INTEGRATED APPROACHES FOR THE MANAGEMENT OF *MYCOGONE PERNICIOSA* CAUSING WET BUBBLE DISEASE

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ABSTRACT

Wet bubble in white button mushroom (*Agaricus bisporus*) incited by *Mycogone perniciosa* Magn. has been reported as one of the serious diseases from almost all the major mushroom growing countries of the world. Bubble or mole, first described from Paris in 1888, was reported in India for the first time in 1978. As the pathogen inflicts serious damage to the crop, various attempts have been made to manage the disease through various means. Interaction between *A. bisporus* and *M. perniciosa* studies conducted in dual, half plate and paired cultures. The average growth of *A. bisporus* and *M. perniciosa* in either dual culture was 16.13 and 28.86 mm, respectively. Growth of *A. bisporus* remain unaffected (16.02 mm) and *Mycogone* enhanced to 36.91mm (21.80% increase) when both grown in dual culture. Pre spawning of casing soil 5-20 days prior to pasteurization resulted in reduced incidence of wet bubble disease. Thermal death point of *Mycogone* observed to be at 44-45 °C. Moisture contents of casing soil less than 60% at the time of pasteurization favour the survival of *M. perniciosa*. *Mycogone* failed to survive in casing soil having moisture contents 60% or above at 60 °C or above temperature. Two bacterial isolates B-9 (*Bacillus*) and B-18 (*Alcaligenes*) proved to be very promising bio control agents for the management of wet bubble disease both under laboratory and mushroom house conditions. Out of five fungicides and two other chemicals tried, carbendazim proved most effective in managing wet bubble disease among all the fungicides/ chemicals tested.

Keywords: *Mycogone perniciosa*, *Agaricus bisporus*, wet bubble, fungicides, control

INTRODUCTION

Wet bubble in white button mushroom incited by *Mycogone perniciosa* Magn. has been reported as one of the serious diseases from almost all the major mushroom growing countries of the world. Bubble or mole (*M. perniciosa*), first described from Paris in 1888, is stated to be responsible for the heaviest losses in mushroom beds in France, England and United States [1]. The disease has also been reported to assume serious proportion in other major mushroom growing countries of the world such as United Kingdom, Netherlands, USA, China, Taiwan, South Africa, Brazil, Hungary, Australia and Poland from time to time. In India, this disease was reported for the first time in 1978 from mushroom farms in Jammu and Kashmir [2]. Later this disease was reported from Himachal Pradesh, Haryana and Maharashtra. Symptoms of wet bubble at different stages of mushroom development have been described by many workers. It has been estimated [3] that qualitative and quantitative losses caused by wet bubble and dry bubble in 70s in Pennsylvania (USA) were 2.2 million lbs and 19.7 million lbs, respectively. Natural incidence of wet bubble disease of button mushroom ranged from 1 to 100 % in North India [4].

MATERIALS AND METHODS

Interaction studies

Mycogone and *A. bisporus* were grown together in paired culture on PDA, mushroom extract agar (MEA) and mushroom extract dextrose agar (MDA) in 90 mm Petri plates to view their interaction *in vitro*. Five mm diameter plugs from the edge of actively growing *A.bisporus* and *M. perniciosa* were inoculated opposite of each other in 90 mm Petri plates. Control consisted of plates of either *A.bisporus* or *M. perniciosa* at both sides of the Petri plates.

Five mm diameter plugs from actively growing *A.bisporus* and *M. perniciosa* were also inoculated in the centre of the bottom of 90 mm, MA Petri plates, to make dual culture. After 48 h the bottom of the Petri plates containing plugs of *A. bisporus* were joined with bottom from Petri plates containing plugs of *M. perniciosa* by taping the respective bottom plates together with insulation tapes. In some cases plates containing *M. perniciosa* plugs were the bottoms of the dual culture and others *A.bisporus* plates were the bottom plates. Control consisted of taped cultures with both top and bottom plates of the dual culture containing *M. perniciosa* or *A. bisporus*, respectively.

In third set of experiment both *M. perniciosa* and *A. bisporus* were grown together on MEA, MDA and PDA, in 90 mm half Petri plates to examine the effect of volatiles on fungal growth and interaction *in vitro.* Five mm diameter plugs from the edge of actively growing *A. bisporus* and *M. perniciosa* were inoculated in separate compartments of 90 mm half Petri plates. Control consisted of half plates of either *M. perniciosa* or *A. bisporus* in both compartments of the plate.

