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Chemical and biological management of major fungal pathogens *ofAgaricus bisporus* (Lange) Imbach

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ABSTRACT: Of the 223 samples examined *Hypomyces rosellus, Fusarium moniliformae, Mycogone perniciosa, Trichoderma* sp., *Vertcillium fangicola* and *Sepedonium chrysosporium* were found in most of the samples. The pathogens *M. perniciosa* and *H. rosellus* proved to be detrimental causing 95.65 to 100 percent and 61.0 to 72.06 percent loss in yield, respectively, when inoculated in casing. Significantly higher yields were recorded when Sporgon (0.075%) was added in casing against *M. perniciosa* and *V. fangicola.* Bavistin (0.075%) was most effective against *Trichoderma.* While Bavistin (0.025%)+formalin (0.20%) was effective in terms of yield against *H. rosellus, F. moniliformae* and *S. chrysosporium* when applied in casing. Five bacterial isolates were selected on the basis of their ineffectiveness to *A. bisporus* and designated as BI (Bacterial Isolates) I, II, III, IV and V. All of these isolates were effective against *V. fangicola* and *F. moniliformae* while BI 1,11 and V were found to be effective against *M. perniciosa* and *S. chrysosporium. Trichoderma* was controlled by BI III. The isolates selected against pathogens in in-vitro studies were further inoculated in the casing. It was found that BI I against *Sepedonium* and *Mycogone* and BI III against *Trichoderma* were effective in terms of yield.

1 INTRODUCTION

White button mushroom, *Agaricus bisporus* (Lange) Imbach cultivation in India is being done either in environment-controlled cropping rooms on pasteurized compost or under natural climatic conditions using unpasteurized (long method) or pasteurized compost. The unpasteurized compost harbors several parasitic and antagonistic fungi. Similarly, in absence of pasteurization facility, casing soil is treated with formaldehyde. The casing mixtures used in our country include Farm Yard Manure (FYM), soil and spent compost (1-2 years old) in absence of standard peat moss. The undertreated or unpasteurized casing invites several soil-borne pathogens *ofA. bisporus.* Among the various factors responsible for low production and productivity of *A. bisporus,* fungal diseases play a major role (Ramsbottom 1953, Kneebone & Merek 1961, Atkins 1971, Fletcher et al. 1986, Sohi 1992, Sharma 1995). The predominant fungal flora isolated from the compost and casing samples recently from India were *Trichoderma viride, Verticillium fangicola, Populospora byssina, Geotrichum* sp., *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp. and *Chaetomium* spp. (Sharma 1991, 1992, Sharma & Vijayl991, 1996).

The management of diseases of mushroom poses problems because, both the host and parasite are fungi. To overcome this problem there is a need to select those fungicides which have least effect on the growth of *A. bisporus.* It has been reported that the microbes present in casing have antagonistic potential against pathogenic fungi of *A. bisporus* (Trogoff & Ricardl976, Gandy et al 1980). Keeping above in view the present studies were undertaken on the chemical and biological management of pathogenic fungi of *A. bisporus.*

2 MATERIALS AND METHODS

2.1 *Laboratory screening of fungicides against A. bisporus and pathogens*

Eighteen different systemic and non-systemic fungicides were evaluated at four different concentrations for studying their inhibitory effects on the mycelial growth of *A. bisporus* by food poison technique of Falk (1907). Percent inhibition over control was calculated by applying the following formula proposed by Vincent (1947).

$$1 = \frac{C - TX100}{C} \quad (1)$$

Where I = Percent Inhibition; C = Growth in check; and T = Growth in treatment.

In the bioassay test, five fungicides, which did not adversely affect the growth of *A. bisporus* to the greater extent, were further evaluated in the laboratory screening to know their inhibitory effect against the fungi encountered viz. *Hypomyces rosellus, Fusarium moniliformae, Mycogone perniciosa, Trichoderma* sp., *Verticillium fungicola var.fungicola* and *Sepedonium chrysosporium.* The fungicides tested were Sporgon, Bavistin, Ridomil, Captaf and Bavistin+formalin at four different concentrations against each pathogen. All the treatments were replicated three times and incubated for 6-10 days at a temperature of 24±lOc.

2.2 *Chemical control of pathogens in mushroom beds*

Trial was laid out by incorporating two different doses of fungicides in casing. The prepared doses of inocula (0.50 & 0.75%) were mixed into the casing, then the casing was applied in the bags having compost of full mycelial spread of *Agaricus bisporus* strain X-13 into it. In both the trials three fungicidal sprays were given at an interval of 14 days. Two checks: one with inoculum and without fungicides and another without inoculum and fungicides,were kept for comparison in yield.

