Short Communication

Effect of *Verticillium fungicola* (PREUSS) HASSEBR inoculation in casing soil and conidial spray on white button mushroom *Agaricus bisporous*  

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Dry bubble disease induced by *Verticillium fungicola* has been observed as an important disease of white button mushroom (*Agaricus bisporus*) in India. The symptoms produced on well differentiated fruit body are localized light brown depressed spots. The adjacent spots coalesce together to form irregular blotches. If the host pathogen infection is established before differentiation, sclerodermoid fruiting bodies appear on casing surface. Disease percent increased with the increased doses of inoculum whether inoculated in compost, casing or sprayed at the time of pinhead initiation. Fresh inoculum of pathogen (*V. fungicola*) was mixed on sorghum grains with sterilized casing soil. Inoculum of pathogen at 0.1, 0.2 and 0.3% per kg casing soil was mixed and applied at the time of casing. The negative effects was observed with increased doses of inoculum in casing. The introduction of *V. fungicola* at 3 g inoculum/kg casing soil delayed pinhead initiation thereby, more disease and less yield. Inoculum suspension prepared from actively growing mycelium when sprayed at the time of pinhead initiation drastically reduced the mushroom yield. Inoculum at 3 g/L resulted highest decrease in yield over control.  

**Key words:** *Agaricus bisporus*, *Verticillium fungicola*, dry bubble, casing soil, inoculums.

INTRODUCTION  

Mushroom, like any other crops, are attacked by several pests and diseases. Since they are grown indoors on specific substrates, their productivity and quality are adversely affected by a large number of biotic and abiotic...
factors. The most common biotic causes include parasitic and antagonistic fungi, bacteria, virus, nematodes, mites and insect pests. Mushrooms are grown on a specially prepared substrate (compost) which favours the selective growth of *Agaricus bisporus* mushroom mycelium. However, if composting is not carried out properly, weed moulds develop, some of which can reduce the yields significantly or even result in complete crop failure depending upon their severity and stage of appearance. This is more so under Indian conditions where significant proportion of the total mushroom production is contributed by seasonal growers who use unpasteurized compost prepared by long method composting (traditional method of preparation of compost) which harbour several parasitic fungi, antagonistic moulds and myceliophagous nematodes. Since the appearance of weed moulds indicates improper composting (unpasteurized), they are also known as indicator moulds.

Weed moulds compete with mushroom mycelium for space, water and nutrients, and are called competitor moulds. The main abiotic factors are temperature, relative humidity, CO$_2$ concentration, excess of moisture in compost and casing mixture, and presence of toxic chemicals in substrate or atmosphere. Most of the growers cultivate button mushrooms under natural climatic conditions taking one to three crops per year without any environmental control and insulation in ordinary rooms, abandoned poultry sheds and thatched huts. Poor hygienic conditions and lack of ‘cook out’ facilities also help in the perpetuation of various pathogens. Sometimes no facilities exist with the growers for measuring moisture, pH and N content of the compost, which are important quality parameters for ideal compost. Casing material used in India is sterilized with formaldehyde and in some cases not treated at all, which also introduces large number of pest and pathogen on compost. Presently, the scenario of mushroom cultivation in India is different from all the mushroom growing countries of the world with respect to substrates casing mixture and system of cultivation and so is the occurrence of disease.

Among the pathogenic fungi dry bubble disease caused by *V. fungicola* is the most dreaded disease of *Agaricus bisporous*. At some places heavy infection have been recorded resulting in failure of mushroom crops. The pathogen has been isolated from compost, casing soil, diseased button mushroom and mushroom house soil etc. Therefore, the present investigations were undertaken to understand the possible source of infection, resistant strains of *A. bisporous* and suitable management measures to minimize the losses. The symptoms produced under Haryana conditions on fully developed sporophores, are localized light brown depressed spots. Adjacent spots coalesce and form irregular brown blotches like those of bacterial blotches but lighter in colour and some what sunken at the center. Diseased caps shrink in blotched areas. If the infection had taken place during spawn run or before pinhead initiation, onion shaped deformed mushrooms produced instead of normal sporophore.

**MATERIALS AND METHODS**

The studies were carried out in the Mushroom Technology Laboratory (MTL), Department of Plant Pathology, CCS Haryana Agricultural University, Hisar.

**Glassware and equipment**

Glassware used in the present study were of Borosil. Polythene bags (30 × 45 cm), polypropylene bags (7.50 × 30 cm) is used for spawn production, mushroom, and 500 ml empty glucose bottles were used for spawn and inoculum preparation.

**Chemicals**

The standard analytical grade chemicals were used in the present study.

**Sterilization of glassware**

Glasswares were sterilized at 180°C for two hours in a hot air oven.

**Maintenance of culture**

Pure cultures of *A. bisporous* and *V. fungicola* were maintained on PDA at ±23°C and ±20°C.

