibition } (\%) = \frac{(C-T)}{C} \times 100$$

where, C = Radial growth of mycelium (mm) in the control
T = Radial growth of mycelium (mm) in the treated sample

Ethanol extract

The botanicals' ethanol extracts were produced using Krishnananda's methodology (Ingle *et al.*, 2017). After washing with tap water and rinsing with sterile distilled water,



Figure 3. Preparation of botanical extracts.

the plants and plant components were dried in the shade. The rhizomes of turmeric and the walnut husk were also dried until brittle. An electric grinder was used to crush the shade-dried plants and plant components into a fine powder for each botanical. After that, the powder was dried and kept in plastic containers for further use.

In a 250 mL conical flask, 20 g of the dried powdered plant components were combined with 200 mL of solvent

(70% ethanol). Additionally, 20 g of crushed turmeric rhizomes were combined with 200 mL of solvent in an electric grinder. Every flask was fitted with sterile cotton plugs that were snugly sealed, its mouth covered with aluminium foil, and it was left on a shaker for thirty-six hours. The suspended plant material was allowed to settle in the flasks for six hours before the extract was decanted, filtered, and centrifuged for fifteen minutes at 5000 rpm. To get a final volume that was one-fourth of the initial volume, the supernatant was collected and the solvent was allowed to evaporate at 40-50ºC. The extract, which was now considered to be 100% standard, was kept for later use in airtight bottles or containers. Using the poisoned food technique, the ethanol extract of each botanical was biotested for fungitoxic action against *A. bisporus* and *M. perniciosa* at concentrations of 1, 2, and 3% (Shivam *et al.*, 2016).

In vivo assessment

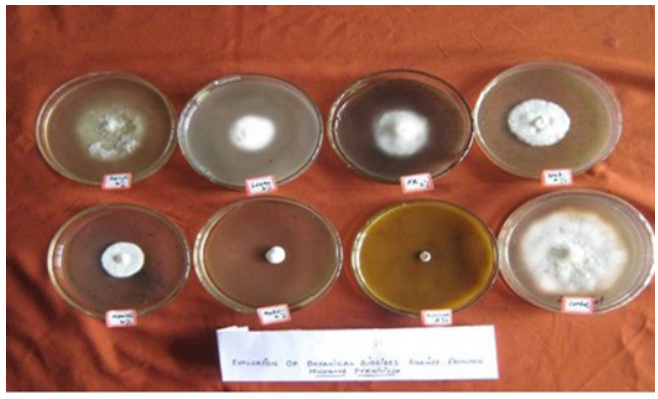
In vivo testing of various potent botanical extracts revealed that *C. longa*, *Artemisia annua*, and *Lavandula officinalis* exhibited strong inhibitory activity against *M. perniciosa*, while exerting minimal inhibition on *A. bisporus*. After being collected, the botanicals were sun-dried until they were brittle, and then they were ground into a fine powder in a grinder. Before the illness was inoculated, the powder was blended at rates of 0.5, 1, and 2 per cent with the casing mixture after passing through two layers of muslin cloth. Before the pathogen was inoculated, the spawn-run compost (10 kg poly bags) was covered with the botanically admixed casing mixture. Three replications were conducted for every treatment, with each replication represented by a single bag. The bags were stored in the production room between 22 and 24°C for four days following casing and between 15 and 180°C for the remaining time till cropping was completed. Control treatments included both pathogen injections and those without any botanical admixture. While other quality characteristics were only examined during the first flush of cropping, the percentage of disease intensity and yield per bag were computed during a one-month cropping period.

Statistical analysis

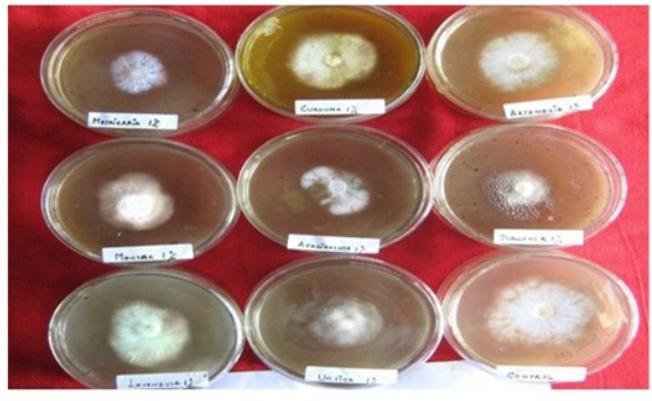
Statistical analysis was performed on the gathered data. The significance of the differences between the treatments in different experiments was evaluated using the techniques recommended by Gomez and Gomez (1984). The computer software "Minitab" was utilised to analyse the data.