2.3 *Isolation of bacterial population from casing soil*

Bacteria from the casing soil were isolated using suitable selective media (King>s B and soil extract). Dilution plate technique (Waksman 1922) was employed for the isolation.

2.4 *Interaction between bacterial isolates and A. bisporus*

A method developed by Weller (1985) with slight modification was used. A 5 mm diameter disc from 21 days old culture of *A. bisporus* was placed at the center of the petriplates containing CDA medium and incubated at 22±1^C for 20 days. Thereafter, bacterial suspension containing lO^cfu ml'l was streaked 1.0 cm away from the growing^, *bisporus* colony. The plates were again incubated for another 7 days. Observations were recorded for growth enhancement / inhibition. Each treatment was replicated thrice along with check.

2.5 *Screening of bacterial isolates as antagonists*

The selected isolates were tested for their antagonistic potential against the pathogens of *A. bisporus.* Dual culture technique, as described by Morton & Stroube (1955), was used to test antagonism. Melted CDA/PDA was poured in 90 mm petriplates. A plug of test fungal growth measuring 5 mm in diameter was taken from the leading edge of the culture and placed in the petriplates. Sterilized filter paper disc (3 mm in diameter, impregnated in bacterial suspension, containing 10"cfu/ml of individual isolates) was placed at a distance of 5-10 mm from the periphery of inoculated plates. The plates were then incubated at 25±20c for 5-10 days and observations were recorded for the radial growth of the test fungus and size.

2.6 *Application of antagonists in mushroom beds*

Five selected bacterial isolates were mass multiplied in nutrient broth. Four hundred ml of each isolate containing 10^ cfu mH was thoroughly mixed with 4.00 kg casing mixture. Inocula of the pathogens prepared on wheat grains were mixed in the casing at 0.75 percent of casing by weight. Then the casing was applied 4 cm in thickness in the bags having compost of full mycelial spread into it. Two checks were kept for comparison. In one check only pathogen was mixed in casing and in other neither pathogen nor antagonists were used. All the treatments were replicated thrice.

3 RESULTS AND DISCUSSION

The pathogens were isolated from the contaminated parts of compost, casing and affected fruiting. On inoculation of pathogens in casing, *Mycogone perniciosa* caused complete crop failure.

3.1 *Laboratory screening of fungicides against A. bisporus and pathogens*

In order to screen out the fungicides that could be safely used to control the pathogens, eighteen fungicides were tested at four different concentrations. It was found that Captaf, Bavistin, Sporgon (25 ppm), Ridomil (200 ppm) did not inhibit the growth of *A. bisporus* upto greater extent. They were selected for further studies with regard to their effect on pathogens. It has been reported earlier that Benomyl and MBC did not exert any adverse effect the growth of mushroom mycelium even at as high concentration as 500 ppm (Jandaik et al. 1978). It has also been reported that sprays of carbendazim, thiophanate methyl *in- vitro* and *in- vivo* at 50 and 500 ppm concentrations had no adverse effect on the mushroom mycelium. It was also observed that the spawn run in treated beds was found to be faster, more whitish and luxuriant as compared with the growth in control, the fungicides were found to be slightly inhibitory to the mycelial growth at 1000 ppm (Thapa & Raina 1989).

Five fungicides (Bavistin, Sporgon, Captaf, Ridomil and formalin) were selected on the basis of their ineffectiveness against *A. bisporus* and a laboratory screening of these selected fungicides at different concentrations was undertaken against the pathogens. Bavistin and Sporgon at 10 ppm concentration caused 100 percent inhibition of the growth of//! *rosellus* and *V. fungicola.* While the growth of all other pathogens was inhibited at 20 ppm concentration. Captaf and Ridomil at 30 and 300 ppm concentrations respectively, checked the growth of all the pathogens completely. A combination of Bavistin and formalin at a concentration of 12+12 ppm checked 100 percent growth of all the pathogens except *Trichoderma,* which was inhibited by a concentration of 15+1 ppm.

3.2 *Chemical control of pathogens in mushroom beds*

The results obtained under *in-vitro* trials revealed that Bavistin, Sporgon, Ridomil, Captaf and formalin were comparatively safer in the sense that they did not adversely effect the growth of *A. bisporus* to any significant extent. These fungicides were sprayed in the mushroom beds at different intervals. The results so obtained are presented in Table la and Ib. It is clear from the data presented in Table la and Ib that the performance of fungicides applied was affected by inoculum load. Significant decrease in yield was obtained with inoculum load. The yield obtained after using the chemicals against pathogens in mushroom beds is as follows:
M. perniciosa'. Significant increase in yield over check I was obtained by the application of all the chemicals. The yield obtained after the treatment by Sporgon at 0.075 percent concentration was at par with the yield of check II at 0.50 percent inoculum load and this was at par with the yield obtained at 0.75 percent inoculum load. It was found to be best in control *of M. perniciosa* followed by Bavistin and Bavistin + formalin. Effective control of M *perniciosa* using Bavistin, formaldehyde and prochloraz (Sporgon) has earlier been reported (Baker 1977, Stroller 1981).

V. fungicola: Sporgon at 0.05 and 0.075 percent concentration was at par in terms of yield with check II at both the inoculum loads. It was followed by Bavistin and Bavistin + formalin. Control of *V. fungicola* in mushroom beds was achieved by spraying with carbendazim at 100, 150 and 200g / lOOm^2, in 100-500 lit. water, immediately after casing (Geijin, 1977) and prochloraz manganese at 60g /100 m2 within 7 days of casing and subsequently at 2 weeks interval (Fletcher & Hims, 1981). However, excellent control of the pathogen through Sporgon as a spray was claimed by Zaayen (1983) and Russel (1984).

H. rosellus: The yield obtained after spray of Sporgon (0.075%) and Bavistin + formalin (0.025+0.20%) was at par with check II at 0.50 percent inoculum load. Yields recorded with Bavistin and Bavistin+formalin were at par at both the concentrations and inoculum loads. It has been reported that application of prochloraz at 1.5-3g / m^2 on 9^{tn} day from casing controlled the pathogen with no marked phytotoxity (Zaayen 1982, Fletcher 1983, Fletcher et al. 1983).

Trichoderma sp.: The best control of the fungus was obtained using Bavistin (0.075 %) at both inoculum loads (0.05 and 0.75 %) and the yield obtained was at par with check II. The effectivity of Bavistin and other systemic fungicides against *Trichoderma* has been reported by various workers (Merck 1961, Thapa & Seth 1977, Shandilya & Guleria 1984, Tiwari & Singh 1991).

S. chrysosporium: Bavistin and Bavistin+formalin proved to be the best treatments at both the concentrations used and were followed by Ridomil and Sporgon. The inhibitory effect of formalin to the growth of pathogen was reported by Sharma et al. (1997).

F. moniliformae: Significantly higher yield was obtained from the treatment Bavistin+formalin (0.015+0.10 and 0.025+0.20 %) at both the inoculum loads. The yield obtained from 0.025+0.20 percent concentration was at par with check II. It was followed by Bavistin. Effectiveness of Bavistin against *Fusarium* in mushroom beds has earlier been reported (Rakwal 1992).

3.3 *Antagonistic effects of Bacterial Isolates against pathogens (in-vitro)*

Out of sixteen bacteria isolated from casing, five were selected on the basis of their ineffectiveness to *A. bisporus* in *in-vitro* tests in dual cultures. The five selected isolates were tested against pathogens. Out of these all the isolates were effective against *V. fungicola* and *F. moniliformae.* These isolates reduced the growth of these pathogens by about 40-60 percent. BI I, II, and V were effective against the pathogen *M. perniciosa* and 50-60 percent reduction in growth was recorded. *Trichoderma* sp. itself is a strong antagonist against soil-borne pathogens of plants (Weller & Cook 1986) was effectively reduced by isolate I. The remaining isolates were ineffective and run- over by *Trichoderma* sp. itself. Radial growth of *Sepedonium* sp. was effectively reduced by isolates I, II and V.

3.4 *Interaction of isolates and pathogens in the beds*

In order to know the effectivity of bacterial isolates against different pathogens in mushroom bed, the bacterial strains found effective against the particular pathogen *in-vitro* were selected to test them for the yield of *A. bisporus.* The data obtained are presented in Table 2. It is clear from the Table that all the bacterial isolates significantly enhanced the yield *of F. moniliformae* and *V. fungicola* compared to check I (with pathogen and without bacteria). However, BI V was found to be the best against *F. moniliformae* and *V. fungicola* in terms of yield. BI I was most effective against *F. moniliformae* and *V. fungicola* in terms of mushroom yield. BI I was most effective against *M. perniciosa* and significantly higher yields were recorded by BI I and V in comparison to check I. *Trichoderma* sp. was very well controlled by BI III resulting 30.45 percent higher yields. Amongst the three isolates (BI I, BI II and BI V) tried to control *S. chrysosporium* in mushroom beds, the BI I proved to be better in terms of higher yields of mushroom. It has earlier been reported that unidentified microorganisms in the casing soil suppressed the development *of M. perniciosa* (Han et al. 1974) whereas amongst more than 1000 bacterial isolates from rhizosphere and rhizoplanes of various plants and mushrooms, seven isolates inhibited the microconidia *of F. oxysporum* (Isaka & Okamotol989). Of 343 bacterial isolates only 12 showed antibiotic activity on casing soil with *M. perniciosa* (Jhune et al. 1990). Some

of the siderophore producing bacteria have been found effective as biocontrol agents against major parasitic fungi (Singh 1998).

