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# Sustainable Management of Wet Bubble Disease (*Mycogone Perniciosa*) in Button Mushroom (*Agaricus Bisporus*) using Botanicals Agents under Temperate Conditions

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***Abstract*****:** *Agaricus bisporus* (Lange) Imbach is the most widely cultivated mushroom globally. Wet bubble disease, mainly caused by *Mycogone perniciosa*, is a major threat to *A. bisporus* production worldwide. In the current study, it was expected to select safe plant extracts that can successfully control wet bubble disease on mushroom due to the biological similarity of *M. perniciosa.* Seven antifungal potentials of some plant extracts namely, *Azadirachta indica, Azadirachta indica* (seed kernel*)*, *Ocimum sanctum, Lanatana camara, Allium sativum, Azadirachta indica +lanatana camara* and *Aloe vera* were evaluated for their efficacy against pathogen *M. pernicosa*, under *in vitro* conditions. The test botanicals were highly effective at 3 per cent concentrations. The ethanol extract of *Azadirachta indica* and *Azadirachta indica + lanatana camara* behaved in a similar way. *M. perniciosa*, *Azadirachta indica* and *Azadirachta indica + lanatana camara* showed highly inhibitory against the pathogen with over all mean mycelium inhibition of 59.35 and 57.37 per cent respectively. The least inhibition of *M. perniciosa* was exhibited by *Allium sativum.* Under *in vivo* conditions, use of *Azadirachta indica* and *Azadirachta indica + lanatana camara* in different concentration resulted in a maximum yield of button mushroom of 401.11g and 360.26g/kg compost.

***Keywords*****:** *Agaricus bisporus,* Compost, Disease control, *Mycogone perniciosa*

*Agaricus bisporus* (Lange) Imbach, also known as the white button mushroom, is the most widely cultivated mushroom worldwide. Given its nutritional value, medicinal value (antitumor activity and hypoglycemic and hypo cholesterol limbic effects) and environmentally friendly status, the white button mushroom constitutes an increasing proportion of the diet (Grube et al 2001, Jeong et al 2010, Du et al 2017). Wet bubble disease of *A. bisporus* is a soil-borne disease mainly caused by *Mycogone perniciosa* (Magnus) Delacroix is reported worldwide wherever *A. bisporus* is grown (Sharma and Singh 2003, Ghazzawi and Beig 2011). *M. perniciosa* can infect the fruiting bodies of *A. bisporus* at various growth stages, causing the massive deformed tissues (Sclerodermoid mushrooms) before the mushroom tissue has differentiated into stalks and caps. *M. perniciosa* can produce two types of spores, phialoconidia and chlamydospores (Gea et al 2010). Both types of conidia can initiate disease, although their roles in dissemination and survival are still unclear (Glamoclija et al 2008). Casing soil is considered the main source of wet bubble disease as *M. perniciosa* releases phialoconidia or chlamydospores into the soil. Since most commercial cultivars of *A. bisporus* are highly susceptible to wet bubble disease control of wet bubble disease mainly involves fungicide disinfection of casing soil, cultural practices, and sanitation. The immediate application of salt to the infected areas is reportedly to be effective against *M. perniciosa* (Pieterse 2005). Chemical control is an effective method for the management of many fungal plant diseases (Shi et al 2020). Considering the serious nature and limited information available on the disease detail investigations on isolation, identification, and proving the pathogenicity of the Wet bubble disease and *In-vitro* and *in- vivo* management strategies.

