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# Evaluation of some botanical extract in controlling dry bubble (*Verticillium fungicola*) disease of button mushroom (*Agaricus bisporus*) under the conditions of sub tropics of Jammu

**Sardar Singh Kakraliya, Sachin Gupta, Sneha Choudhary, Stanzin Diskit, Shazia Paswal, Devanshi Pandit and Sheikh Saima Khushboo**

**Abstract**
The experiment was conducted under *in vitro* evaluation of different botanicals *viz.*, *Azadirachta indica* (neem leaf), *Azadirachta indica* (neem seed cake), *Allium sativum* (garlic), *Aloe vera* (aloevera), *Lanatana camara* (lantana), *Ocimum sanctum* (tulsi) and *Azadirachta indica* + *Lantana camara* were used of ethanol extract of different concentrations 5 and 10 per cent were tested by poisoned food technique. The results reveals that *Azadirachta indica* was found most effective and resulted in maximum inhibition of 33.18 and 63.04 per cent followed by *Azadirachta indica* + *Lantana camara* which showed 30.71 and 55.95 per cent inhibition at 5 and 10 per cent concentrations, respectively. Plant extracts showing maximum efficacy and minimum inhibition against *Veticillium fungicola* were further evaluated against *Veticillium fungicola* infection in mushroom crop room (*in-vivo* test). In *in-vivo* study, the polybags which receive *Azadirachta indica* showed maximum mean disease control (55.48%) and exhibited minimum mean disease incidence (28.92%). Ecofriendly management practice, i.e., use of botanicals was studied both *in vitro* and *in vivo* which gave batter results and these practices can be economical, long lasting and free from residual side effects.

**Keywords:** *Agaricus bisporus*, dry bubble disease, plant extracts, *Verticillium fungicola*

## Introduction
Mushroom production represents one of the commercially important microbial technologies for large scale recycling of agro wastes. Among commercially cultivated mushrooms, *Agaricus bisporus* popularly known as white button mushroom or European mushroom is extensively cultivated throughout the world. In the present scenario of economy, it has opened up new vistas of export earnings. Button mushroom contains high amounts of protein, minerals, vitamin B group, vitamin D and K and also A and C vitamins. The amount of fat, calorie, sodium and cholesterol levels are low in button mushroom (Saiqa *et al.*, 2008) [10]. *A. bisporus*is one of the most important mushrooms that is cultivated in the world (Toker *et al.*, 2007) [17]. *Verticillium fungicola* var. *fungicola*– the causal agents of dry bubble disease is important fungal pathogens of the button mushroom. Symptoms of dry bubble, caused by *Verticillium fungicola* var. *fungicola*, vary depending on the time of infection. Infection at an early stage in mushroom development results in the production of undifferentiated masses of mushrooms. If maturing mushrooms are infected, then spotting symptoms develop (Potocnik *et al.*, 2008) [9]. White button mushroom is the most popular mushroom, grown all over in India. In Jammu commercial cultivation of button mushroom introduced by number of organizations since long and a good amount of reasonable work has been done on various aspects of this mushroom in SKUAST- Jammu. The cultivation of button mushroom has increased in the Jammu division due to easy cultivation process. But the major constraint in the speedy popularization of this crop is diseases and pests. The diseases and pests happen to be devastating and perpetuate easily from one season to another. *Verticillum fungicola* is responsible for causing a serious disease called dry bubble which major fungal problem for mushroom growers. The pathogen inhibits the growth of mushrooms and hinders the production of fruiting bodies. It is a common contaminant, badly hindering the production of mushroom in the Jammu division. Some workers have recommended bio-control and fungicides for the treatment of dry bubble. But growers hardly use the fungicides for the treatment of this havoc disease. They often found fungicidal treatment as non-economical (Shah and Nasreen, 2011) [11].

The present study was carried out to develop economically viable and eco-friendly management of this devastating disease through the application of botanicals.

## Materials and Methods

### *In vitro* evaluation botanicals

In this experiment, ethanol extract of 7 botanicals *viz.*, *Azadirachta indica* (neem leaf), *Azadirachta indica* (neem seed cake), *Allium sativum* (garlic), *Aloe vera* (aloevera), *Lanatana camara* (lantana), *Ocimum sanctum* (tulsi) and *Azadirachta indica* + *Lantana camara* were evaluated in the laboratory for their efficacy against *Vertricillium fungicola*. The plant extracts were evaluated *in-vitro* through poison food technique (Nene and Thapliyal, 2000) [8].

