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Efficacy of Fungicides against *Trichoderma* spp. Causing Green Mold Disease of Oyster Mushroom (*Pleurotus sajor-caju*)

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ABSTRACT

Green mold (*Trichoderma* spp.) is a devastating disease in the crop production of mushrooms. In India, it has been reported to cause serious crop losses. It is also common contaminant, occurring in mushroom houses in the Kashmir valley. The aim of the present study was to check the *in vitro* and *in vivo* efficacy of fungicides against Green mold (*Trichoderma harzianum*) associated with the cultivation of *Pleurotus sajor-caju*. *Pleurotus sajor-caju* is the third most commercially important edible mushroom worldwide. Five fungicides namely Carbendazim, Bitertanol, Hexaconazole, Captan and Mancozeb were evaluated *in vitro* against Green mold (*Trichoderma harzianum*) and against the mushroom mycelium as well, by following Poison food technique. The results revealed that the maximum average inhibition of *Trichoderma harzianum* was recorded in Carbendazim (90.8%), followed by Bitertanol (40.0%), Captan (36.6%) and Hexaconazole (16.1%). The least inhibition (11.7%) of *Trichoderma harzianum* was exhibited by Mancozeb. It was further observed that Carbendazim exhibited the least inhibition (24.9%) of *Pleurotus sajor-caju,* followed by Captan (45.5%), Bitertanol (63.0%) and Hexaconazole (74.5%). The maximum inhibition (87.4%) of *Pleurotus sajor-caju* was exhibited by Mancozeb. Fungicides showing maximum efficacy against the pathogen (*Trichoderma harzianum*) and minimum efficacy against mushroom (*Pleurotus sajor-caju*) were further tested against *Trichoderma harzianum* during an *in vivo* test in mushroom house. It was observed that all tested fungicides reduced the disease intensity of *Trichoderma harzianum* and produced more yield than control polybags. Maximum increase in yield (36.9%) over control and minimum mean disease incidence (9.3%) was recorded in treatment which received Carbendazim as the fungicide. Carbendazim was found to be best fungicide, against the infection of Green mold (*Trichoderma* spp.) disease of mushrooms.

Key words: Edible, worldwide, inhibition, pathogen, treatment, incidence

INTRODUCTION

Mushrooms have been used by humans for millennia. Edible mushrooms are appreciated as a delicacy and are integral part in the cuisines of various cultures. Mushrooms are commonly found on compost in wooden trays, in polybags, in bottles, on shelves and on beds in production rooms at low temperatures with high humidity (Hall *et al*., 2003). The commonly cultivated mushrooms

include *Agaricus, Lentinus, Flammulina, Pleurotus* and *Volvariella*. In India, Dhingri mushroom (*Pleurotus sajor-caju*) is the most widely cultivated mushroom after *Agaricus*. *Pleurotus* spp. are one of the most efficient lignocellulose decomposing types of mushrooms (Baysal *et al*., 2003). So many industrial and agricultural wastes can be utilized as substrates for cultivation of Dhingri mushroom. However, an emerging problem faced by growers in mushroom houses is the development of Green mold that frequently causes huge losses in mushroom industry (Jaklitsch *et al*., 2006; Samuels *et al*., 2002). The genus *Trichoderma*, commonly called as weed mould in mushroom industry is responsible for causing Green mold disease of mushrooms. This pathogenic fungus completely colonizes the substrates and in certain cases, grow on the surface of the emerging mushrooms. One of the major diseases of the Dhingri mushroom (*Pleurotus sajor-caju*) worldwide is caused by *Trichoderma harzianum* (Bayer *et al*., 2000).

Trichoderma spp. in its initial stage, produces a dense pure white mycelium which resembles mushroom mycelium, which often becomes difficult for growers to distinguish between the two. Mycelial growth of *Trichoderma* spp. on the substrates gradually gets converted to a green colour because of the heavy sporulation of *Trichoderma* spp. producing a characteristic symptom of the Green mold disease (Danesh *et al*., 2000). The mycelia of *Trichoderma* covering substrates, competes with mushroom mycelium for space and nutrients and results in the reduction in the production of mushroom fruit bodies (Bayer *et al*., 2000). The symptoms of Green mold disease is characterized by dense green sporulation on the substrates, resulting in a huge reduction in mushroom yield (Anderson *et al*., 2000). *Trichoderma* species produces various mycotoxins, which retards the growth of mushroom mycelium. Some mushrooms show symptom of wateriness, shrinking and drying of fruiting bodies as well. The species also secrete hydrolytic enzymes like chitinases, b-glucanases and cellulases which lyse the fungal cell walls and play a role in the mycoparasitic activity of this fungus (Danesh *et al*., 2000).

Trichoderma species have created havoc in the mushroom houses of Kashmir valley. They are most nuisance species responsible for producing Green mold in mushroom houses. Green mold disease of *Pleurotus sajor-caju* is considered to be one of the most devastating disease as its occurrence assumes greater significance resulting in the huge losses of mushroom crop. Hence, an attempt was made to evaluate different fungicides against the pathogen to manage this devastating disease.