## MATERIAL AND METHODS

Isolation and Identification of wet bubble disease The study was conducted during 2019-20 to 2020-2021 at Division of Plant Pathology, SKUAST Jammu, Wet bubble disease (*M. perniciosa*) was isolated from infected casing and infected sporophores, displaying typical symptoms, by routine pathological techniques of Holliday (1980). The isolated *M. perniciosa* was tested for its aggressiveness towards the button mushroom under *in vivo* conditions. In the first experiment, conidial suspension (1x 104/mL SDW) of isolated *M. perniciosa*, inoculated in sterilized casing mixture at the time of casing. In the second experiment, isolated *M. perniciosa* was inoculated on healthy pinheads and fruit-bodies via conidial suspension (1 ×104/mL SDW) and the mycelial discs from the active culture plate to observe bubble symptom development (Fig. 1). After inoculation, the bags

were kept in an isolated room at a temperature of 21 ± 1$^0$C with relative humidity of 87 per cent. Poly bags without pathogen inoculation, maintained under similar conditions, served as a control and were kept apart to avoid contamination. Both the experiments of pathogenicity tests were closely monitored for symptom development. The morphological cultural characteristics, *viz.,* mycelial, colour and growth; shape, size, color and septation of hyphae and conidiophores, conidia and phialides of the isolated *M. perniciosa* on host *A. bisporus* and in artificial culture were examined. The isolated micro-organism was identified on the basis of its morphological and cultural characteristics of comparing it with the standard descriptions of *M. perniciosa* given by Hus and Han (1981) and Singh and Sharma (2002). To further support the identification, *M. perniciosa* was also reconfirmed from CSIR- Indian Institute of Integrative Medicinal Jammu and the culture deposited under accession No. 1060.

**In-*vitro* evaluation:** Ethanol extract of six botanicals (Table 1) were evaluated in the laboratory for their efficacy against mycelial growth of *M. pernisiona* through poison food technique (Nene and Thapliyer 1993). Each the ethanol extract of the botanicals was prepared by the method of Deshpande et al (2004). The plants/plant parts were washed with tap water and rinsed with sterile distilled water followed by shade drying. Leaves of neem, lantana, tulsi, and garlic cloves were also dried till brittle. The shade dried plant/plant parts were ground with the help of electric grinder to obtain fine powder of each botanical. The dried powder was then stored in plastic containers for further use. The dried powder of the plant parts (20g) was mixed with 200 ml solvent (70% ethanol) in 250 ml conical flask. The flasks were tightly plugged with sterile cotton plugs wrapped with aluminum foil

**Table 1.** Botanicals against the wet bubble disease of button mushroom (*A. bisporus*)

| Treatment | Common name | Botanical name | Part used |
|---|---|---|---|
| $Trt_1$ | Neem | *Azadirachta indica* | Leaves |
| Trt2 | Neem cake | *Azadirachta indica* | Seeds |
| $Trt_3$ | Garlic | *Allium sativum* | Bulbs |
| $Trt_4$ | Tulsi | *Ocimum sanctum* | Leaves |
| Trt5 | Lantana | *Lantana camara* | Leaves |
| $Trt_6$ | Neem +lantana | *Azadirachta indica* +*Lantana camara* | Leaves |
| $Trt_7$ | Control | - | - |