RESULTS

In vitro assays were conducted to evaluate the antifungal activity of eight botanicals—*Matricaria* sp., *C. longa*, *M. longifolia*, *Artemisia annua*, *J. regia*, *Lavandula officinalis*, *Azadirachta indica*, and *U. dioica* against both *M. perniciosa* and *A. bisporus* mycelium (Figure 4a).



a) Evaluation of Botanical Biocides against pathogen *Mycogone perniciosa*



b) Evaluation of Botanical Biocides against host *Agaricus bisporus*

Figure 4 (a-b). Evaluation of botanical extract against *M. perniciosa and Agaricus bisporus.*

In vitro assessment of antifungal activity against the pathogen *Mycogone perniciosa*

Aqueous extract

The test botanicals had substantial inhibitory effects on the mycelial growth of *M. perniciosa* when their aqueous extract was assessed at concentrations of 1, 2, and 3% using the poisoned food technique (Table 2, Figure 4(a). *Matricaria* sp., *L. officinalis*, and *C. longa* aqueous extracts showed, on average, the highest levels of inhibition (59.94-65.19%) of *M. perniciosa* mycelium. The next highest level of inhibition (58.94%) was found in the aqueous extract of *A. annua*, whereas the average inhibition for *A. indica* and *M. longifolia* was between 45.67 and 47.83%. Of all the investigated botanicals, the test fungus *U. dioca* showed the least inhibitory activity (14.96%) against *M. perniciosa*. At the lowest test concentration of 1%, the minimum average inhibition of 34.28% was observed, and at the highest test concentration of 3%, the maximum average inhibition of 65.79%. These results indicate a considerable rise in the mycelial growth inhibition with an increase in the extract concentration. A notable interaction was found between the type of botanical used and its concentration in aqueous

Table 2. Assessment of the antifungal activity of aqueous plant extracts against *M. perniciosa,* responsible for wet bubble disease in *Agaricus bisporus*

Conc. (%) / Botanical	Per cent inhibition over control			Mean
	1	2	3	
Curcuma longa	54.16 (47.38)	64.58 (53.48)	76.84 (61.24)	**65.19 (54.03)^{a}**
Matricaria spp.	51.15 (45.66)	59.48 (50.47)	74.99 (60.00)	**61.49 (52.04)^{b}**
Artemisia annua	47.91 (43.80)	59.02 (50.19)	69.90 (56.73)	**58.94 (50.24)^{c}**
Juglans regia	27.08 (1.34)	54.16 (47.38)	63.88 (53.05)	**48.37 (43.92)^{d}**
Lavandula officinalis	46.29 (42.87)	63.88 (53.05)	69.67 (56.59)	**59.94 (50.83)^{c}**
Azadirachta indica	28.23 (32.08)	36.11 (36.93)	72.68 (58.48)	**45.67 (42.49)^{d}**
Urtica dioica	5.55 (13.55)	11.11 (19.44)	28.23 (2.08)	**14.96 (21.69)^{e}**
Mean	**34.28 (34.81)^{a}**	**50.97 (45.17)^{b}**	**65.79 (54.38)^{c}**	
	S.E±		**CD(p=0.05)**	
Botanical	0.53		0.89	
Concentration	0.33		0.55	
Botanical x Concentration	0.93		1.56	

Values represent the means of three replications. Figures in parentheses indicate angular-transformed values. Means followed by the same letter(s) are not significantly different at the 5% significance level (α = 0.05). *S.E.*± denotes the standard error of the mean, and *CD* (p = 0.05) represents the critical difference at the 5% level of significance.

Table 3. Assessment of antifungal activity of ethanol extract of botanicals against *M. perniciosa* causing wet bubble disease of *Agaricus bisporus*

Conc. (%) / Botanical	Per cent inhibition over control			Mean
	1	2	3	
Matricaria spp.	85.41 (67.54)	90.27 (71.83)	93.74 (75.53)	**89.80 (71.63)^{b}**
Curcuma longa	97.91 (81.77)	98.60 (83.36)	100.00 (90.00)	**98.83 (85.04)^{a}**
Mentha longifolia	44.44 (41.80)	53.24 (46.85)	68.05 (55.59)	**55.24 (48.08)^{e}**
Artemisia annua	49.53 (44.73)	63.42 (52.78)	78.94 (62.68)	**64.96 (53.39)^{d}**
Juglans regia	35.18 (36.37)	51.84 (46.05)	70.09 (56.84)	**52.37 (46.42)^{f}**
Lavandula officinalis	51.38 (45.79)	71.17 (57.52)	76.42 (60.94)	**66.32 (54.75)^{c}**
Azadirachta indica	26.39 (30.89)	76.38 (60.94)	91.66 (73.25)	**64.81 (55.02)^{c}**
Urtica dioica	1.84 (7.70)	4.62 (12.39)	9.72 (18.13)	**5.39 (12.74)^{g}**
Mean	**49.01 (44.57)^{C}**	**62.72 (53.37)^{B}**	**73.57 (61.62)^{A}**	
	S.E±		**CD(p=0.05)**	
Botanical	(0.53)		(0.89)	
Concentration	(0.33)		(0.55)	
Botanical x concentration	(0.93)		(1.56)	