4 CONCLUSION

The incidence of pathogens was higher in the samples collected from the seasonal mushroom farms. *Mycogone perniciosa* caused 100 percent loss in yield when inoculated in casing. Significantly higher yields were recorded when Sporgon (0.075%) was sprayed in casing against *Mycogone perniciosa* and *Verticillium fungicola.* Bavistin (0.075%) was most effective against *Trichoderma.* While Bavistin (0.025%) + formalin (0.20%) was effective in terms of yield

Table la. Effect of different fungicides on the yield of *A. bisporus* at 0.50 and 0.75% inoculum load of pathogens

Fungicide	Dose	Yield in kg per quintal compost Pathogens and their inoculum loads (%)					
		M. perniciosa		V. fungicola		H. rosellus	
		0.50	0.75	0.50	0.75	0.50	0.75
Sporgon	0.050	14.75	13.95	15.50	14.95	14.25	13.75
	0.075	16.50	15.75	16.75	16.25	15.38	14.00
Bavistin	0.050	13.90	11.90	12.75	12.25	13.75	12.95
	0.075	14.30	12.75	15.36	13.75	14.70	13.25
Captaf	0.050	10.25	8.25	12.75	11.95	10.00	9.25
	0.075	10.75	9.00	13.05	12.25	10.88	10.75
Ridomil	0.10	12.50	10.75	10.25	9.75	8.25	8.35
	0.15	12.75	10.95	11.92	9.00	8.70	8.95
Bavistin+ formalin	0.015+ 0.10	12.00	11.95	14.25	13.75	14.75	14.25
	0.025+ 0.20	13.90	12.75	15.30	12.95	15.20	14.75
Check I	-	0.00	0.00	7.25	6.33	7.75	6.01
Check II	-	16.85	16.85	16.85	16.85	16.85	16.85
CD at 5%			0.787		1.97		1.90

Check I: With inoculum and without fungicide
Check II : Without inoculum and fungicide

Table Ib. Effect of different fungicides on the yield of *A. bisporus* at 0.50 and 0.75 % inoculum load of pathogens

Fungicide	Dose (%)	Yield in kg per quintal compost Pathogens and their inoculum loads (%)					
		Trichoderma sp.		*Sepedonium* sp.		*Fusarium* sp.	
		0.50	0.75	0.50	0.75	0.50	0.75
Sporgon	0.050	11.90	10.75	12.25	11.95	10.75	9.75
	0.075	12.78	11.25	12.72	13.75	12.58	10.00
Bavistin	0.050	14.70	14.00	14.25	13.25	12.75	11.25
	0.075	15.58	15.25	15.05	13.75	14.17	13.75
Captaf	0.050	12.75	11.35	10.90	9.25	9.00	9.25
	0.075	13.45	12.00	11.91	9.75	11.34	10.75
Ridomil	0.10	13.00	12.75	12.75	10.95	9.70	10.25
	0.15	13.06	13.25	13.05	11.25	12.34	10.75
Bavistin+	0.015	11.70	10.90	15.00	14.75	15.00	15.25
formalin	0.10	12.41	9.75	15.20	15.25	16.25	15.75
Check I	-	9.57	8.95	9.33	8.00	9.16	8.75
Check II	-	16.85	16.85	16.85	16.85	16.85	16.85
CD at 5%			1.85		1.35		1.53

Check I : With inoculum and without fungicide
Check II : Without inoculum and fungicide

Table 2. Effect of bacterial isolates on the yield of *A. bisporus*

Bacterial isolates	Av. yield of *A. bisporus* (kg / qtl compost)				
	Fusarium sp.	*Mycogone* sp.	*Trichoderma* sp.	*Verticillium* sp.	*Sepedonium* sp.
BIT	10.25	7.50		10.08	12.58
Bill	9.00	6.17		9.25	9.50
BIIII	10.92	-	12.58	10.17	-
BIIV	9.58			8.59	-
BIV	11.84	5.08		10.58	10.25
Check I	7.44	0.00	8.75	6.88	7.65
Check II	16.50	16.50	16.50	16.50	16.50
CD at 5%	0.902	0.870	1.042	1.106	1.12

Check I: With inoculum and without bacteria
Check II: Without inoculum and bacteria

against *Hypomyces rosellus, Fusarium moniliformae* and *Sepedonium chrysosporium* when applied in casing. All five bacterial isolates were effective against *V. fungicola* and *F. moniliformae.* While BI I, II and V were found effective against *M. perniciosa* and *S. chrysosporium. Trichoderma* was controlled by BI III. In the mushroom beds BI V against *Fusarium* and *Verticillium,* BI I against *Sepedonium* and *Mycogone* and BI III against *Trichoderma* were effective in terms of yield.

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