MATERIALS AND METHODS

In the present study, samples of pathogen (*Trichoderma* spp.) were collected from the Green mold infected substrate blocks of *Pleurotus sajor-caju*. A few samples were also obtained from Green mold infected spawn bottles. All the samples were collected from Mushroom Research Training Center (MRTC), SKAUST-K Srinagar Kashmir during August 2007 to November 2007.

In this study, five fungicides 3 systemic: Carbendazim, Bitertanol and Hexaconazole each at 25, 50, 100, 250, 500 and 1000 μg mL^{-1} concentrations and two non-systemic fungicides: Captan and Mancozeb, each at 100, 250, 500, 1000, 2000 and 3000 μg mL^{-1} concentrations were evaluated to estimate their comparative efficacy on the growth of *Trichoderma harzianum* and *Pleurotus sajor-caju* (Table 1).

***In vitro* evaluation:** The poisoned food technique was adopted for *in vitro* testing of fungicides (Nene and Thapliyal, 2000). Istly, the required amount of distilled water was poured in conical flasks (100 mL). The flasks were plugged and then autoclaved at 15 lbs pressure/$inch^2$ for 15 min.

Table 1: Fungicides used in the study

Common name	Trade name	Chemical name
Carbendazim	Bavistin	Methyl benzimidazole-2-yl carbamate
Bitertanol	Baycor	1-(biphenyl-4-yloxy)-3, 3-dimethyl-1 (1H-1, 2, 4-triazol- 1yl) butan-2-ol
Hexaconazole	Anvil	(RS)-2-(2, 4-dichlorophenyl)-1-(1H-1, 2, 4 triazol-1-yl) hexan-2-ol
Captan	Captain	N-trichloromethylthio-4- cyclohexane -1, 2- dicarboximide
Mancozeb	Diathane	M-45 Manganese ethylene bisdithio carbamate

The calculated quantities of fungicides were aseptically added to the cooled sterile distilled water in conical flasks, so as to get the desired concentration of active ingredient of each fungicide separately. The flasks were shaken gently to ensure the proper mixing of chemicals in distilled water. Then 2 mL from each flask was dispensed in petriplates with the help of sterilized pipettes. After that, 20 mL of molten and cooled Potato Dextrose Agar (PDA) was poured in each petriplate. The petriplates were rotated gently to ensure the uniform distribution of fungicide solution in the media. After solidification of media, mycelial discs (5 mm) were cut from the edges of 6 days old culture of both pathogen (*Trichoderma harzianum*) and *Pleurotus sajor-caju* with the help of sterile cork borer. Each disc of both fungi was transferred aseptically to the center of each petriplate separately. Three replications per treatment were maintained. The PDA plates without fungicide were also inoculated with the discs and maintained as controls. The plates were incubated at 27±1°C until the petriplate in control treatments were fully covered with mycelial growth. The radial colony growth was measured and the efficacy of fungicides was expressed as per inhibition of mycelial growth over control, calculated by using formula suggested by Vincent (1947):

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

***In vivo* evaluation:** In this study, the three selected fungicides, Bavistin, Baycor and Captan were evaluated using *in vivo* testing. The three fungicide concentrations were tested: 250, 500, 1000 μg mL^{-1}. The cultivation trials were laid during 2009. The calculated quantities of fungicides were weighed and added to the water in plastic tubs. The chemicals were properly mixed with water in tubs. The paddy straw after proper washing and draining was soaked in different aqueous fungicide solutions in tubs. The straw was kept in solutions for 5 h. After that, straw was drained properly keeping moisture content of 60%. The treated straw was then filled in poly bags at the rate of 1 kg dry substrate per bag. The bags were inoculated with the spawn of *Pleurotus sajor-caju* at the rate of 1% per bag, in layers. Un-treated (no fungicide), but spawned bags were kept as control. After spawning, the mouths of the bags were closed properly and 10-15 pin holes were made all over the bags for proper aeration. All the treatments including control were replicated six times in CRBD (Complete Randomized Block Design). The bags were kept for spawn running in the dark cropping room for 10-15 days till complete colonization of compost by mushroom mycelium (El-Kattan and El-Hadded, 1998). The temperature (28°C max and 25°C min) and relative humidity of 80% was maintained in cropping room. After the mycelia had completely covered the substrate, the poly bags were cut and prepared doses of inocula of 3 mL spore suspension of *Trichoderma harzianum* @ 1×10^{8} spores mL^{-1} was inoculated in the middle of the substrate block, with the help of syringe.

Fruiting and Harvest: After the completion of spawn running in all treated and untreated bags, the temperature was dropped to 15-17°C, relative humidity was raised to 90-95%. Fresh air was given to growing room by exhaust fan to lower the carbon dioxide, for 3-4 h. Pin heads appeared and mushroom harvesting was done at maturity, the three mushroom flushes were picked for 1 month.