Values represent the means of three replications. Figures in parentheses indicate angular-transformed values. Means followed by the same letter(s) are not significantly different at the 5% significance level (α = 0.05). *S.E.*± denotes the standard error of the mean, and *CD* (p = 0.05) represents the critical difference at the 5% level of significance.

extract form. Among the botanicals tested*, C. longa* and *Matricaria* sp. at a 3% concentration showed the most effective inhibition of *M. perniciosa*, reducing its growth by 74.9% to 76.8%. *Azadirachta indica* followed closely, achieving 72.6% inhibition at the same concentration. Other botanicals, including *M. longifolia, Artemisia annua,* and *Lavandula officinalis*, also demonstrated considerable antifungal activity at 3% concentration, with growth suppression ranging between 69.6% and 70.1%. In contrast, *U. dioica*, tested at 1% concentration, was the least effective, resulting in only 5.5 % inhibition of fungal growth.

Ethanol Extract

The antifungal efficacy of ethanol-based botanical extracts against *M. perniciosa* was assessed using the poisoned food technique, with extract concentrations tested at 1%, 2%, and 3% levels. The results showed that all of the botanicals demonstrated significantly different inhibitory effects at each concentration level (Table 3, Figure 4(a). Comparatively speaking, the highest mycelial growth inhibition was shown by *C. longa* and *Matricaria* sp., at 98.83% and 89.80%, respectively. *Lavandula officinalis* and *Azadirachta indica* exhibited relatively low inhibition percentages of 66.32% and 64.81%, respectively. *U. dioica* showed the least antifungal activity with only 5.39% inhibition, while *J. regia* recorded an inhibition rate of 52.37% against the test pathogen. Minimum inhibition was observed at lower concentrations (49.01%), while maximal inhibition was observed at higher values (73.57%). There was also a notable interaction between the concentrations of the ethanol extracts and the botanicals. At a concentration of 3%, *C. longa* demonstrated the highest level of inhibition, and at a concentration of 2%, the same botanical showed 98.60% growth inhibition. *Matricaria* sp., evaluated at a 3% concentration, achieved 93.74% inhibition of *M. perniciosa* growth, whereas *C. longa* demonstrated superior efficacy, providing 97.91% inhibition even at a lower concentration of 1%. The least pathogen-inhibiting botanical*, U. dioica,* barely inhibited growth by 1.84% at a 1% concentration.

In vitro assessment of antifungal activity against the *Agaricus bisporus* mycelium

Aqueous extract

Botanical aqueous extracts were tested for their impact on the mycelial growth of *A. bisporus* using the poisoned food technique, with concentrations set at 1%, 2%, and 3%. The results showed that the test botanicals had significant inhibitory effects (Table 4, Figure 4(b). The water extracts of *A. longa, A. annua,* and *A. indica* showed, on average, the least amount of inhibition (6.52-87.71%) of *A. bisporus* mycelium. The least inhibitory substance was found in the *L. officinalis* aqueous extract (11.09%), while the highest average inhibition of the *A. bisporus* mycelium was found in the *U. dioica* extract (40.96%). At the lowest test concentration of 1%, the least average inhibition of 12.35% was observed, and at the highest test concentration of 3%, the maximum average inhibition of 28.74%. These results indicate a considerable rise in inhibition with an increase in extract concentration. There was also a notable relationship between the concentrations of botanical aqueous extracts and their interactions. Among the botanicals tested, *Artemisia annua* exhibited the lowest inhibition of *A. bisporus* mycelial growth, with inhibition ranging from 3.95% to 6.76% at concentrations up to 2%. Similarly, *Lavandula officinalis* and *Azadirachta indica* at 1%, *Matricaria sp.* at 1%, *A. indica* at 2%, and *C. longa* and *A. annua* at 3% showed limited inhibitory effects, with mycelial suppression between 7.80% and 10.93%. In contrast, *U. dioica* consistently caused the highest level of mycelial inhibition across all tested concentrations.