**Effect of pathogen inoculated in casing on dry bubble disease incidence**

Fresh inoculum of the pathogen multiplied on sorghum grain was mixed with disinfected casing soil at 0.1, 0.2 and 0.3%. In check 2.0 g of M-140 spawn was mixed with disinfected casing mixture. This casing mixture was used in place of normal casing and five replicates of each treatment were kept.

**Observation:** At the time of picking diseased fruiting bodies were discarded and weight of the normal mushrooms was recorded and the data was expressed in percent decrease in yield over control.

**Effect of inoculum spray on disease development**

Culture of pathogen *V. fungicola* was prepared on broth media. Actively growing culture was harvested from the broth and different dilutions of inoculum sprays were prepared by using 1.0, 2.0 and 3.0 g of mycelial mat per litre of the sterilized distilled water. The suspension was homogenized in electric blender. Twenty milliliter of above inoculum (0.1, 0.2 and 0.3% concentration) dilutions per bag were sprinkled at pin head initiation condition of white button mushroom as per treatment and 20 ml sterilized distilled water was sprinkled per bag in control. On subsequent days normal water was sprayed for maintaining the desired humidity.

**Observation:** At the time of picking diseased fruiting bodies were discarded and the weight of normal mushrooms was recorded and the data were expressed in percent decrease in yield over control.
Table 1. Effect of *Verticillium fungicola* inoculation in casing on dry bubble disease.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Inoculum dose (g/kg casing soil)</th>
<th>Pin head initiation/ First picking (days)</th>
<th>Total yield (kg/100 kg compost)</th>
<th>Percent decrease in yield over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1</td>
<td>30/34</td>
<td>31/35</td>
<td>11.2</td>
</tr>
<tr>
<td>2.</td>
<td>2</td>
<td>34/38</td>
<td>34/38</td>
<td>9.5</td>
</tr>
<tr>
<td>3.</td>
<td>3</td>
<td>35/39</td>
<td>36/40</td>
<td>5.6</td>
</tr>
<tr>
<td>4.</td>
<td>Control</td>
<td>28/32</td>
<td>29/33</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>CD at 5%</td>
<td></td>
<td></td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table 2. Effect of *Verticillium fungicola* spray on the yield of *A. bisporus*

<table>
<thead>
<tr>
<th>S/N</th>
<th>Inoculum concentration (g/L)</th>
<th>Total yield (kg/100 kg compost)</th>
<th>Percent decrease in yield over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1</td>
<td>6.34</td>
<td>5.98</td>
</tr>
<tr>
<td>2.</td>
<td>2</td>
<td>4.20</td>
<td>3.94</td>
</tr>
<tr>
<td>3.</td>
<td>3</td>
<td>1.80</td>
<td>1.52</td>
</tr>
<tr>
<td>4.</td>
<td>Control</td>
<td>14.30</td>
<td>14.82</td>
</tr>
<tr>
<td></td>
<td>CD at 5%</td>
<td>1.95</td>
<td>1.83</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Effect of pathogen inoculated in casing on dry bubble disease

The production of button mushroom largely depends on a top dressing fungicides after the mushroom compost has been fully colonized with mushroom mycelium. To see the effect of casing material on infection with *V. fungicola*, that experiment was conducted. It was found that loss of mushroom yield increase with the increased doses of inoculum and up to 62.16% decrease in yield was recorded. It is presumed that yield loss may further increase with increase inoculum dose. The casing material as a source of pest inoculum was recognized by various scientists. Mantal (1973) recommended chemical sterilization of casing soil with formalin whereas Shandilya et al. (1976) advocated integrated sterilization of casing mixture that is, steam sterilization at 60°C for 1 h + Benlate 240 g/100 m². Jandaik and Gularia (2002) isolated *V. fungicola* from 12 month old spent compost and recommended proper sterilization of spent compost if used for casing (Table 1).

Effect of inoculum spray on disease development

Smith (1924) reported that mushroom beds inoculated by spraying or sprinkling with a suspension of *Mycogone* spp. spores in sterile water produced mushrooms with an external symptoms of wet bubble disease in addition a considerable number of sclerodermoid mushroom. It has been suggested that *Mycogone* attack the spawn and the parasitic hyphae mingle and grow side by side the mushroom. It is very improbable that the parasite could grow in content with the mushroom hyphae for any length of time as *Mycogone* produces enzymes which rapidly break down the hyphae of the mushroom.

In the present experiment first flush was almost normal, but in subsequent flushes of mushroom were more and more heavily infected until finally they were practically deformed with zero market value and as the bags became older all the mushroom were sclerodermoid. This may be due to broken strands of mycelium due to harvesting of mushroom. These broken ends frequently round off and form new button which are readily attacked by the pathogen (Table 2).

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES