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Full Length Research Paper

Chemical control of dry bubble disease induced by *Verticillium fungicola* [Preuss] Hassebr 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. All the four fungitoxicants tested *in vitro* by poisoned food technique inhibited the growth of *V. fungicola* and *A. bisporus.* Carbendazim gave highest percent growth inhibition of pathogen and host followed by Thiophanate-methyl, Dithane Z-78 and Dithane M-45. In all the fungitoxicants when tested in bed condition, Carbendazim was observed to be most effective in reducing the disease incidence

Key words: *Agaricus bisporus, Verticillium fungicola,* dry bubble, carbendazim, thiophanate-methyl, dithane Z-78 and dithane M-45.

INTRODUCTION

White button mushroom is cultivated throughout the world, contributing about 40% of total world production of mushroom (Flegg, 1992). In India, white button mushroom is being cultivated in majority of the states both under seasonal and controlled conditions with an annual production of approximately 42,500 tons (Dandge, 2012). Haryana has become one of the leading states in white button mushroom production with 5312 tons/annum (Tiwari, 2004). Mushroom production is adversely affected by a large number of biotic and abiotic factors. Among the

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Table 1. Compost substrate without chicken manure.

Wheat straw compost		
Wheat straw	300	300
Calcium ammonium nitrate	09	09
Urea	03	03
Wheat bran	15	15
Murate of potash	03	03
Single super phosphate	03	03
Gypsum	30	30
Molasses	05	05

several biotic factors associated with reduction in yield of mushroom, fungal diseases significantly affect the mushroom production and yield.

Most common fungal diseases of white button mushroom are cobweb, dry/wet bubble, false truffle and green mould (Sharma, 1995). Of these diseases, dry bubble caused by *Verticillium fungicola* (Preuss) Hassebr is prevalent in all mushroom growing areas and has 25-50% incidence (Sharma, 1995). Dry bubble disease of white button mushroom causing brown spots was reported for the first time by Malthouse in 1901. He found a species of *Verticillium* associated with this disease. Two types of symptoms were observed. Initially, fungal growth appeared on the casing soil which later spread and turned grayish yellow. After that, light brown superficial spots appeared on the caps which finally coalesced to become large brown blotches. This disease is transmitted by contaminated compost, casing soil (Kumar et al., 2014), human beings and splash of water (Fekete, 1967; Cross and Jacobs, 1969). Mushrooms infected by *V. fungicola* shows typical thickening of stem, resulting in onion shaped fruiting bodies. However, the symptoms vary with the age of the mushroom and the stage of development at which the infection takes place. When mushrooms are infected by this fungus at an early stage, symptoms appear as small undifferentiated masses of tissue up to 2 cm diameter. Fruiting bodies are not properly formed and caps are partially differentiated. When infected at a later stage the stipes are distorted and have tilted caps. Infected mushroom show the presence of grey white mycelial growth and become discoloured and dry but do not rot. They show small pimple like outgrowth or brown grey spots (1-2 cm diameter) on the surface. Such spots often have a yellow or bluish grey halo around them.

In Haryana, the white button mushroom is being cultivated on compost prepared by long method in low cost mushroom houses under seasonal conditions. These factors coupled with poor sanitation and persistence of *V. fungicola* in soil pose a serious threat to the future of mushroom cultivation in Haryana.

MATERIALS AND METHODS

Glassware and equipment

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

Chemicals

Standard analytical grade chemicals were used in the present study.

Sterilization of glassware

Glasswares were sterilized at 180°C for 2 h in a hot air oven.

Maintenance of culture

Pure cultures of *A. bisporus* and *V. fungicola* were maintained on PDA at 20±1°C.

Preparation of compost

Two methods of composting viz., long method of composting (LMC) and short method of composting (SMC) were followed: Six types of composts were used: Wheat straw compost (LMC); Wheat straw compost with chicken manure (LMC); Wheat straw compost (SMC); Wheat straw compost with chicken manure (SMC); Brassica straw compost with chicken manure (LMC); Brassica straw compost with chicken manure (SMC). These composts were prepared for conducting the experiment.