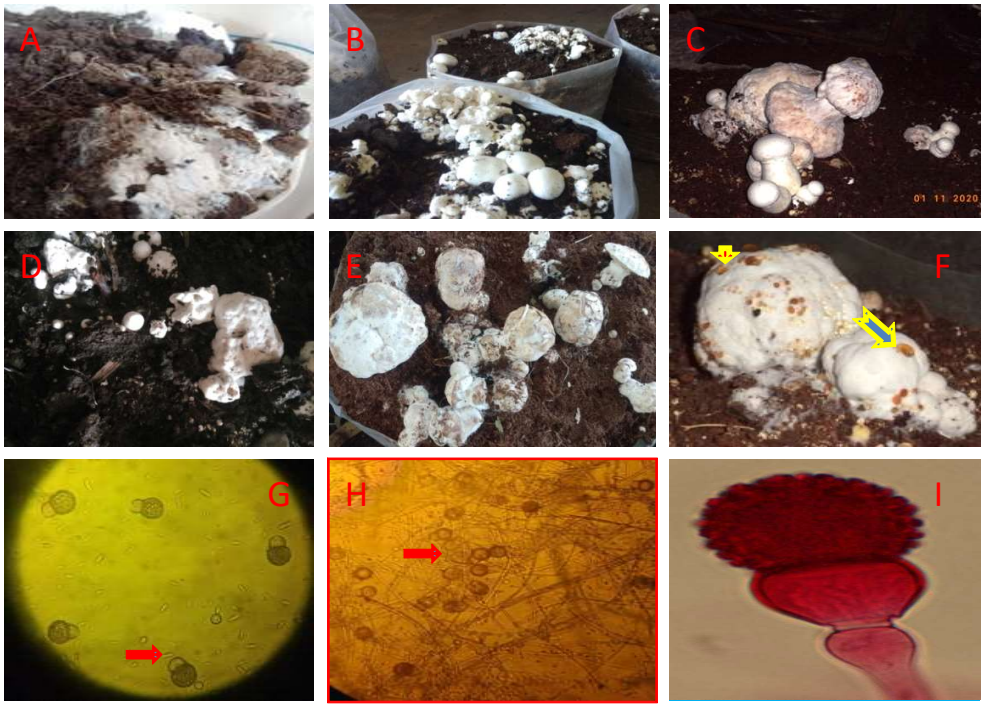


**Fig. 1.** Wet bubble symptoms and microscopic photograph of *M. perniciosa.*A) Early stage of disease turns into a shapeless mass. B) Later stage of amber exudation of fluffy mycelium. C) Sporophores mushroom became malformed with stipes, deformed caps .D) Infected pin head showing amorphic shapes. E) Cauliflower like distortion of infected fruit bodies. F) Little droplets of liquid amber in color appear on the surface of fruit bodies. G) Read arrow indicates single conidia. H) Single and Bio-cellular chamydospore. F) Terminal chamydospore

and kept on a rotator shaker for 36 hours and kept undisturbed for 6 hours to allow suspended plant material to settle down. The extract was decanted, filtered and centrifuged at 500 rpm for 15 minutes. The supernatant was collected and the solvent was evaporated at 40-50°C to make final volume 1/4$^{th}$ of the original volume. The test concentrations of 1, 2, and 3% were achieved by adding appropriate amounts of sterile, distilled water to the standard solution (100%). Two ml from each extract was dispensed in petriplates (90mm) and then 20 ml of molten PDA was poured gently containing extract solution. After solidification, inoculations were done with 5 mm disc of mycelial cut from 6 days old cultures of M.*pernisiona* isolated pathogens separately. The media without the plant extract served as control. The plates were incubated at 27±1°C till the complete growth was observed in control plates. Percent inhibition in growth was calculated in relation to growth in control.

$$\text{Growth inhibition} = \frac{\text{Radial growth in control - Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

***In vivo* evaluation of botanicals:** In this study, effects of the botanicals which showed maximum inhibitory to *M. perniciosa* were evaluated against the wet bubble disease in cultivation trials during the month of September-March of 2019-20 and 2020-21 in Mushroom Cropping Room, Division of Plant Pathology, SKUAST-Jammu. Mushroom compost was prepared according to the method of Mantel et al (1972 ) followed by the Long Method, using different constituents (wheat straw, 500 kg; rice bran, 50 kg; chicken manure, 200kg; mustard oil cake ,25 kg, gypsum, 15 kg and Urea 4 kg). After that added the different supplement *viz*. Neem, neem cake, Tulsi, Lantana and Garlicin crushed into a fine powder with the help of the grinder. The wet substrate and supplements were mixed thoroughly of the compost at 1, 2 and 3% (w/w) and the polythene bags of 22.5 cm × 30 cm size were filled with 1 kg of prepared compost. Spawn of *A. bisporus* was added at 10-12g/kg of compost. The untreated bags (devoid of botanicals) were kept as control. All the treatments including control were replicated five times. The bags were then incubated inside the Mushroom Cropping Room in dark for 10-15 days and the temperature was maintained at (22-24°C) till complete colonization of the compost with fungal mycelium was observed (El-Kattan and El-Hadded, 1998). After complete colonization on compost with mycelium of *A. bisporus*, the bags were inoculated with 3ml spore suspension of *M. perniciosa* separately with a spore load of $1\times10^3$ spores ml$^{-1}$ in the middle of the bag with the help of syringe. The untreated bags (devoid of botanicals) with the same inoculums load were kept as control. Once the bags attained full spawn growth, casing layer (1.5 inches) was applied and the temperature was reduced to 15-18°C and humidity (80-85%). Observations on days for complete spawn run, days for pin head initiation, per cent increase in yield over control and disease incidence were recorded.

## RESULTS AND DISCUSSION

**Effect of ethanol extract of botanicals against of *M. perniciosa in vitro:*** The botanicals at each level of concentration significantly varied in inhibitory effects (Table 2, Fig. 2). *Azadirachta indica* exhibited maximum inhibition of 67.65% followed by *Azadirachta indica* + *Lantana camara* at 3% concentration 64.60%).

**Days for complete colonization/Spawn-run**: There was significant difference between the effects of plant extracts on time taken for complete colonization by mycelium of *A. bisporus*. The average number of the days required for spawn run in *A. bisporus* was significantly less in Trt1 followed by Trt6 at 3 per cent concentration. The next best treatments were Trt1 and Trt6 at 2 % and 1 % concentration. The average number of the days for spawn-run was significantly more in control, devoid of plant extracts. The time taken for pin head formation by *A. bisporus* showed no significant difference between the effect of botanicals and concentrations. All the botanicals slightly reduced the days for pin formation as compared to control (8.70 days)

**Effect of botanicals on yield and disease incidence:** The yield of mushrooms was maximum in Trt1 (401.11/ kg compost) followed by Trt6 (360.26g/ kg compost). The lowest yield was in Trt3 (337.84g/kg compost) and to check untreated control (323.38/ kg compost). The different concentrations of botanicals showed significant reduction in disease incidence caused by *M. perniciosa*. The botanical Trt1 was most effective (29.28%), followed by Trt6, Trt5 and Trt4 . The highest disease incidence (71.23%) was in control. Significant disease control of mushrooms in different treatments was observed when compared with inoculated-unsprayed control. Trt1 sprayed bags recorded maximum reduction in disease incidence and statistically superior over other botanical treatments followed by Trt6 and Trt4. Wet bubble is widely distributed in mushroom growing countries of the world and generally appears in substrate rich in carbohydrates and deficient in nitrogen (Sharma 1999). The use of biological management tools such as botanicals agents is necessary for the successful and effective management of wet bubble disease. The botanicals agents were tested both *in vitro* as well as *in vivo* against *M. perniciosa* to choose the most effective for disease management. Botanical extracts not only inhibit mycelial growth of the pathogen but also significantly influence the

yield and disease control of button mushroom. *In vitro* evaluation of botanicals *Azadirachta indica* showed the highest mycelial growth inhibition of 59.35% . Furthermore, under *in vivo* evaluation, the application of Trt1 resulted in disease control of 58.88%. The study is also in line with the work of Shaiesta et al (2011) and Kumar et al (2017). Antifungal properties of neem against moulds of mushroom have been reported by Grewal (1988). Sharma and Jandaik (1994) also observed that *Azadirachta indica, Eucalypyus, Tegetus erectus* and garlic extract inhibited the growth of various weed fungi of *A. bisporus*. Haq et al (2010) reported that bioactive components of *Azadirachta indica, Eucalyptus camaldulensis*, *Cymbopogon marginatus,* and citrus lemon are capable of enhancing the potential yield of mushrooms and slow down the progression of pathogenic microbes in oyster mushroom cultivation. Singh et al (2015) and Szumigaj et al (2012) also advocated the integrated use of botanicals where *A. indica* was the most effective botanical and showed maximum inhibition against *M. perniciosa* without inhibiting the mushroom mycelium. The findings indicate the potential usefulness of *Azadirachta indica* and *Azadirachta indica* + *lantana camara, Lantana camara, Ocimum sanctum* and