In this *in vivo* test, the observations on days to spawn run, first pin formation, percent (%) increase in yield over control and percent (%) disease incidence were recorded. Yield of oyster mushroom was expressed as gram fresh fruiting body/bag of compost (1 kg) in three flushes of mushroom.

Percent increase in yield in treated bags over control (untreated) was calculated using the formula:

$$\text{Percent increase in yield over control}(\%) = \frac{\text{Yield treatment} - \text{Yield control}}{\text{Yield treatment}} \times 100$$

Disease incidence was scored on a 0-5 scale with 0 = disease free, 1 = 1-20% area covered by disease, 2 = 21-40% area covered by disease, 3= 41-60% area covered by disease, 4 = 61-80% area covered by disease and 5 = 81-100% area covered by the disease (Bhardawaj, 1992). Percent disease incidence (PDI) was calculated as follows:

- PDI = Sum of numerical values×Grades×100
- Total No. of observations×Maximum grade

Statistical analysis: For *in vivo* trial, Completely Randomized Block Design (CRBD) was applied. The experiment was conducted in (CRBD) with three replications in each treatment. All the experiments were analyzed statistically by the Analysis of Variance (ANOVA) for drawing the conclusions from the data. The value calculated, was compared with the tabulated value at 0.05% level of probability.

RESULTS

In vitro evaluation of fungicides on mycelial inhibition of *Trichoderma harzianum* indicated that all the fungicides, both systemic and non-systemic were significantly inhibiting, more or less, the radial growth of *T. harzianum*. Among the tested systemic fungicides, Carbendazim was found to be superior at all the selected concentrations in comparison to the other fungicides. The maximum average inhibition (90.8%) was recorded in Carbendazim, followed by Bitertanol (40.0%). The least percent inhibition of mycelial growth of *T. harzianum* (16.1%) was recorded in Hexaconazole. Carbendazim completely inhibit the radial growth of *T. harzianum* at 500 and 1000 μg mL^{-1} concentrations (Table 2). It was followed by (89.6%) at 250 μg mL^{-1}, (88.0%) at 100 μg mL^{-1}, (84.7%) at 50 μg mL^{-1} and (82.9%) at the lowest dose of 25 μg mL^{-} . It was observed that Carbendazim exhibited the least inhibition (24.9%) of *Pleurotus sajor*-caju, followed by Bitertanol (63.0%) and Hexaconazole (74.5%) (Table 3). The percent inhibition in mycelial growth of *P. sajor-caju* at the most effective concentrations of 500 and 1000 μg mL^{-1} of Carbendazim (against *T. harzianum*), recorded was (26.2%) and (31.4%). It is obvious from the Table 3, that growth of *Pleurotus* was better in media amended with Carbendazim at the other concentrations of 250, 100, 50 and 25 μg mL^{-1}, showing 26.2, 25.2, 22.2 and 18.1% growth

Table 2: *In vitro* evaluation of various systemic fungitoxicants against *Trichoderma harzianum*

	*Percent inhibition in mycelial growth over control						
Treatment	0.0025	0.005	0.001	0.025	0.05	0.1	Mean
Carbendazim	82.9 (65.5)**	84.7 (66.9)	88.0 (69.7)	89.6 (71.1)	100.0 (90.0)	100.0 (90.0)	90.8 (75.5)
Bitertanol	20.7 (27.0)	28.4 (32.2)	37.7 (37.8)	45.5 (42.4)	51.4 (45.8)	56.2 (48.5)	40.0 (38.9)
Hexaconazole	0.0	0.73 (4.9)	8.8 (17.2)	20.3 (26.7)	31.4 (34.0)	35.5 (36.5)	16.1 (19.8)
Mean	34.5 (30.8)	37.9 (34.0)	44.8 (41.5)	51.8 (46.6)	60.9 (56.6)	63.9 (58.3)	

SOV	S.Em±	C.D (p = 0.05%)
Treatment	0.68	1.14
Concentration	0.96	1.62
Treatment×Concentration	1.67	2.82

*Mean of three replications. **Value in parenthesis are Arc sine transformed values

Table 3: *In vitro* evaluation of various systemic fungitoxicants against *Pleurotus sajor-caju* (Dhinghri mushroom)

	*Percent inhibition in mycelial growth over control						
Fungicide	0.0025	0.005	0.001	0.025	0.05	0.1	Mean
Carbendazim	18.1 (25.1)**	22.2 (28.1)	25.5 (30.3)	26.2 (30.7)	26.2 (30.7)	31.4 (34.0)	24.9 (29.8)
Bitertanol	52.1 (46.2)	59.5 (50.4)	62.9 (52.4)	65.5 (54.0)	67.3 (55.1)	71.1 (57.4)	63.0 (52.5)
Hexaconazole	63.6 (52.8)	68.8 (56.0)	75.5 (60.3)	76.2 (60.8)	78.1 (62.0)	85.1 (67.2)	74.5 (59.8)
Mean	44.6 (41.3)	50.2 (44.8)	54.6 (47.6)	55.9 (48.5)	57.2 (49.3)	62.5 (52.9)	

SOV	S.Em±	C.D (p = 0.05%)
Fungicide	0.45	0.76
Concentration	0.40	0.67
Fungicide x Concentration	1.22	N.S

*Mean of three replications. **Value in parenthesis are Arc sine transformed values, N.S: Non significant

inhibition, as compared to other fungicides. The maximum inhibition of *T. harzianum* (56.2%) by Bitertanol was recorded at highest concentration of 1000 μg mL^{-1} and minimum percent inhibition recorded was 20.7% at the lowest dose of 25 μg mL^{-1}. The percent inhibition of *P. sajor-caju* by Bitertanol ranges from 52.1% at the lowest concentration of 25 μg mL^{-1} and 71.1% at the highest concentration of 1000 μg mL^{-1}. The maximum inhibition of *T. harzianum* (35.5%) by Hexaconazole was recorded at 1000 μg mL^{-1} concentration. *T. harzianum* remained un-effected at the lowest dose of 25 μg mL^{-1} of Hexaconazole. The same fungicide inhibited mycelial growth of *P. sajor-caju* by 63.6 to 85.1%. There was significant difference between the concentrations of fungicides in inhibiting the radial growth of both *T. harzianum* and *P. sajor-caju*, i.e. with the increase in concentration, the percent inhibition also increased. It was further observed that the interaction between the treatment (fungicide) and concentration was recorded as significant in case of *T. harzianum*, but there was no significant difference in the interaction between the fungicides and concentration in case of *P. sajor-caju*.

It is evident from Table 4 and 5, that there was significant difference between the efficacy of non-systemic fungicides on radial growth of *T. harzianum* and *P. sajor-caju*. Among the non-systemic fungicides, the maximum inhibition in radial growth of *T. harzianum* (36.6%) was observed in Captan (Table 4). The least inhibition (11.7%) of *T. harzianum* was exhibited by Mancozeb. Captan at highest dose of 3000 μg mL^{-1}, exhibited the maximum inhibition of (54.4%), followed by (42.9%) at 2000 μg mL^{-1}, (37.3%) at 1000 μg mL^{-1}, (34.7%) at 500 μg mL^{-1}, (30.7%)

Table 4: *In vitro* evaluation of Non-systemic fungitoxicants against *Trichoderma harzianum*

	*Percent inhibition in mycelial growth over control						
Treatment	0.001	0.025	0.05	0.1	0.2	0.3	Mean
Captan	19.6 (26.2)**	30.7 (33.6)	34.7 (36.0)	37.3 (37.6)	42.9 (40.9)	54.4 (47.5)	36.6 (36.9)
Mancozeb	2.9 (9.8)	4.0 (11.5)	6.2 (14.4)	15.1 (22.8)	18.4 (25.4)	23.6 (29.0)	11.7 (18.8)
Mean	11.2 (17.9)	17.3 (22.5)	20.5 (25.2)	26.2 (30.2)	30.7 (33.1)	39.0 (38.2)	
SOV			S.Em±				C.D (p = 0.05%)
Treatment			0.40				0.68
Concentration			0.7				1.19
Treatment x Concentration			0.98				1.67

*Mean of three replications. **Values in parenthesis are Arc sine transformed values

Table 5: *In vitro* evaluation of Non systemic fungitoxicants against *Pleurotus sajor-caju* (Dhingri mushroom)

Conc (%)	*Percent inhibition in mycelial growth over control						
Treatment	0.001	0.025	0.05	0.1	0.2	0.3	Mean
Captan	29.9 (33.1)**	36.9 (37.4)	41.4 (40.0)	44.7 (41.9)	53.6 (47.0)	66.6 (54.6)	45.5 (42.3)
Mancozeb	78.0 (62.0)	80.3 (63.6)	82.2 (65.0)	84.0 (66.4)	100.0 (90.0)	100.0 (90.0)	87.4 (72.8)
Mean	54.0 (47.5)	58.6 (50.5)	61.8 (52.5)	64.4 (54.1)	76.8 (68.5)	83.3 (72.3)	
SOV			S.Em±				C.D (p = 0.05%)
Treatment			0.33				0.56
Concentration			0.58				0.99
Treatment x Concentration			0.82				1.40