Ethanol extract

Aqueous extracts of various botanicals were assessed for their impact on the mycelial growth of *A. bisporus* through the poisoned food technique, employing extract concentrations of 1%, 2%, and 3%. The results showed that all of the botanicals demonstrated significantly different inhibitory effects at each concentration level (Table 5, Figure 4(b). Overall, *A. bisporus* mycelial growth was inhibited least by *C. longa, A. annua,* and *L. officinalis* (7.70-9.22%), with *A. indica* coming in second (9.89%). The lowest inhibition that *U. dioca* showed was 35.48%. Again, the higher dosages showed maximum inhibition of 28.18%, while the lower concentrations showed minimal inhibition of 12.42% on average. A significant interaction

Table 4. *In vitro* assessment of aqueous botanical extracts on the mycelial growth of host *Agaricus bisporus*

Conc. (%) / Botanical	Per cent inhibition over control			Mean
	1	2	3	
Matricaria spp.	8.33 (16.47)	19.26 (26.00)	44.78 (42.00)	**24.12 (28.16)^{c}**
Curcuma longa	04.16 (11.38)	06.24 (14.40)	09.16 (17.56)	**06.52 (14.44)^{a}**
Mentha longifolia	20.30 (26.74)	29.16 (32.68)	41.14 (39.89)	**30.20 (33.10)^{d}**
Artemisia annua	04.68 (12.04)	06.76 (14.98)	10.93 (19.18)	**07.45 (15.40)^{a}**
Juglans regia	21.35 (27.49)	33.33 (35.25)	43.74 (41.40)	**32.80 (34.71)^{d}**
Lavandula officinalis	03.95 (11.09)	14.58 (22.35)	17.18 (24.76)	**11.90 (19.40)^{b}**
Azadirachta indica	04.29 (11.86)	07.80 (16.13)	14.06 (22.00)	**8.71 (16.66)^{a}**
Urtica dioica	31.76 (34.28)	42.18 (40.49)	48.95 (44.39)	**40.96 (39.72)^{e}**
Mean	**12.35 (18.92)^{a}**	**19.91 (25.28)^{b}**	**28.74 (31.40)^{c}**	
	S.E±		**CD(p=0.05)**	
Botanical	(1.12)		(2.26)	
Concentration	(0.68)		(1.38)	
Botanical x concentration	(1.94)		(3.91)	

Values represent the means of three replications. Figures in parentheses indicate angular-transformed values. Means followed by the same letter(s) are not significantly different at the 5% significance level (α = 0.05). *S.E.*± denotes the standard error of the mean, and *CD* (p = 0.05) represents the critical difference at the 5% level of significance.

Table 5. *In vitro* evaluation of ethanol extracts from various botanicals on the mycelial growth of host *Agaricus bisporus*

Conc. (%) / Botanical	Per cent inhibition over control			Mean
	1	2	3	
Matricaria spp.	21.14 (27.69)	28.12 (32.01)	44.26 (41.70)	**31.17 (33.80)^{c}**
Curcuma longa	05.20 (12.97)	07.18 (15.54)	13.01 (21.09)	**8.46 (16.53)^{a}**
Mentha longifolia	16.14 (23.68)	32.80 (34.92)	43.74 (41.40)	**30.89 (33.33)^{c}**
Artemisia annua	03.33 (10.29)	06.76 (14.98)	13.01 (21.09)	**07.70 (15.45)^{a}**
Juglans regia	20.72 (27.39)	35.31 (36.41)	37.49 (37.75)	**31.17 (33.85)^{c}**
Lavandula officinalis	02.70 (09.09)	10.41 (18.74)	14.57 (22.36)	**09.22 (16.73)^{ab}**
Azadirachta indica	03.12 (09.95)	10.93 (19.27)	15.62 (23.26)	**09.89 (17.49)^{b}**
Urtica dioica	27.08 (31.34)	35.63 (36.75)	43.74 (41.40)	**35.48 (36.50)^{d}**
Mean	**12.42 (19.05)^{C}**	**22.13 (27.19)^{B}**	**28.18 (31.25)^{A}**	
	S.E±		**CD(p=0.05)**	
Botanical	(0.96)		(1.93)	
Concentration	(0.58)		(1.18)	
Botanical x concentration	(1.66)		(3.35)	

Values represent the means of three replications. Figures in parentheses indicate angular-transformed values. Means followed by the same letter(s) are not significantly different at the 5% significance level (α = 0.05). *S.E.*± denotes the standard error of the mean, and *CD* (p = 0.05) represents the critical difference at the 5% level of significance.