Compost preparation

Six types of compost were used. The compost was prepared with or without chicken manure in both methods. Wheat and *brassica* straw were spread separately on a pucca floor and wetted thoroughly with clean water for 48 h to attain 70-75% moisture content. Wheat bran was dry mixed, with chemical fertilizer, moistened with water, covered with polythene sheets and kept overnight to facilitate adsorption of chemical fertilizer on the bran. The mixture was evenly spread on wet wheat and *Brassica* straw, mixed and stacked to make a compact rectangular pile. Seven turnings were given to the pile using the turning schedule of 0, 6, 10, 13, 16, 19, 22, 25 and 28 days. At each turn, approximately 30 cm layer was separated from all the exposed surface of the pile and moistened, if necessary. The remaining pile was also dismantled and mixed well. The material was restacked in such a way that the outer portion of the previous pile was in the center of the new pile. Molasses was mixed at first turn, gypsum at the third. Two days after the last turn, the pile was dismantled and the contents were mixed thoroughly. The compost was checked for desirable characteristics, that is, dark brown colour, pH (7-8), absence of ammonia smell and appropriate moisture content (68-72%). The composition for LMC and SMC of the substrates are given in Table 1.

Spawning

Thorough spawning was done at 1% spawn before filling of compost in the polythene bags. In further studies, wheat straw compost with chicken manure prepared by LMC was used (Table 2).

Table 2. Compost substrate with chicken manure.

Wheat/Brassica straw compost		
Ingredient	**LMC (kg)**	**SMC (kg)**
Wheat/ *Brassica* straw	300	300
Chicken manure	100	130
Urea	08	04.00
Wheat bran	15	15
Murate of potash	03	-
Single super phosphate	03	-
Gypsum	30	30
Molasses	05	05

The compost was made separately.

Table 3. Disease appearance on cut-mushrooms.

Treatment	Number of fruit bodies	Number of fruit bodies infected			Infected percentage (%)		
		After hours			hours		
		24	48	72	24	48	72
Inoculated cut mushroom	10	0	6	10	0	60	100
Uninoculated cut mushroom	10	0	0	0	0	0	0

This table show pathogenicity test for dry bubble disease in white button mushroom caused by *Verticillium fungicola.*

Spawn run

After spawning, bags were covered with newspaper sheets sterilized with formalin (4%) and water was sprinkled to keep moist. Temperature was maintained 24 ± 2°C with relative humidity of 85-90%. After pin head initiation, temperature was lowered down to 16± 2°C and RH of 90%. Fresh air was circulated for 3-4 h daily during cropping.

Casing

Casing soil was prepared by mixing well decomposed (16-18 month old) farm yard manure and burnt rice husk (4:1 v/v). The casing mixture was disinfected with 4% formalin solution using 600 ml formalin (36%) diluted to 5 L for 100 kg of casing material. The formalin treated moistened casing material was kept covered with polythene sheets for at least 48 h followed by frequent turnings to evaporate formalin fumes. Before casing, the newspaper sheets were removed from the spawn impregnated compost and the surface was covered with disinfected casing material (4 cm) for uniform thickness.

Cropping

Adequate humidity (85-90%) was maintained inside the growing room by spraying water on the walls and the floor. Water was sprayed on the bags twice a day, very little or no ventilation was provided until the first appearance of pin heads. Thereafter, intermittent cross ventilation was given for a total 4-6 h per day. The mushrooms were harvested by gentle twisting of the fruit body. The depressions created in the casing layer were filled with fresh disinfected casing soil. The lower part of the pileus of harvested fruit bodies were trimmed off and yield was recorded.

Yield data and statistical analysis

The yield data was recorded for upto 45 days of cropping period. A daily record of the number of fruit bodies and their weight (g) per bag per treatment was maintained and the yield data was expressed as kg mushroom per 100 kg compost. The critical difference (CD at 5%) was calculated from the replicate data using factorial experiment and in common complete randomized design (CRD).

Isolation of pathogen

The diseased mushroom pileus showing typical symptoms of dry bubble disease were cleaned gently by wiping the outer surface with sterile cotton moistened with distilled sterilized water. Pieces of infected cut mushroom pileus were planted on PDA slants and incubated at 20±1°C. To suppress the bacterial contamination, the medium was amended with streptocyclin at a concentration of 50 ppm. The subculturing was done periodically at regular intervals during the course of present investigations (Table 3).

Identification

Pathogen was identified as *V. fungicola* (Preuss) Hassebr on the basis of culture, colour, microscopic studies and type of sporulation with the help of mycologist in the Department of Plant Pathology, CCS HAU, Hisar.

Pathogenicity (Koch postulates)

The pathogenicity was proved by placing actively growing mycelia agar bit (5 mm diameter) of the *V. fungicola* on cut healthy white button mushroom (*A. bisporus*) fruit bodies incubated at 20±1°C

Table 4. List of fungitoxicants and their active ingredient test against *V. fungicola*.