*Mean of three replications. **Values in parenthesis are Arc sine transformed values

at 250 μg mL^{-1} and the least inhibition (19.6%) was recorded at the lowest dose of Captan. The maximum percent inhibition of *T. harzianum* by Mancozeb (23.6%) was recorded at the highest dose of 3000 μg mL^{-1}, followed by (18.4%) at 2000 μg mL^{-1} and (15.1%) at 1000 μg mL^{-1}. The least inhibition of *T. harzianum* by Mancozeb (2.9%) was recorded at 100 μg mL^{-1}, followed by (4.0%) at 250 μg mL^{-1} and (6.2%) at 500 μg mL^{-1}. The maximum inhibition (87.4%) of *P. sajor-caju* was exhibited by Mancozeb, followed by Captan (45.5%) (Table 5). The least inhibition of *P. sajor-caju* (29.9%) was recorded at the lowest dose of Captan i.e.; 100 μg mL^{-1}. There was no growth of *P. sajor-caju* at 2000 and 3000 μg mL^{-1} concentration of Mancozeb. The lowest dose of Mancozeb i.e., 100 μg mL^{-1} inhibit *P. sajor-caju* by 78.0%. It was further observed that there was significant difference between the concentrations of fungicides in inhibiting the radial growth of both *T. harzianum* and *P. sajor-caju*, i.e., with the increase in concentration, the percent inhibition of both fungi also increased. The interaction between the fungicides and concentration was recorded as significant in both *T. harzianum* and *P. sajor-caju*.

Carbendazim, Bitertanol and Captan were selected for *in vivo* trial, as all of them show good inhibitory effect on *T. harzianum* and least inhibition of *P. sajor-caju* in comparison to the other fungicides. The results obtained from *in vivo* evaluation of fungicides against *P. sajor-caju* and *T. harzianum* is presented under the following heads:

- Time taken for complete colonization/Spawn-run

Table 6 reveals that there was significant difference between the effects of fungicides on the time taken for complete colonization by mycelium of *P. sajor-caju*. The average number of the days

Table 6: Influence of various fungicides on time taken for complete colonization by mycelium of *Pleurotus sajor-caju*

	*Time taken for Spawn-run (Days)			
Fungicide	0.025	0.05	0.1	Mean
Carbendazim	16.5	16.1	15.3	15.9
Bitertanol	16.5	16.0	16.0	16.1
Captan	16.3	16.1	15.5	15.9
S.Em±	0.43			
C.D @ 0.05%	0.72			
Control	17.3 days			

*Mean of six replications

Table 7: Influence of various Fungicides on time taken for pin formation by *Pleurotus sajor-caju*

	*Days taken for pin formation			
Conc. (%)				
Fungicide	0.025	0.05	0.1	Mean
Carbendazim	7.5	7.1	6.3	6.9
Bitertanol	7.3	7.0	6.8	7.0
Captan	7.0	6.8	6.3	6.7
S.Em±	0.28			
C.D @ 0.05%	0.46			
Control	7.0 days			

*Mean of six replications

required for spawn-run in *P. sajor-caju* was significantly less (15.9 days) in both Carbendazim and Captan. It was followed by Bitertanol (16.1 days). These were found statistically identical with each other. The average number of the days for spawn-run was significantly more (17.3 days) in control, devoid of fungicides in comparison with treatments:

- Days taken for pin head formation

It is evident from the (Table 7) that there was statistically significant difference between the effects of fungicides on time taken for pin head formation by *P. sajor-caju*. Minimum time (6.7 days) was taken by Captan treatment followed by Carbendazim (6.9 days) and Bitertanol (7.0 days) which were found statistically identical with each other. It was further observed that with the increase in concentration of fungicides, the number of the days for pin head formation decreased. Maximum time (7.5 days) was taken in treatments which received Carbendazim as fungicide, (7.3 days) in Bitertanol and (7.0 days) at lowest dose of 0.025% concentration, which were statistically identical. It was followed by (7.1 days) in Carbendazim, (7.0 days) in Bitertanol, (6.8 days) by Captan at 0.05% concentration, which were statistically identical with each other. It was followed by both Carbendazim and Captan (6.3 days) and (6.8 days) in Bitertanol at highest concentration of 0.1%. It took (7.0 days) for Pin head formation in control:

- Percent increase in yield over control

It was revealed that there was a significant difference between the influences of the fungicides on the effect of total yield of *P. sajor-caju* (Table 8). Maximum increase in yield (36.9%) over control was recorded in treatment which received Carbendazim as fungicide. It was followed by Captan (16.1%). Minimum increase in yield (5.5%) over control was obtained in the treatment which

Table 8: Influence of Fungicides on yield of *Pleurotus sajor-caju* during one month cropping period

		*Yield (g) during 1 month			
Fungicide	Conc. (%)	Control value	Treatment	% increase in yield over control	Mean fungicides
Carbendazim	0.025	655.0	986.6	33.6	
	0.05	-	1050.0	37.6	36.9
	0.1	-	1083.3	39.5	
Bitertanol	0.025	-	661.6	1.0	
	0.05	-	708.3	7.5	5.5
	0.1	-	713.3	8.1	
Captan	0.025	-	731.6	10.4	
	0.05	-	800.0	18.1	16.1
	0.1	-	818.3	19.9	
S.Em±	9.73				
C.D @ 0.05%	16.2				