was also observed between the type of botanicals and the concentrations of their ethanol extracts. At 1% concentration, *Artemisia annua*, *Lavandula officinalis*, and *Azadirachta indica* demonstrated minimal inhibition of *A. bisporus* mycelial growth, with suppression ranging from 2.70% to 3.33%. Slightly higher inhibition was observed with *C. longa* (up to 2%) and *A. annua* at 2%, showing growth inhibition between 5.20% and 7.18%. In contrast, at 3% concentration, *Matricaria sp.*, *M. longifolia*, and *U. dioica* achieved the highest levels of mycelial inhibition, ranging from 43.74% to 44.26%.

In vivo evaluation

Effect on disease development

Comparing all of the botanical treatments to the untreated check-I, the data (Table 6) showed that all of them reduced the percentage of disease intensity. Incorporating *C. longa* or *A. annua* at 3% into the casing layer effectively reduced wet bubble disease to 1.85-3.70%, corresponding to a disease control rate of 81.50-90.75%, compared to 20.0% disease incidence in the untreated control (check-I). The next most effective treatments were *C. longa* at 2.0% and *Lavandula officinalis* at 3.0%, which resulted in disease intensities ranging from 5.55% to 6.29% and provided 68.55% to 72.25% control of wet bubble disease. The least effective treatments included *C. longa* and *Artemisia annua* at 1% concentration, along with *Lavandula officinalis* at 2%, which resulted in wet bubble disease intensities of 9.27-10.37% and corresponding disease control ranging from 48.15% to 53.70%. In contrast, the pathogen-free, untreated check-II yielded a completely disease-free crop with no incidence of wet bubble.

Table 6. Effect of botanicals incorporated into casing material on the percent intensity of wet bubble disease (*M. perniciosa*) in white button mushroom (*Agaricus bisporus*) during spring 2009 and 2010

Treatment		Spring 2009	Spring 2010	Mean	Diseases control (%)
C. longa	0.5%	11.11 (19.40)	9.63 (18.05)	10.37 (18.72)^{f}	48.15
	1.0 %	7.41 (15.75)	5.18 (13.09)	6.29 (14.42)^{de}	68.55
	2.0%	2.22 (8.56)	1.48 (6.66)	1.85 (7.61)^{b}	90.75
A. annua	0.5%	10.37 (18.76)	10.37 (18.69)	10.37 (18.69)^{f}	48.15
	1.0%	6.67 (14.96)	7.41 (15.75)	7.04 (15.36)^{e}	54.80
	2.0%	4.44 (12.16)	2.96 (9.06)	3.70 (10.61)^{c}	81.50
L. officinalis	0.5%	14.81 (22.62)	14.07 (22.01)	14.44 (22.31)g	27.80
	1.0%	9.63 (18.05)	8.89 (17.34)	9.26 (17.69)^{f}	53.70
	2.0%	5.92 (14.02)	5.18 (12.82)	5.55 (13.42)^{d}	72.25
Check-I		22.96 (23.73)	17.04 (23.34)	20.00 (24.03)^{g}	-
Check-II		0.0 (2.86)	0.0 (2.86)	0.00 (2.86)^{a}	-
S.E±		**0.77**	**1.23**	**0.96**	
CD(p=0.05)		**1.57**	**2.50**	**11.91**	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical. Level of significance: α = 0.05.
(Note: S.E± represents the standard error, and CD (p = 0.05) denotes the critical difference at a significance level of 0.05).

Impact on crop productivity and related metrics:

Table 7 clearly shows that treatment with *Lavandula officinalis, Artemisia annua*, and *C. longa* at a 2.0% concentration resulted in the lowest fruit body counts, yielding between 86.0 and 86.6 fruit bodies per kilogram of mushrooms. Slightly higher yields were observed with *L. officinalis* and *C. longa* at the same concentration, producing 88.0 and 89.0 fruit bodies per kilogram, respectively. These values were still lower than those recorded in both the untreated control (94.1 fruit bodies/kg) and the uninfested-untreated control (92.83 fruit bodies/kg).

Effect on yield and yield components

The application of different botanical treatments significantly influenced the average fruit-body weight, yield, and quality of *Agaricus bisporus*.