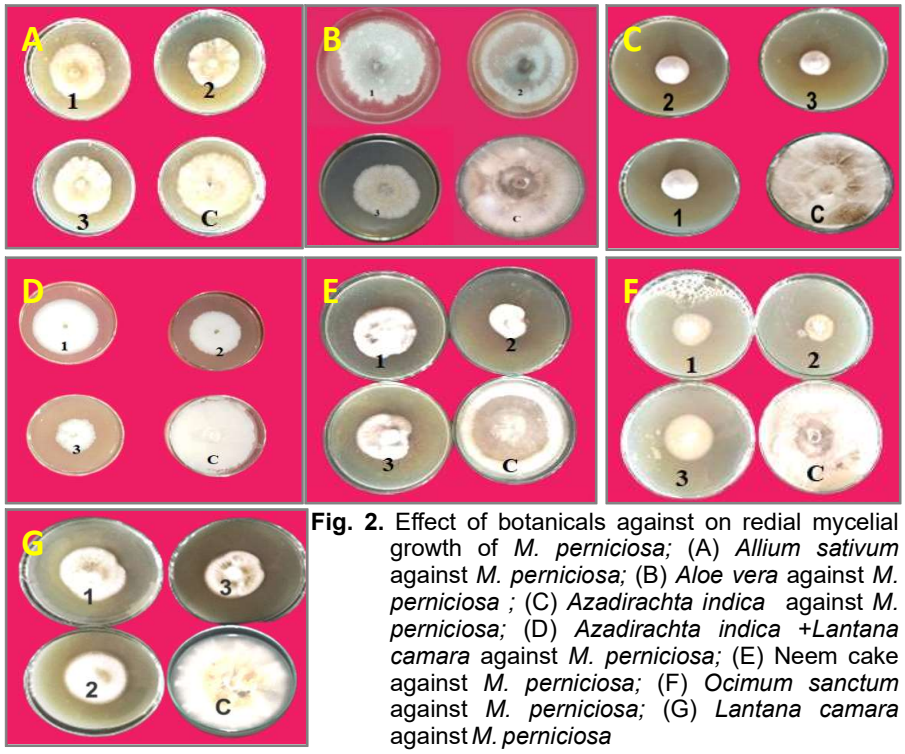


**Fig. 2.** Effect of botanicals against on redial mycelial growth of *M. perniciosa;* (A) *Allium sativum* against *M. perniciosa;* (B) *Aloe vera* against *M. perniciosa ;* (C) *Azadirachta indica* against *M. perniciosa;* (D) *Azadirachta indica +Lantana camara* against *M. perniciosa;* (E) Neem cake against *M. perniciosa;* (F) *Ocimum sanctum* against *M. perniciosa;* (G) *Lantana camara* against *M. perniciosa*

**Table 2.** *In vitro* evaluation of ethanol extract of botanicals against *Mycogone perniciosa* of button mushroom

| Treatments | Mycelical growth different at concentrations (%) | | | Mycelical growth inhibition at different concentrations (%) | | | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 | |
| *Azadirachta indica* | 46.44 | 34.19 | 29.11 | 48.40 | 62.01 | 67.65 | 59.35 |
| *Aloe vera* | 52.42 | 50.87 | 47.19 | 41.75 | 43.47 | 47.56 | 44.26 |
| *Azadirachta indica +Lantana camara* | 47.03 | 36.68 | 31.86 | 47.74 | 59.78 | 64.60 | 57.37 |
| *Lantana camara* | 51.62 | 37.67 | 32.00 | 42.64 | 58.14 | 64.44 | 55.07 |
| *Ocimum sanctum* | 51.32 | 40.18 | 32.00 | 42.97 | 55.35 | 64.40 | 54.24 |
| *Allium sativum* | 61.91 | 56.18 | 51.28 | 31.21 | 37.57 | 43.02 | 37.27 |
| Neem cake | 62.26 | 55.25 | 49.20 | 30.82 | 38.61 | 45.33 | 38.25 |
| Control | 90.00 | 90.00 | 90.00 | - | - | - | |
| Source | Botanical (B) | | Concentrations (C) | | Botanical × Concentration | | |
| CD (p=0.05) | 0.90 | | 0.98 | | 2.66 | | |