Test fungicides	Active ingredient	Group	Common name
Bavistin	50% WP (2-methoxy-carbamoyl-benzimidazole	Benzimidazole	Carbendazim
Mancozab	75% WP (Zinc manganous ethylene) bis thiocarbamate	-	Dithane M-45
Zineb	75% WP (Zinc ethylene) bis di thiocarbamate	-	Dithane Z-78
Topsin –M	50% WP (Thiophanate methyl)	Thiophanate	Thiophanate methyl

Table 5. Effect of different fungitoxicants on mycelial growth of *V. fungicola in vitro.*

Fungitoxicants	Colony diameter (mm)			Percent growth inhibition		
	Concentration (ppm)					
	1	10	100	1	10	100
Carbendazim	80.23	60.11	10.5	10.86	33.22	88.33
Thiophanate-Methyl	81.93	61.42	12.74	8.97	31.76	85.84
Dithane Z- 78	84.78	63.22	18.31	5.81	29.76	79.65
Dithane M- 45	88.01	72.41	30.35	1.33	19.55	66.28
Control	90.00	90.00	90.00	0.00	0.00	0.00
CD at 5%	1.53	1.23	0.832	1.65	1.23	1.46

and 85% humidity. The observations were recorded for disease appearance. Reisolated pathogen was compared with the original one. In the case of control, only agar bit was placed on the cut healthy fruit bodies.

Effect of different fungitoxicants on *V. fungicola* and *A. bisporus*

Four fungitoxicants namely Carbendazim, Mancozeb, Thiophanate-methyl and Zineb were evaluated at different concentations (1, 10 and 100 ppm) against *V. fungicola* and *A. bisporus* using poisoned food technique (Schmitz, 1930).

To evaluate the effectiveness of fungitoxicants in controlling dry bubble disease of white button mushroom, the fungitoxicants namely, Carbendazim. Dithane M-45, Dithane Z-78 and Thiophanate-methyl were used at 1, 10, 100 ppm concentrations *in vitro.* Treatment without any fungitoxicants served as control.

For this purpose, double strength fungitoxicants were added to the double strength PDA media to get the desired concentrations. The PDA amended with test fungitoxicant was poured in Petri-plates (20 ml/plate) (Table 4). After solidification, the poisoned medium was seeded with 5 mm mycelial agar bit of actively growing *V. fungicola* and *A bisporus* separately. The three replications of each treatment were kept (Table 5 and 6).

Observations were recorded at regular intervals for radial growth of *V. fungicola* till whole plate (in control) was covered with mycelial growth of this pathogen (14 days) of incubation at 20±1°C. Growth inhibition (%) was calculated with the growth of the test fungus in control (devoid of fungitoxicant).

Observations were recorded for radial growth of pathogen.

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

where C = Diameter of colony in the control; T = diameter of the colony in the treatment

Effect of different fungitoxicants on the development of dry bubble disease

The test fungitoxicants were mixed in compost to get desired concentration. In the case of Carbendazim and Thiophanate methyl, 400 mg each was dissolved in 4.0 L of water and sprayed on 20 kg of compost, spread over a clean polythene sheet. 800 mg each of Dithane M-45 and Dithane Z-78 was dissolved in 4.0 L of water sprayed over the 20 kg compost. After mixing the compost thoroughly, spawning with M140 of *A. bisporus* (1%) and *V. fungicola* (0.3%) was done and filled in polythene bags weighing 5.0 kg each, four replication of each treatment were kept. In the control, water without fungitoxicant was sprayed before spawning. The yield of mushroom was recorded upto 45 days and compared with control treatment. The different concentrations of different fungicides were used so as to see comparable results and which was cheaper than the control of the disease. The spawn of *A. bisporus* (1%) is necessary for better results and for the inoculum of *V. fungicola* (0.3%), if percent inoculum taken is more, the more disease will occur (Table 7).

RESULTS AND DISCUSSION

Isolation, purification and identification of dry bubble pathogen and pathogenicity

Isolation of pathogen was made on PDA from diseased sporophores suspected of having Verticillium infection. The pathogen cultures were further purified and incubited at 20±1°C. For morphological studies, pure culture was transferred in Petri dishes and incubated at desired temperature. The colony growth charactesrtics were recorded.

The colonies were white in appearance, under part of the plate was colorless to yellow and had scalloped

Table 6. Effect of different fungitoxicants on mycelial growth of *A. bisporus*.

Fungitoxicant	Colony diameter (mm)			Per cent growth inhibition		
	Concentration (ppm)					
	1	10	100	1	10	100
Carbendazim	88.80	83.32	58.17	1.33	7.42	47.75
Thiophanate-methyl	89.18	85.62	60.90	0.91	4.86	43.65
Dithane Z-78	89.40	87.24	52.74	0.66	3.06	40.89
Dithane M-45	89.70	88.50	64.19	0.33	1.66	38.72
Control	90.00	90.00	90.00	0.00	0.00	0.00
CD at 0.05%	NS	1.11	1.16	NS	1.42	2.10

Table 7. Efficacy of different fungitoxicants in controlling dry bubble disease on *Agaricus bisporus*.