### Collection of plant materials and preparation of extracts

Ethanol extract of the botanicals was prepared by the method adopted by Deshpande *et al*. (2004) [2]. The plants/plant parts were washed with tap water and rinsed with sterile distilled water followed by shade drying. Leaves of neem, lantana, tulsi, aloevera 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 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$^{o}$ C to make final volume 1/4$^{th}$ of the original volume. The extract now taken as 100 per cent standard extract was stored in air tight containers/bottles for further use. The ethanol extract of each botanical was bioassay for toxic activity at different concentration against *Verticillium fungicola* by employing poisoned food technique.

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

### *In vivo* evaluation of botanicals

In this study, effects of the botanicals were evaluated against *Vertricillium fungicola* under *in vivo* conditions during the month of September-March of 2019-20 and 2020-21 in Mushroom Cropping Room, Division of Plant Pathology, SKUAST-Jammu. Wheat straw based compost was used as substrate for cultivation of button mushroom. The botanicals were dried under shade after collection and crushed into a fine powder with the help of the grinder. The powder was passed through double layered muslin cloth and mixed with the mixture of the compost at 1, 2 and 3 per cent (w/w) and the polythene bags of 22.5 cm × 30 cm size were filled with 1 kg of prepared compost. Spawn of *Agaricus bisporus* was added at 10g kg $^{-1}$ 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$^{o}$C) till complete colonization of the compost with fungal mycelium was observed (El-Kattan and El-Hadded, 1998) [3]. After complete colonization on compost with mycelium of *Agaricus bisporus*, the bags were inoculated with 3 ml spore suspension of *Verticillium fungicola* 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 with the same inocula load were kept as control (Shah *et al.*, 2012) [16]. Once the bags attained full spawn growth, casing layer (1.5 inches) was applied and the temperature was reduced to 15-18 $^{o}$C and humidity (80-85%). While carrying the above experiment, observations on days for disease control and disease incidence were recorded.

$$\text{Per cent disease control (PDC)} = \frac{C - T}{T} \times 100$$

Where, C= Disease incidence control, T = Disease incidence in treatment

$$\text{Disease incidence} = \frac{\text{Number of infected trays/ bags}}{\text{Total number of trays /bags}} \text{ x } 100$$

### Analysis of data

In the *in-vitro* experiments, complete randomized design was applied. In *in-vivo* trial, Factorial design (RBD) was applied. All the experiments were analyzed statistically by Analysis of Variance (ANOVA). The calculated value was compared with tabulated value at 0.05% level of probability for the appropriate degree of freedom.

## Results and Discussion

The results obtained on inhibition of mycelia growth of *Verticillium fungicola* by food poisoning plate technique were presented in Table 1. All the botanical treatments were significantly effective over control in checking the growth of *Verticillium fungicola* at the both test concentrations (5 and 10%). The results revealed that, out of all botanical extracts maximum growth inhibition (63.04%) was however recorded at 10 per cent followed by 5 per cent recording (33.18%) growth inhibition, followed by *Azadirachta indica* + *Lantana camara* recording (55.95%) growth inhibition at 10 per cent followed by 5 per cent recording (30.71%) growth inhibition whereas lowest growth inhibition of (12.32%) was exhibited by *Aloe vera* at 5% concentration and (17.10%) inhibition was recorded at 10 per cent concentration. Similar observations have been reported by Jatav *et al*. (2014) [4] who found *Azadirachta indica* as the best treatment which effectively control *Verticillium fungicola* among all the selected botanicals. Singh *et al*. (2015) [14] observed that the maximum inhibition was recorded by onion (31.2%) followed by neem (28.0%), garlic (23.2%) and eucalyptus (12.0%), respectively at 100 per cent concentration. Sharma and Jarial (2000) [13] tested neem leaves for the management of false truffle disease in *Agaricus bisporus* and observed good results to manage this disease under *in vitro* condition. Mishra (2009) [6] also used neem for the control of *Trichoderma viride* and recoded similar results. Narzari *et al*. (2007) [7] used 0.4% *Allium sativum* and recorded complete suppression of *Trichoderma harzianum* growth and Lirrio *et al.* (1998) has also reported antibacterial activity in *Allium cepa* against *Erwinia carotovora* pv.carotovora, *Xanthomonas campestris pv.compestris* and *Pseudomonas solanacearum*. Similar results have been reported by Chakraborty *et al*. (2013) [1], who found neem as the best treatment in controlling different contaminants associated with *Pleurotus sajor-caju* and

*Lentinla edodes*. Shah *et al.*, (2011) [12] used eight plant extracts for control of *Trichoderma harzianum* in mushroom crop and recorded neem to be the best among the tested botanicals.