Table 7. Effect of botanical biocides incorporated into *M. perniciosa*-infested casing on fruit body number, weight, and button yield during spring 2009-2010 (pooled data)

Botanical		No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield kgs/q compost
C. longa	0.5%	91.33^{d}	10.63^{de}	8.38^{e}
	1.0 %	89.00^{c}	10.70^{d}	9.09^{d}
	2.0%	86.66^{ab}	10.90^{c}	10.84^{b}
A. annua	0.5%	91.83^{d}	10.47^{f}	8.67^{e}
	1.0%	88.50^{c}	10.88^{c}	9.75^{cd}
	2.0%	86.16^{a}	11.16^{a}	11.06^{b}
L. officinalis	0.5%	92.00^{de}	10.52^{ef}	7.33^{f}
	1.0%	88.00^{bc}	10.87^{c}	8.42^{e}
	2.0%	86.00^{a}	11.20^{a}	9.21^{de}
Control I (Infested-untreated)		94.16^{f}	10.68^{d}	6.18^{g}
Control II (uninfested - untreated)		92.83^{ef}	10.93^{c}	12.84^{a}
SE±		**0.68**	**0.06**	**0.45**
CD (p=0.05)		**1.37**	**0.13**	**0.90**

Means of three replications; means followed by similar letter(s) are statistically identical.
Level of significance: α = 0.05.
(Note: S.E± represents the standard error, and CD (p = 0.05) denotes the critical difference at a significance level of 0.05).

Average fruit-body weight

The average weight of individual fruit bodies varied notably among treatments. *Artemisia annua* and *Lavandula officinalis*, both applied at 3.0% concentration, resulted in the highest average fruit-body weights, ranging from 11.16 to 11.20 g. These were followed by *C. longa* and *L. officinalis* at 2.0% concentration, which recorded average weights between 10.87 g and 10.90 g.

Button mushroom yield

Button yield also showed significant improvement with botanical applications. *C. longa* and *A. annua* at 2.0% concentration each produced the highest yields, ranging from 10.84 to 11.06 kg per quintal of compost, compared to 6.18 kg in the untreated check and 12.84 kg in the uninfested-untreated control. Among the subsequent effective treatments, *C. longa* and *Artemisia annua* at 1.0%, along with *Lavandula officinalis* at 2.0%, yielded between 9.09 and 9.75 kg of mushrooms per quintal of compost. In contrast, the lowest yield was observed with *L. officinalis* at 0.5%, which produced only 7.33 kg per quintal of compost.

Influence on sporophore quality metrics

Pileus weight

In the untreated check, the average pileus weight was 5.32 g, which significantly increased to 6.14-6.17 g in treatments with *A. annua* or *C. longa* at 2.0% concentration. Treatments with *C. longa* at 0.5% and 1.0%, and *Artemisia annua* or *Lavandula officinalis* at 1.0%, yielded 5.7-5.9 g pileus weight.

Pileus diameter

The maximum pileus diameter (3.62 cm) was observed with *A. annua* at 2.0% concentration. This was followed by treatments with *A. annua* at 1.0%, *C. longa*, and *L. officinalis* at 2.0%, which exhibited diameters between 3.50 and 3.57 cm, compared to 3.42 cm in the untreated check.

Stipe weight

Stipe weight in the infested, untreated check was 4.29 g, which significantly increased to 4.83-4.95 g in treatments with *Lavandula officinalis*, *Artemisia annua*, or *C. longa* at 2.0%. These botanicals at 1.0% concentration also showed improved stipe weights of 4.73-4.81 g, compared to 4.26 g in the uninfested-untreated control.

Stipe diameter

The maximum stipe diameter (1.35 cm) was recorded in the treatment with *A. annua* at 2.0%. This was followed by 1.31 cm diameters in treatments with *A. annua* at 1.0%, *C. longa*, and *L. officinalis* at 2.0%, compared to 1.23 cm in the untreated check (Table 8).

Table 8. Effect of botanical biocides incorporated into *M. perniciosa*-infested casing on quality parameters of white button mushroom (*Agaricus bisporus*) during spring 2009-2010

Botanical		Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
C. longa	0.5%	5.81^{b}	3.34^{f}	4.71^{d}	1.25^{d}
	1.0 %	5.95^{ab}	3.44^{e}	4.81^{bc}	1.28^{c}
	2.0%	6.17^{a}	3.53^{bc}	4.95^{a}	1.31^{b}
A. annua	0.5%	5.31^{d}	3.42^{e}	4.64^{e}	1.27^{c}
	1.0%	5.75^{b}	3.50^{cd}	4.76^{cd}	1.31^{b}
	2.0%	6.14^{a}	3.62^{a}	4.90^{a}	1.35^{a}
L. officinalis	0.5%	5.60c^{d}	3.43^{e}	4.83^{b}	1.24^{d}
	1.0%	5.70b^{c}	3.47^{de}	4.59^{b}	1.27^{c}
	2.0%	6.07^{a}	3.57^{ab}	4.73^{d}	1.31^{b}
Control I (infested-untreated)		5.32^{d}	3.42^{e}	4.29^{f}	1.23^{d}
Control II (uninfested-untreated)		6.26^{a}	3.56^{b}	4.26^{f}	1.25^{d}
SE±		**0.16**	**0.02**	**0.02**	**0.01**
CD (p=0.05)		**0.32**	**0.05**	**0.05**	**0.02**

Means of three replications; means followed by similar letter(s) are statistically identical.