Thermal death point of mycelium under dry conditions

Each culture was grown in Petri plates on malt extract medium. A bit of 4 mm actively growing mycelium of *Mycogone perniciosa* was transferred to empty 200 µl PCR tubes (dry condition) as well as PCR tubes containing 100µl sterile water (wet condition) in such a way so that the culture bit got submerged in the water under aseptic conditions in laminar flow. The culture containing PCR tubes were loaded in the gradient PCR (ASTEC) machine where different temperature starting from 38.0 to 47.9 °C in the gap 0.9 °C was maintained for 10 minutes. After giving the heat shock treatment the culture bits were transferred to fresh slants of malt extract agar (MEA) medium under aseptic conditions and incubated at 25 °C to observe the viability of the culture.

Management studies

Compost prepared by short method was used to raise button mushroom crop. Strain (U-3) was used. Ten kg ready compost was filled in each polythene bag in a way to give the surface area of 0.1 m² and thorough spawning was done. The bags were incubated in cropping rooms at 25±1 °C. After the colonization of compost by the mushroom mycelium after 18 days, 2 cm layer of casing mixture (FYM + spent compost, 50:50 w/w) was applied. The temperature was gradually reduced from 25 °C to 16 °C in the next 8-10 days. Watering was done daily and aeration was provided as per the requirement of the crop. The fruit bodies were harvested and data on yield was recorded.

Prespawning studies

Prespawning of casing mixture was done with *A.bisporus* spawn @ 0.5%. and incubated at 25±1 °C for 20, 15, 10 and 5 days. Control consisted of unspawned casing mixture. After incubation the casing mixture was pasteurized at 65-67 °C for 8h and used as casing material for the experiment.

In one set of experiment various moisture levels viz., 50, 55, 60, 65 and 67% of the casing mixture were maintained. Casing mixture was inoculated with 0.1% of *M. perniciosa* wheat grain based inoculum.Three sets of casing mixture with different moisture levels were maintained. After incubation at 25±1 °C for 5 days, one set was pasteurized at 60-65 °C for 8h, another at 65-67 °C for 8h and third at 67-70 °C for 6h.

Management studies

Two bacterial spp (*Bacillus* sp and *Alcaligenes* sp.) and an actinomycete, *Streptomyces* sp isolated from casing mixture were multiplied on nutrient broth at 28 °C for 24h. One ml bacterial culture containing 10^6-10^7cfu/ml diluted in 50 ml of water was sprayed on each bag after two days of casing application.

Different fungicides viz., Prochloraz manganese (1-N-propyl-N-2(2,4,6- trichlorophenoxy) ethyl) carbomoyl imidazole + Manganese), Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), mancozeb (Manganese ethylene bisdithiocarbamte), formalin (formaldehyde),carbendazim (Methyl-2 benzimidazolecarbamate), K_2HPO_4 and $CaCl_2$ were sprayed at three different concentration concentrations one day after casing. A fungicidal solution of 50 ml / 0.1 m² was sprayed at each bag.

RESULTS AND DISCUSSION

Interaction studies

In Dual culture: The average growth of *A. bisporus* and *M. perniciosa* in either dual culture was 16.13 and 28.86 mm, respectively (Table 1a&b). The growth of *A. bisporus* remain unaffected (16.02 mm) and *Mycogone* enhanced to 36.91mm (21.80% increase) when both grown in dual culture.

Table 1a. Interaction of *Mycogone* and *Agaricus* in paired and half plate culture

Paired culture	**Growth of mycelium in petriplates (in nm) in pairs**					
	***Agaricus* Control (mm)**		***Mycogone* Control (mm)**		**Interaction(mm)**	
Medium	**A with A**		**M with M**		**A with M**	
MA	10.75	10.85	21.2	21.5	11.07	22.15
MDA	9.62	10.1	23.7	24.2	11.6	24.07
PDA	10.45	10.47	23.72	23.95	12.02	24.02
Mean	**10.37**		**23.84**		**11.56**	**23.41**
Half Plate Culture						
MA	10.46	11	29.23	29.76	10.9	30.06
MDA	10.2	10.46	28.53	29.4	9.9	28.8
PDA	9.8	10.06	20.96	20.83	9.33	19.26
Mean	10.33		26.45		10.04	26.04

Table 1b: Interaction of *Mycogone* and *Agaricus* in dual plate technique

	***Agaricus* Control (mm)**		***Mycogone* Control (mm)**		**Interaction(mm)**			
Medium	**A**	**A**	**M**	**M**	**A (bottom)**	**A (Top)**	**M (Top)**	**M (Bottom)**
MA	15.96	16.30	28.70	29.03	15.88	16.16	36.63	37.20
Mean	16.13		28.86		16.02		39.91	
CD(0.05)	0.7		0.8		0.8		1.2	

A = *Agaricus*; M = *Mycogone*

Interaction between *Mycogone* and *A. bisporus* in paired culture on three media (MEA, MDA and PDA) was studied. On all the three media *A. bisporus* grew more (111.56 mm, mean) in paired culture as compared to its alone culture (10.37 mm, mean) Whereas the growth of *M. perniciosa* was at par in paired (23.84 mm, mean) and alone culture (23.41 mm, mean) in all the three media.