**Table 1:** *In vitro* evaluation of ethanol extract of botanicals against *Verticillium fungicola* of button mushroom

| Treatment | Mycelial growth (mm) * | | Mycelical growth inhibition (%) | |
|---|---|---|---|---|
| | 5% | 10% | 5% | 10% |
| *Azadirachta indica* | 54.19 | 30.36 | 33.18 | 63.04 |
| Neem cake | 70.12 | 67.11 | 13.54 | 18.31 |
| *Lantana camara* | 60.11 | 43.16 | 25.89 | 47.46 |
| *Ocimum sanctum* | 57.12 | 39.19 | 29.57 | 52.30 |
| *Allium sativum* | 68.14 | 62.28 | 15.99 | 24.19 |
| *Aloe vera* | 71.11 | 68.12 | 12.32 | 17.10 |
| *Azadirachta indica* + *Lantana camara* | 56.20 | 36.19 | 30.71 | 55.95 |
| Control | 81.11 | 82.16 | - | - |
| SE(m) ± | 01.95 | 01.12 | | |
| CD (0.05%) | 05.91 | 04.75 | | |

In this experiment the botanicals extract which displayed the maximum efficacy against *Verticillium fungicola* under *in vivo* conditions; they were incorporated in casing soil before being applied to the compost bags. The results presented in Table 2 depicted that different concentrations of various botanicals *viz*., *Azadirachta indica*, Neem seed kernel, *Allium sativum*, *Ocimum sanctum*, *Lantana camara* and *Azadirachta indica* + *Lantana camara* displayed varying efficacy against *Verticillium fungicola*, the causal organism of dry bubble. *Azadirachta indica* was found to be best where the disease incidence was recorded (28.63 and 29.20%) followed by *Azadirachta indica* + *Lantana camara* (34.99 and 30.50%), *Lantana camara* (37.75 and 38.44%), *Ocimum sanctum* (44.44 and 45.55%), *Allium sativum* (52.09 and 50.97%) and Neem seed kernel exhibited (53.85 and 54.04%). However, highest disease incidence of (65.19 and 64.76%) was recorded under control during the both the years.

It was also found that with increase in concentrations of selected botanicals, the disease incidence was reduced. The minimum incidence (22.17 and 21.19%) was recorded with *Azadirachta indica* 3 per cent followed by 2 per cent (29.41 and 30.21%) and 1 per cent (34.32 and 36.22%) during both the years of study. The maximum disease incidence (56.46 and 57.46%) was recorded in Neem seed kernel 1 per cent followed by 2 per cent (54.23 and 53.24%) and 3 per cent (50.87 and 51.43%) while *Allium sativum* recorded (58.31 and 57.74%) with 1 per cent (50.54 and 51.12%) with 2 per cent and (47.41 and 44.04%) with 3 per cent during both the years of study.