DISCUSSION

The cultivation of button mushrooms (*A. bisporus* L.) is heavily impacted by wet bubble disease, a fungal infection caused by *M. perniciosa* Magn. This pathogen leads to significant yield losses and recurrent crop failures, particularly in the Kashmir Valley, where mushroom farming is a key agricultural activity.

In vitro evaluation of extracts from locally available botanicals against *M. perniciosa* (the pathogen) and *A. bisporus* (the host) showed that all the botanicals, to varying degrees, inhibited the growth of *M. perniciosa*.

The botanicals tested were highly effective at a 3 percent concentration. *In vitro* evaluation of aqueous extracts from eight locally available plant species revealed that *C. longa*, *Matricaria* sp., and *Lavandula officinalis* exerted minimal inhibitory effects on *A. bisporus* mycelium, while demonstrating strong inhibition of *M. perniciosa* mycelial growth. Similarly, ethanol extracts of *C. longa* and *Matricaria* sp. demonstrated comparable results. Notably, the ethanol extract of *Azadirachta indica* also significantly inhibited *M. perniciosa* while causing minimal inhibition to *A. bisporus*.

In an *in vitro* study, Chandhrapati (2021) assessed the potential of neem-based treatments, specifically neem leaves and neem cake, for controlling False Truffle disease (*Diehliomyces microsporus*) in *Agaricus* spp., and observed promising disease suppression.

Similar findings regarding the fungicidal properties of botanicals have been reported by other researchers, such as Grewal and Grewal (1988), (Altaf *et al*., 2022), (Drobnjaković, *et al.*, 2023) highlighted the varying fungicidal properties of leaf extracts from *Azadirachta indica*, *Chrysanthemum indicum*, and *Tagetes erecta* against weed molds affecting mushrooms.

Similarly, Saleh *et al*. (2006) reported the antifungal properties of *Artemisia herba-alba* against *Pencillium citrinum* and *Mucor rouxii*.

The successful management of wet bubble disease using *C. longa*, *Lavandula officinalis*, and *Artemisia annua*, as observed in the present study, aligns with the findings of Ghimire *et al.* (2021) and Singh and Singh (2012).

The results highlight the potential of *C. longa*, *Matricaria* sp., and *Azadirachta indica* for use as foliar sprays or as amendments in compost or casing soil to effectively suppress wet bubble disease.

The findings of this study support the use of *C. longa*, *Matricaria* sp., and *Azadirachta indica* as part of an integrated disease management strategy, applied either as foliar sprays or incorporated into compost and casing soil, to achieve effective suppression of wet bubble disease.

The demonstrated efficacy of these botanicals at a 3 per cent concentration highlights their potential as practical and sustainable options for controlling *M. perniciosa* in mushroom cultivation.

Furthermore, future research and field trials may be warranted to validate the effectiveness of these botanicals under different environmental conditions and cultivation practices. Continuous monitoring and refinement of botanical-based interventions can contribute to the development of eco-friendly and economically viable approaches to combat wet bubble disease and enhance the resilience of mushroom crops in the Kashmir valley and beyond.

CONCLUSION

The *in vitro* evaluation of botanical extracts against *M. perniciosa* (pathogen) and *A. bisporus* (host) yielded promising results for the biological control of wet bubble disease. All tested botanicals exhibited varying levels of growth inhibition against *M. perniciosa*, with the most pronounced effects observed at a 3 per cent concentration. Among the eight plant species analysed, *C. longa*, *Matricaria* sp., and *Lavandula officinalis* were promising for their strong antifungal activity against *M. perniciosa* while causing minimal impact on *A. bisporus* mycelium. Similar selective inhibition was noted with ethanol extracts of *C. longa* and *Matricaria* sp. Moreover, the ethanol extract of *Azadirachta indica* showed significant suppression of *M. perniciosa* with negligible effect on *A. bisporus*, highlighting the potential of these botanicals as eco-friendly, sustainable alternatives for managing wet bubble disease in mushroom cultivation.