Fungitoxicant	Concentration (ppm)	Spawn run (days)		Pin head initiation/first picking (days)		Yield (kg/100 kg compost)		Per cent in increase yield over control	
		First year	Second year	First year	Second year	First year	Second year	First year	Second year
Carbendazim	100	13	14	30/34	29/33	12.30	15.40	67.80	75.84
Thiophanate methyl	100	15	16	31/35	31/35	09.26	12.26	57.23	69.65
Dithane Z-78	200	17	17	32/36	32/36	07.10	10.13	44.22	63.27
Dithane M-45	200	18	19	34/38	34/38	06.54	09.67	39.75	60.80
Control		20	20	35/39	36/40	03.96	03.72		
CD at 5%						0.43	0.41		

edges .Old cultures developed light purple colouration starting from the center of the colony and spreading outwards. The colony characterctics of the pathogen isolated resembled the characters desired by the Nair and McCauley (1987), Calonje et al. (2000), Khanna et al. (2003) and Justyna et al. (2011). So it was concluded that our pathogen was *V. fungicola* after proving the Koch's postulates. The pure culture was maintained and used in further experiment.

Effect of different fungitoxicants on *Verticillium fungicola*

Four fungitoxicants (two systemic and two contact) viz, Carbendazim, Thiophanate –methyl, Dithane Z-78, and Dithane M-45 when screened *in vitro* by poisoned food technique and were found quite effective. Maximum percent growth inhibition was recorded in Carbendazim followed by Thiophanate–methyl, Dithane Z-78 and Dithane M-45, respectively. Our findings gets support from the reports of Sinden (1949), Hu and Dough (1965), Smith (1970), Fletcher (1971) and Gea et al. (1977, 2011, 2012) who observed that different isolate of *V. fungicola* were sensitive to Prochlorage Mn complex followed by Prochlorage + Carbendazim.

Effect of different fungitoxicants on *Agaricus bisporus*

Fungitoxicant have been reported to have some inhibition effect on the host (mushroom) though they are targeted at the pathogen. Thus, use of fungitoxicant may have bearing on the growth of mushroom being a fungus. Result of *in vitro* screening against *A. bisporus* revealed that 100 ppm concentration of Carbendazim inhibited growth of *A. bisporus* up to 47.75%, followed by Thiophanate –methyl (43.65%), Dithane Z-78 (40.89%) and Dithane M-45 (38.72%). The present results are in agreement with work done by Gandy (1985) who also reported that Carbendazim fungicides were less toxic to basidiomycetes than to other pathogen. Seth and Bharadwaj (1989) have shown that Benlate inhibited the growth of *A. bisporus* least followed by Bavistin during *in vitro* studies. Similarly, the present studies support the work of Dhar and Kapoor (1990) and Navarro et al. (2011) who stated that use of Bavistin can control the fungal pathogens and competitors of white button mushroom and it also had less inhibitory action on mushroom mycelium. Thus, it may be inferred that to minimize damage to *A. bisporus*, extra care should be taken in the selection of fungicides for application to manage the disease. Bhalla (1998) reported that the inhibition percent of *A. bisporus* by

Bavistin, Benlate, Sporogon, Dithane M-45 and Dithane Z-78 ranged from 18.18 to 100% at 50, 100, 200 and 500 ppm concentration.

Effect of different fungitoxicants on dry bubble disease

Bhatt and Singh (2002) reported Sporogon (0.075%) to be effective against V. fungicola. Maximum yield and number of fruit bodies were obtained by using Bavistin. Earlier, Bavistin has been tested against brown plaster mould and found to be effective (Dhar, 1978; Arora et al., 1990; Sharma, 1995). Bhatt (1992) reported that Bavistin at 100 ppm showed 40.50% increase yield. Efficacy of Bavistin against *Papulaspora byssina*, *Trichoderma viride*, *V. fungicola* and *Thermomicrobium roseum* has been reported by Sharma and Vijay (1996) Navarro et al. (2011), Sharma and Satish (2012) and Gea et al. (2012), Bavistin increased the number of fruit bodies and yield. Results of present study are in agreement with that which revealed that Carbendazim advanced spawn run by 6-7 days, pinhead initiation by 5 days resulting in 67.80 to 75.84% increase in yield. Other chemicals, Thiophanate–methyl Dithane Z-78 and Dithane M- 45 also significantly increase the mushroom yield, shortened spawn run period and pinhead initiation as compared to the control.

Conflict of Interests

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

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