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Evaluation of different botanicals against *Verticillium fungicola* causal pathogen of dry bubble disease of button mushroom

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Abstract
An investigation was conducted to study the management of dry bubble disease of white button mushroom (*Agaricus bisporus*) using twenty one different botanicals. The *in vitro* evaluation of leaf extract of twenty one different locally available plant extracts indicated that all the tested botanicals inhibited growth of the pathogenic fungus but *Allium sativum, Metricaria* sp., *Phytolac* sp., *D. stromonium, M. alba and Conium maculatum* exhibited maximum inhibition of mycellial growth with inhibition percentage of 100% per cent proved to be the best botanical. The test botanicals were highly effective at 3 per cent concentration. The integration of one or more of these botanicals reduced the disease to a level significantly lower than check and appreciably enhanced the early pinhead formation, weight and number of healthy fruit bodies and the ultimate mushroom yield, as comparedto check.

Keywords: *Verticillium fungicola,* mushroom, *Agaricus bisporus*

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) [13]. *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 *V. 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) [8]. Various botanicals have proved useful source of fungitoxic substances that are rather harmless compared to synthetic chemical fungicides, which often impose undesirable side effects. Several plants have been reported to posses' substances, which are toxic to microbial pathogens and serve as protective barrier to infection. Keeping above in view, an effort was made to evaluate the efficacy of leaf extracts of angiospermic plants as compared to control of pathogenic fungi of mushrooms under field conditions.

Materials and Methods
The present investigation was carried out to ascertain the status of dry bubble disease of white button mushroom, *Agaricus bisporus*. The frequency of prevalence of dry bubble disease was recorded on the basis of the number of mushrooms infected out of the totals under study as per the method adopted by Singh and Sharma (2002) [12]. For working out the disease incidence for each mushroom farm, the number of infected bags/trays out of the total number of bags/trays observed was recorded, and the per cent disease incidence was calculated by the formula given by (Fletcher *et al.*, 1983) [3]. Fresh leaves of 21 different plant species (Table1) were collected to observe their efficacy in control of pathogenic fungi viz. *Verticillium fungicola* var. *fungicola* of button mushroom. The pathogens were isolated from infected mushrooms, casing

mixture and compost by standard methods. Pure culture from single spore was maintained on Potato Dextrose Agar medium form growth. The fresh plant leaves were washed in distilled water and were separately homogenized with sterile distilled water at 1:1 w/vin a pestle and mortar separately of each plant and filtered through a muslin cloth. This formed 100percent plant extract solution. In sterile Petri plates, 20 ml of PDA was poured and 2 wells of 5 mm were dug at two sides. Diluted plant extracts (100 micro liters) were added to these wells using sterile micropipette. Fungal discs (5mm) were punched from 5 days old culture of *Verticillium fungicola* and were placed in the centre of Petridish to evaluate the efficiency of the plant extracts. Petridishes containing the growth medium with fungal inoculum at 1.5% concentration alone served as control. All the cultures were incubated at room temperature (28±2 °C).The area of inhibition zone (mm^2) at 3% was recorded after a week. Also, the per cent inhibition of spore germination was noticed over control at 1.5%.

$$\text{Disease incidence} = \frac{\text{No. of infected trays/bags/}}{\text{Total No. of trays/bagsx 100}}$$

Results and Discussions

The aqueous extract of twenty one different botanicals were evaluated for their inhibitory effect son mycelial growth at 1.5 and 3 per cent concentrations. On an average, the aqueous extracts of *Allium sativum, Metricaria* sp., *Phytolac* sp., *D. stromonium, M. alba and Conium maculatum* exhibited maximum inhibition (100%) of mycellial growth. Aqueous extract of *Z. officinalis* showed next maximum inhibitory effect (94.66%).The aqueous extract of the botanicals viz., *Salix alba, M. longifolia, P. vulgaris, L. officinalis and Coriander* recorded minimum inhibition (0.00%) over control. The mycellial growth inhibition significantly increased with increase in the extract concentration. The minimum average inhibition (27.99%) was noticed at the lowest test concentration of 1.5%.These findings indicate usefulness of the *Allium sativum, Metricaria* sp, *Phytolaca, D. stromonium, M. alba and Conium maculatum*as sprays or as amendments in compost or casing soil to keep the dry bubble disease at low ebb. Similar results have also been reported by Sharma and Kumar (2005) [11]. These findings are in complete agreement with those of Sabharwal and Kapoor, (2014) [9] who reported that botanical extracts and oils like neem, citrullina, clove, olive and castor used in different concentration effectively controlled the mycelial growth of mushroom mycopathogen. Similarly, Tanovic *et al*. (2009) also observed inhibition of V. fungicola by using botanicals from aromatic and medicinal plants. Several botanicals have been evaluated against many fungal pathogens throught the world by various researchers (Jahan *et al*., 2013) [5] found that botanicals like garlic and henna also reduced the radial colony diameter of pathogen appreciably at different concentrations. Pattnaik *et al*. (2012) [7] reported that the application of A. indica extract resulted in reduction of leaf spot diseases up to extent of 40% in Lycopersicum esculentum. Similarly, Minz *et al*. (2012) [6] observed that mycelia growth in hibitiont was upto extent of 58-99% when thirteen plant extracts were used for antifungal assay against wilt disease in ginger. The bio-efficacy of botanicals against pathogens attributed to the fact that they have active compounds such as azadirachtin, allicin, Salicin, etc. which were antifungal, antibacterial and anti-insecticidal in nature (Bohra *et al*., 2006) [1]. Similar, findings were also obtained against fusarium wilt of carnation by application of different botanicals (Chandel and Tomar, 2008) [2]. Thus, botanicals are a promising option for the cultivation of pesticide-free mushrooms, avoiding inflicting harm to either man or environment. However, the two aspects of cost-effectiveness and application efficiency must be taken into account, which justify the necessity for greater research into the practical utilization of botanicals in mushroom cultivation.

Botanicals	Inhibition of mycelia growth (area of inhibition zone mm^2 at 3% conc.)	Percent inhibition of spore germination over control at 1.5% conc.
Caestina sativa	8.63	0.00
Juglansregia	132.19	54.66
Salix alba	10.67	0.00
Artemisia annua	75.98	35.92
Allium sativum	42.23	100
Arctium lappa	33.59	0.66
Merticaria	98.28	100.0
Urticadioca	53.53	26.56
Zingiber officinale	186.67	94.66
Mentha longifolia	14.75	0.00
Phytolaca	267.52	100.0
Atropa belladonna	33.28	14.80
Prunella vulgaris	10.67	0.00
Lavandula officinalis	39.87	0.00
Coriander	36.26	0.00
Allantium nigrum	226.23	45.11
Equisetum arvensis	36.26	27.90
Datura stramonium	289.50	100
Morus alba	208.49	100
Conium maculatum	36.57	100
Anisomeles	55.90	18.35

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