*Mean of three flushes

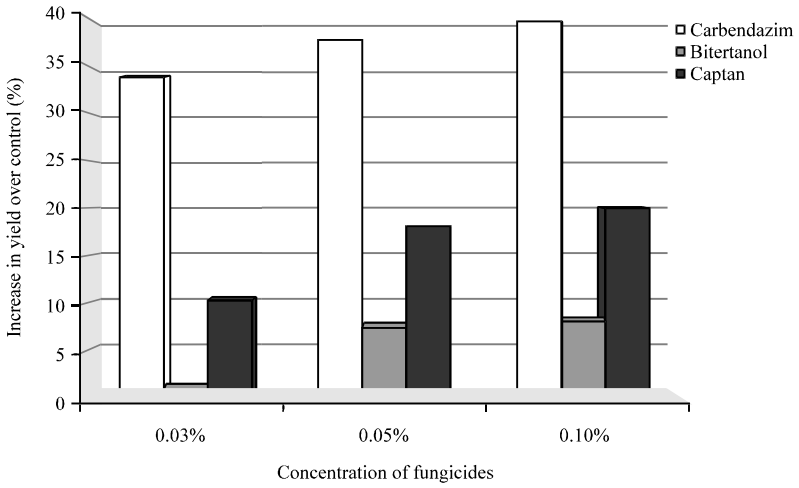

Fig. 1: Effect of fungicides on the yield of *Pleurotus sajor-caju*

received Bitertanol as fungicide. It was further observed that with the increase in concentration (0.025%, 0.05%, and 0.1%) of fungicides, the yield also increased. Maximum increase in yield (39.5%) over control was recorded in treatment which received Carbendazim as fungicide at a concentration of 0.1%, followed by Carbendazim (37.6%) at the concentration of 0.05% and Carbendazim (33.6%) at the lowest dose of 0.025%, which were found statistically identical with each other. It was followed by Captan (19.9%) at the concentration of 0.1%, followed by Captan (18.1%) at 0.05%, Captan (10.4%) at 0.025%, Bitertanol (7.5%) at 0.05%, Bitertanol (8.1%) at 0.1%, which were found statistically identical with each other. Minimum increase in yield (1.0%) over control was obtained in case of Bitertanol at the lowest dose of 0.025% (Fig. 1).

Percent disease incidence: It is evident from the Table 9 that all the three fungicides at all concentrations (0.025, 0.05, 0.1%) were significantly effective in reducing the Green mold of *P. sajor caju* as compared to the control. The minimum mean disease incidence was recorded in Carbendazim (9.3%) followed by Bitertanol (18.5%) and Captan (25.9%). The disease incidence

Table 9: Effect of Fungicides on disease incidence of *Trichoderma* sp. in *Pleurotus sajor-caju* (Dhinghri mushroom) cultivation

Fungicide	Conc (%)	*Disease incidence (%)	Mean fungicides
Carbendazim	0.025	22.2 (28.1)**	
	0.05	5.7 (13.8)	9.3 (14.9)
	0.1	0.25 (2.86)	
Bitertanol	0.025	27.8 (31.8)	
	0.05	16.7 (24.1)	18.5 (25.1)
	0.1	11.2 (19.5)	
Captan	0.025	44.4 (41.7)	
	0.05	22.2 (28.1)	25.9 (29.7)
	0.1	11.2 (19.5)	
Control (no fungicide)	-	88.8 (70.4)	
S.Em±	9.7		
C.D @ 0.05 %	16.1		

*Mean of six replicatious. **Figures in parenthesis are Transformed angular values

recorded in control was (88.8%). It was observed that, with the increase in concentration of fungicides (0.025, 0.05 and 0.1%), the disease incidence was reduced. The disease incidence of *T. harzianum* was not observed in case of bags treated with Carbendazim at the highest concentration of 0.1%. Minimum disease incidence (5.7%) was recorded in Carbendazim at the concentration of 0.05%, followed by Bitertanol and Captan (11.2%) at highest concentration of 0.1%. It was followed by Carbendazim (22.2%) at 0.025%, Captan (22.2%) at 0.05% and Bitertanol (27.8%) at 0.025% concentration. Maximum disease incidence (44.4%) was recorded in Captan at the lowest concentration of 0.025%.