**Table 3.** Impact of selected botanicals on time taken for complete mycelium run, pin head inhibition, yield and disease incidence of button mushroom under in *vivo*

| Treatment | Concentration (%) | Complete colonization (days) | | Pin head initiation /first picking (days) (Pooled) | Yield (g)/kg (Pooled) | Disease incidence (%) (Pooled) | Disease control (%) (Pooled) |
|---|---|---|---|---|---|---|---|
| | | 2019-20 | 2020-21 | | | | |
| Trt1 | 1 | 17.10 | 17.32 | 6.55 | 374.22 | 34.77 | 51.92 |
| | 2 | 16.02 | 16.67 | 6.30 | 392.23 | 30.81 | 56.77 |
| | 3 | 15.12 | 15.10 | 6.00 | 436.88 | 22.26 | 68.05 |
| | Mean | 16.08 | 16.36 | 6.28 | 401.11 | 29.28 | 58.88 |
| Trt2 | 1 | 19.28 | 19.39 | 7.85 | 340.74 | 57.42 | 19.38 |
| | 2 | 19.03 | 19.07 | 7.60 | 343.01 | 55.23 | 22.46 |
| | 3 | 18.78 | 18.78 | 7.45 | 350.99 | 48.60 | 31.75 |
| | Mean | 19.03 | 19.08 | 7.63 | 344.91 | 53.75 | 24.53 |
| Trt3 | 1 | 19.02 | 18.89 | 8.05 | 331.17 | 56.05 | 21.28 |
| | 2 | 18.78 | 18.44 | 7.85 | 337.28 | 51.44 | 27.77 |
| | 3 | 18.00 | 17.89 | 7.65 | 345.07 | 47.37 | 33.51 |
| | Mean | 18.60 | 18.41 | 7.85 | 337.84 | 51.62 | 27.51 |
| Trt4 | 1 | 18.67 | 18.29 | 7.35 | 335.71 | 49.27 | 30.80 |
| | 2 | 18.19 | 18.01 | 7.05 | 356.39 | 46.04 | 35.34 |
| | 3 | 17.32 | 17.19 | 6.85 | 365.94 | 41.73 | 41.37 |
| | Mean | 18.06 | 17.83 | 7.08 | 352.68 | 45.68 | 35.83 |
| Trt5 | 1 | 17.55 | 17.89 | 7.10 | 350.59 | 44.65 | 37.27 |
| | 2 | 16.23 | 16.78 | 6.85 | 358.21 | 40.60 | 42.97 |
| | 3 | 15.54 | 15.87 | 6.45 | 367.88 | 31.99 | 55.05 |
| | Mean | 16.44 | 16.85 | 6.80 | 358.89 | 39.08 | 45.09 |
| Trt6 | 1 | 17.34 | 17.47 | 6.95 | 351.44 | 43.33 | 39.12 |
| | 2 | 16.11 | 16.20 | 6.55 | 359.74 | 37.94 | 46.71 |
| | 3 | 15.34 | 15.47 | 5.80 | 369.61 | 29.59 | 58.42 |
| | Mean | 16.26 | 16.38 | 6.43 | 360.26 | 36.95 | 48.08 |
| Trt7 | (Untreated inoculated) | 20.45 | 20.11 | 8.70 | 323.38 | 71.23 | |

*Allium sativum* as amendments or sprays in soil or compost casing to reduce wet bubble disease to low levels.

## CONCLUSIONS

The ethanol extracts of *Azadirachta indica* and *Azadirachta indica* + *Lantana camara* showed significant mycelial inhibition of *M. perniciosa* at a concentration of 3% was most effective in inhibiting the wet bubble disease while having the least effect on host mycelium and a positive effect on mushroom yield. Therefore, the present study reveals that a treatment of botanicals agents on compost and soil casing may cause substantial increases in yield by subduing wet bubble disease.

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