REFERENCES

Altaf, S., Jan, S. K., Basu, U., Ahanger, S. A., Dave, A., Kakraliya, S. S., Mushtaq, M. 2022. Sustainable management of green mold disease of white button mushroom using botanicals and biocontrol agents under temperate conditions. *Horticulturae*, **8**(9): 768.

Amin, Z., Wani, F. F., Gulzar, H., Dar, W. A., & Sheikh, P. A. 2021. Diseases of White Button Mushroom (Agaricus bisporus) — A Potential Threat to Mushroom Industry. *International Journal of Current Microbiology and Applied Sciences*, 10(2), 2076-2085.

Arora, C., Kaushik, R. D., Kumar, A., and Garg, G. K. 2003. Fungicidal potential of Kumaon and Tarai region plants against mushroom fungal pathogens. *Allelopathy J*, **11**(1): 63-70.

Chandhrapati, A., Singh, S., Kumar, V., and Andrews, A. 2021. Biological management of competitor moulds and diseases of mushrooms. *J Pharm Innov*, **10**(5): 1169-1175.

Drobnjaković, T., Luković, J., Milijašević-Marčić, S., Todorović, B., Stepanović, M., Potočnik, I., and Rekanović, E. 2023. Impact of neem cake amendment in the casing soil on control of *Trichoderma aggressivum* Samuels & W. Gams and *Lycoriella ingenua* (Dufour) and mushroom yield. *Pesticidi i Fitomedicina*, **38**(3): 111-121.

Fletcher, J. T., Drakes, G. D., and Talent, C. J. W. 1975. The control of wet bubble disease of mushrooms caused by *Mycogone perniciosa*. *Ann. Appl. Biol.*, **79**(1): 35-41. https://doi.org/10.1111/j.1744-7348.1975.tb01519.x

Gea, F. J., Navarro, M. J., Santos, M., Diánez, F., and Carrasco, J. 2021. Control of fungal diseases in mushroom crops while dealing with fungicide resistance: A review. *Microorganisms*, **9**: 585. https://doi.org/10.3390/microorganisms9030585 PMid:33809140 PMCid:PMC8000694

Ghimire, K. R. P., Joshi, Y. R., Sobita, S., and Subedi. 2021. Major fungal contaminants of mushrooms and their management. *Int J Appl Sci Biotechnol*, **9**(2): 80-93. https://doi.org/10.3126/ijasbt.v9i2.37513

Gomez, K. A., and Gomez, A. A. 1984. *Statistical procedures for agricultural research*. John Wiley and Sons, New York.

Grewal, P. S., and Grewal, S. K. 1988. Selective fungicidal properties of some plant extracts to mushroom weed moulds. *Phytopathol Mediterr*, 27: 112-114.

Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P., and Khelurkar, V. C. 2017. Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. *J Pharm PhytoChem*, **6**(1): 32-36.

Saleh, M. A., Belal, M. H., and El-Baroty, G. 2006. Fungicidal activity of *Artemisia herba-alba* Asso (Asteraceae). *J Environ Sci, Part B*, **41**(3): 237-244. https://doi.org/10.1080/03601230500354774 PMid:16484084

Shauket, A. P., Abdul, W., and Riyaz, A. M. 2013. Evaluation of the yield performance of *Pleurotus sajor-caju* on different agro-based wastes. *Afr J Agric Res*, **8**(23): 3025-3028.

Sharma, N., Vaidya, M., Kayastha, R., and Kumari, M. 2017. Economic analysis of the production of white button mushroom (*Agaricus bisporus*) in Himachal Pradesh: A case study of Shimla district. *Int J Chem Sci*, **6**: 97-100.

Singh, S., Lal, A. A., Singh, A., Yaduman, R., and Murmu, R. 2016. Evaluation of some plant extracts in the management of dry bubble (*Verticillium fungicola*) disease of white button mushroom [*Agaricus bisporus* (Lange) Imbach]. *J Appl Nat Sci*, **8**(3): 1205-1209. https://doi.org/10.31018/jans.v8i3.941

Singh, M., and Singh, R. P. 2012. Management of mushroom pathogens through botanicals. *Ind Phytopathol*, **58**(2): 189.

Vincent, J. M. 1974. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **159**: 239-241.

Singh, S., Gupta, S., Choudhary, S., Diskit, S., Paswal, S., Pandit, D., and Khushboo, S. S. 2022. Evaluation of some botanical extracts in controlling dry bubble (*Verticillium fungicola*) disease of button mushroom (*Agaricus bisporus*) under the conditions of the sub-tropics of Jammu. *J Pharm Innov*, **11**(1): 1456-1459.