DISCUSSION

In vitro evaluation of the fungicides against both *T. harzianum* and *P. sajor-caju* was carried out. Among the systemic fungicides tested by food poisoning technique, Carbendazim exhibited the maximum inhibition of pathogen (*T. harzianum*) followed by Bitertanol. The least inhibition of mycelial growth of pathogen was expressed by Hexaconazole. Systemic fungicides were tested against *P. sajor-caju* also and it was found that Carbendazim exhibit minimum efficacy against mushroom, indicating that *P. sajor-caju* is slightly resistant to this fungicide. Among the two non-systemic fungicides tested, Captan was found to be effective against *T. harzianum*. The minimum inhibition against *T. harzianum* was exhibited by Mancozeb and it showed the maximum inhibition of mushroom mycelium. Out of five fungitoxicants tested *in vitro*, Carbendazim, Bitertanol and Captan were further evaluated for *in vivo* trail in mushroom house. The selected fungicides expressed the minimum inhibition of mushroom mycelium but strong inhibition of pathogen as compared to the other fungicides. Carbendazim was found to be most inhibitory against *T. harzianum*, but it expressed least inhibitory potential against *P. sajor-caju*. This finding is in partial agreement of Thapa and Seth (1977) who reported that best control of *T. viride* was given by Carbendazim, without affecting the growth of mushrooms. Rai and Vijay (1992) reported that Carbendazim stimulated the mycehal growth of *Pleurotus sajor-caju* at low concentration but inhibited it at higher concentrations. Gupta *et al.* (1995) studied *in vitro* evaluation of different chemicals against *Trichoderma viride* isolated from button mushroom. They reported that Mancozeb and Carbendazim inhibited *Trichoderma viride* isolates *in vitro*. Domondon and Poppe (2000) reported that benomyl, prochloraz and imazalil inhibited the growth of *Trichoderma*. Kresoxim-methyl and azoxystrobin inhibited sporulation. Rinker and Alm (2008) evaluated four fungicides, benomyl, chlorothalonil, thiabendazole and thiophanate-methyl against ten isolates of

Trichoderma. Thiabendazole was the most effective, followed by benomyl and thiophanate methyl. Chlorothalonil was ineffective. Parvez *et al.* (2009) evaluated formalin, bavistin and combination of formalin and bavistin against mycoflora of oyster mushroom substrates. The combination of formalin and bavistin (500 mL+75 ppm) was found to be the best in inhibiting the radial growth of all the identified fungi.

In vivo evaluation of Carbendazim, Bitertanol and Captan against *T. harzianum* in mushroom house indicated that all the fungicides slightly reduced the time taken for complete spawn-run as compared to control. It took (15.9 days) for spawn-run in the treatments of Carbendazim and Captan. It was followed by Bitertanol (16.1 days). Time taken for pin formation was also slightly reduced as compared to control, with (6.7 days) in Captan followed by Carbendazim (6.9 days) and Bitertanol (7.0 days). It was further observed that with the increase in concentration, the number of days for pin formation decreased. It was observed that fungicides had a significant effect on yield as well. Carbendazim was found to be superior in expressing the maximum increase in yield (36.9%) over control, followed by Captan (16.1%). Bitertanol exhibited the minimum increase in yield (5.5%) over control. It was further observed that the percent increase in yield over control was increased with the increase in concentration of fungicides. Similarly fungicides were significantly effective in reducing the incidence of Green mold, as compared to control. Carbendazim recorded the minimum disease incidence (9.3%) followed by Bitertanol (18.5%) and Captan (25.9%). Disease incidence was reduced with the increase in concentration of fungicides. Carbendazim at the highest concentration of (0.1%) completely controlled the infection of Green mold. Kim (1975) reported, that in *in vivo* tests, Benomyl and BCM gave better control of mushroom pathogens; *Verticillium malthousei*, *Mycogone perniciosa* and *Trichoderma viride* than Maneb. Benomyl or BCM at 0.5 g a.i. m^{-2} applied 3 days after casing gave satisfactory disease control. Shandliya and Guleria (1984) reported that Carbendazim was the most effective fungicide for the treatment of Green mold which strongly supports the present study. Jhune *et al.* (1990) reported that maximum control of Green mold was obtained when 2 or 5 g m^{-2} of thiabendazole was applied to the substrate before pasteurization. Grogan *et al.* (1996) reported that Carbendazim applied to spawn grains gave the best control of *Trichoderma harzianum*, responsible for causing serious yield reductions of *Agaricus bisporus*. Bhatt and Singh (2000) reported that Carbendazim (0.075%) was most effective against *Trichoderma* spp. Lovkesh Beniwal and Pahil (2006) reported that growth inhibition of *Trichoderma viride* and the *Pleurotus* species increased with an increase in the concentration of different fungitoxicants. Maximum yield was obtained when carbendazim was used. This finding again supports the results, that increase in concentration of carbendazim enhances the yield of mushroom and reduces the disease incidence of pathogen.

CONCLUSION

The present study clearly indicates that Carbendazim was the most promising fungicide that was able to suppress the Green mold infection associated with the dhingri mushroom cultivation, both *in vitro* and *in vivo* treatments. Moreover this fungicide expressed the less inhibition of mushroom mycelium. It suggests that Carbendazim can be employed for controlling Green mold havoc in mushroom industry without having any adverse effect on mushroom crop.

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REFERENCES

Anderson, M.G., D.M. Beyer and P.J. Wuest, 2000. Using spawn strain resistance to manage *Trichoderma* green mold. Sci. Cultivation Edible fungi: Mushroom Sci., 2: 641-644.