**Table 2:** Efficiency of botanicals on disease incidence of dry bubble disease of button mushroom

| Treatment | Concentration (%) | Disease incidence (%) | | | Disease control (%) | | |
|---|---|---|---|---|---|---|---|
| | | 2019-20 | 2020-21 | Pooled | 2019-20 | 2020-21 | Pooled |
| *Azadirachta indica* | 1 | 34.32 | 36.22 | 35.27 | 47.35 | 44.07 | 45.71 |
| | 2 | 29.41 | 30.21 | 29.81 | 54.88 | 53.35 | 54.11 |
| | 3 | 22.17 | 21.19 | 21.68 | 65.99 | 67.49 | 66.63 |
| | Mean | 28.63 | 29.20 | 28.92 | 56.07 | 54.97 | 55.48 |
| Neem seed kernel | 1 | 56.46 | 57.46 | 56.96 | 13.39 | 11.85 | 12.32 |
| | 2 | 54.23 | 53.24 | 53.74 | 16.81 | 11.27 | 17.28 |
| | 3 | 50.87 | 51.43 | 51.15 | 21.96 | 20.58 | 21.27 |
| | Mean | 53.85 | 54.04 | 53.95 | 17.38 | 14.56 | 16.96 |
| *Allium sativum* | 1 | 58.31 | 57.74 | 58.025 | 10.55 | 10.84 | 10.68 |
| | 2 | 50.54 | 51.12 | 50.83 | 21.60 | 21.06 | 21.76 |
| | 3 | 47.41 | 44.04 | 45.72 | 27.27 | 31.99 | 29.62 |
| | Mean | 52.09 | 50.97 | 51.53 | 34.04 | 29.66 | 20.69 |
| *Ocicum sanctum* | 1 | 47.33 | 49.10 | 48.22 | 27.21 | 24.18 | 25.78 |
| | 2 | 44.87 | 45.21 | 45.04 | 31.17 | 30.18 | 30.67 |
| | 3 | 41.12 | 42.33 | 41.73 | 36.92 | 34.63 | 36.38 |
| | Mean | 44.44 | 45.55 | 45.00 | 31.76 | 29.66 | 30.94 |
| *Lantana camera* | 1 | 42.65 | 44.65 | 43.65 | 34.57 | 31.05 | 32.81 |
| | 2 | 40.26 | 42.01 | 41.14 | 38.24 | 35.12 | 36.67 |
| | 3 | 30.34 | 28.65 | 29.49 | 53.45 | 55.75 | 54.60 |
| | Mean | 37.75 | 38.44 | 38.09 | 42.08 | 40.64 | 41.36 |
| *Azadirachta indica* + *Lantana camera* | 1 | 41.12 | 42.54 | 41.12 | 36.92 | 34.31 | 36.70 |
| | 2 | 36.65 | 35.23 | 35.94 | 43.17 | 45.59 | 44.63 |
| | 3 | 27.21 | 25.76 | 26.48 | 58.26 | 59.24 | 59.23 |
| | Mean | 34.99 | 30.50 | 34.51 | 46.11 | 46.38 | 46.85 |
| Control (Untreated inoculated) | | 65.19 | 64.76 | 64.97 | - | - | - |

The pooled data revealed that the different concentrations of botanicals showed significant reduction in disease incidence of dry bubble caused by *Verticillium fungicola*. The botanical *Azadirachta indica* was found to be the most effective treatment where the disease incidence was recorded (28.92%) whereas *Azadirachta indica* + *Lantana camara* recorded (34.51%) incidence followed by *Lantana camara* (38.09%), *Ocimum sanctum* (45.00%), *Allium sativum* (51.53%) and neem seed kernel (53.95%). The highest disease incidence (64.97%) was recorded under control.

The investigations also revealed that all the botanicals treatment were significantly effective in control of dry bubble disease. The disease control ranged between (17.38-56.07%) during 2019-20 and (14.56-54.97%) during 2020-21. However, *Azadirachta indica* at 3 per cent was found to be most effective treatment for maximum disease control (65.09 and 67.49%) followed by (58.26 and 59.24%) with 3 per cent *Azadirachta indica* + *Lantana camara* (56.67 and 53.43%)

with 3 per cent *Lantana camara*, (53.45 and 55.75%) with 3 per cent *Ocimum sanctum* and (27.27 and 31.99%) with 3 per cent *Allium sativum*. Minimum disease control (21.96 and 20.58%) was recorded by 3 per cent neem seed kernel.

The pooled data revealed that the different concentrations of botanicals showed significant reduction in disease incidence caused by *Verticillium fungicola*. The botanical *Azadirachta indica* was found to be most effective treatment where the incidence was recorded (55.48%), whereas the *Azadirachta indica* + *Lantana camara* recorded (46.85%) followed by *Lantana camara* (41.36%), *Ocimum sanctum* (30.94%), *Allium sativum* (20.69%) and neem seed kernel (16.96%) during the both years 2019-20 and 2020-21, respectively. Similar observations have been reported by Singh and Singh (2017) who reported that the plant extract of *Azadirachta indica* was found to be the best treatment followed by *Lantana camara* and *Allium cepa*. Maximum efficacy against *Verticillium fungicola* show maximum mean increase in yield (43.46%) over control and exhibited minimum mean disease incidence (27.7%).

## Conclusion

Currently, the use of inhibitory botanicals is one of the most possible methods for controlling some plant diseases. It is reasonable to collect and screen foe more plants with high activity to suppress the dry bubble infection. This study has found two most promising botanicals; *Azadirachta indica* and *A. indica + Lantana camara* were able to inhibit the infection of dry bubble disease of white button mushroom under both *in vitro* and *in vivo* conditions. In *in vivo* study incorporation of these botanicals into the compost reduces the disease incidence and enhances the yield. When compared to the check (without botanical). However are still several further studies to be carried out.

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