Bayer, D.M., P.J. Wuest and J.J. Kremser, 2000. Evaluation of epidermilolgical factors and mushroom substrate characteristics influencing the occurrence and development of *Trichoderma* green mold. Sci. Cultivation Edible Fungi: Mushroom Sci., 15: 633-640.

Baysal, E., H. Peker, M.K. Yalinkilic and A. Temiz, 2003. Cultivation of oyster mushroom on waste paper with some added supplementary materials. Bioresour. Technol., 89: 95-97.

Bhardawaj, S.C., 1992. Effect of carbon, nitrogen and trace element sources on growth and sporulation of *Trichoderma viride*. Plant Dis. Res., 7: 79-82.

Bhatt, N. and R.R. Singh, 2000. Chemical and biological management of major fungal pathogens of *Agaricus bisporus* (Lange) Imbach. Int. Soc. Mushroom Sci., Vol. 15,

Danesh, Y.R., E.M. Goltapeh and H. Rohani, 2000. Identification of *Trichoderma* species causing green mold in button mushroom farms, distribution and their relative abundance. Proceedings of the 15th International Congress on the Science and Cultivation of Edible Fungi, May 15-19, 2000, Maastricht, Netherlands, pp: 653-659.

Domondon, D. and J. Poppe, 2000. Prevention of yield loss as influenced by *Trichoderma* in mushroom cultivation. Meded. Fac. Landbouwkd. Toegep. Biol. Wet. Univ. Gent., 65: 771-781.

El-Kattan, M.H. and S.A. El-Hadded, 1998. Regional Training Course on Mushroom Production. Mushroom Biology and Spawn Production, FOA/UNESCO/ARC/TMF, Cairo.

Grogan, H.M., R. Noble, R.H. Gaze and J.T. Fletcher, 1996. Control of *Trichoderma harzianum* a weed mould of mushroom cultivation. Pests Dis., 1: 337-342.

Gupta, A., P.N. Kerni and A. Gupta, 1995. *In vitro* evaluation of different chemicals against *T. viride* isolated from button mushroom. Res. Dev. Rep., 12: 44-47.

Hall, I.R., S.L. Stephenson, P.K. Buchanan, W. Yun and A.L.J. Cole, 2003. Edible and Poisonous Mushrooms of the World. Timber Press, Portland, Cambridge, ISBN: 0881925861, pp: 371.

Jaklitsch, W.M., G.J. Samuels, S.L. Dodd, B.S. Lu and I.S. Druzhinina, 2006. *Hypocrea rufa/Trichoderma viride*: A reassessment and description of five closely related species with and without warted conidia. Stud. Mycol., 55: 135-177.

Jhune, C.S., C.H. You, D.Y. Cha and G.P. Kim, 1990. Effects of thiabendazole on green mould, *Trichoderma* spp. during cultivation of oyster mushroom, *Pleurotus* spp. Korean J. Mycol., 18: 89-95.

Kim, G.P., 1975. The toxicity of benomyl and BCM to some edible fungi and pathogenic organisms causing major diseases of *Agaricus bisporus*. Plant Prot. Mycol., 17: 137-147.

Lovkesh Beniwal, J. and V.S. Pahil, 2006. Physiological and fungistatic interaction studies between *Pleurotus* spp. and *Trichoderma viride*. Crop Res. Hisar, 32: 499-503.

Nene, Y.L. and P.N. Thapliyal, 2000. Poisoned Food Technique. Fungicides in Plant Disease Control. 3rd Edn., Oxford and IBH Publishing Company, New Delhi, pp: 531-533.

Parvez, Z., M.K.A. Bhuiyan and M.S. Islam, 2009. *In vitro* control of associated mycoflora of oyster mushroom substrates by the application of fungicides. Bangladesh Res. Pub. J., 2: 737-741.

Rai, R.D. and B. Vijay, 1992. Effect of carbendazim on mycelial growth and extracellular enzymes of *Pleurotus sajor caju* and *Trichoderma viridae*. Indian Phytopathol., 45: 207-212.

Rinker, D.L. and G. Alm, 2008. Management of casing trichoderma using fungicides. Int. Soc. Mushroom Sci., Vol. 17.

Samuels, G.J., S.L. Dodd, W. Gams, L.A. Castleburry and O. Petrini, 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia, 94: 146-170.

Shandliya, T.R. and D.S. Guleria, 1984. Control of green mold (*Trichoderma viride*) during the cultivation of *Agaricus bitorquis*. Indian J. Plant Pathol., 2: 7-12.

Thapa, C.D. and P.K. Seth, 1977. Occurrence of green mold (*Trichoderma viride*) in mushroom beds causing *Trichoderma* spot or blotch of mushroom (*Agaricus bisporus* (Lange)). Indian J. Mushrooms, 3: 26-30.

Vincent, J.M., 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 159: 850-850.