In half Petridish culture the mean growth of *A. bisporus* was 10.33 mm in alone culture against its 10.04 mm growth in paired culture. Growth of *M. perniciosa* was 26.45 mm in alone culture against its 26.04 mm growth in paired half plate culture.

Thermal death point

Thermal death point of mycelium of *Mycogone perniciosa* was observed to be at 45.2 °C under dry conditions whereas mycelium failed to recover at 44.3 °C under wet conditions clearly indicating that the thermal death point of *M. perniciosa* is between 44-45 °C (Table 2).

Table 2. Thermal death point of mycelium of *Mycogone*

Temperature of Incubation	Under Dry conditions (10 min)	Under wet conditions (10 min)
38.0	+	+
38.9	+	+
39.8	+	+
40.7	+	+
41.6	+	+
42.5	+	+
43.4	+	+
44.3	+	-
45.2	-	-
46.1	-	-
47.0	-	-
47.9	-	-

Under dry conditions thermal death point of *M. perniciosa* was 44.3 °C and under wet conditions the thermal death point was 43.4 °C. Since the culture consisted mainly of mycelium and condia, so the thermal death point observed in the present case may not hold true for cultures having chlmydospoes. In that case thermal death point might be on higher side. Wuest and Moore [5] suggested that aerated steam at 54.4 °C for 15 minutes can eliminate *M. perniciosa* from casing soil.

Effect of pre spawning of casing soil on the survival of *Mycogone perniciosa*

Interaction studies between *A. bisporus* and *M. perniciosa* indicated that *A. bisporus* enhances the growth of *M. perniciosa*. Since the pathogen attack host only in the casing layer it might be possible that the volatiles released by *A.bisporus* stimulate germination/ growth of *Mycogone* spores/ mycelia in the casing layer. Pre spawning experiment aimed to study such effect revealed that pre spawning of casing soil 5-20 days prior to pasteurization resulted in reduced incidence of wet

Table 3. Effect of pre spawning on *Mycogone* inhibition

Duration (Days)	Treatments	Yield
20 Days prior	Spawned +Inoculated	13.06
	Inoculated (Only *Mycogone*)	11.08
	Unspawned + Uninoculated	13.98
15 Days prior	Spawned +Inoculated	12.56
	Inoculated (Only *Mycogone*)	10.14
	Unspawned + Uninoculated	10.80
10 Days prior	Spawned +Inoculated	13.74
	Inoculated (Only *Mycogone*)	10.41
	Unspawned + Uninoculated	12.53
5 Days prior	Spawned +Inoculated	11.65
	Inoculated (Only *Mycogone*)	8.46
	Unspawned + Uninoculated	13.57
CD (0.05)		0.78

bubble disease (Table 3). Pre spawning of casing soil with *A. bisporus* stimulated the germination of *M. perniciosa* inoculmn in the casing soil and after pasteurization resulted in death of active inoculmn i.e. mycelial form.

Effect of casing soil moisture contents and pasteurization temperature on the survival of *M. perciosa*

Wet conditions leads to coagulation of microbial proteins at lower temperature. With this intention the present experiment concerning effect of different moisture levels and temperature on the survival of *M. perniciosa* was laid which reveals that lower moisture contents of casing soil at the time of pasteurization favours the survival of *M. perniciosa. Mycogone* failed to survive in casing soil having moisture contents 60% or above at 60 °C or above temperatures (Table 4). Results explicitly indicated the role of moisture contents in the survival of inocula in relation to moisture contents in the casing soil at the time of pasteurization.

Table 4. Effect of casing soil moisture contents and pasteurization temperature on the survival of *Mycogone perniciosa*

Moisture (%)	Pasteurization temp. (°C) and duration	Uninoculated	Inoculated
50	60-65 /8h	11.39	6.82
55		12.97	7.66
60		10.52	8.41
65		12.81	10.17
67		12.60	13.82
70		13.69	13.40
50	65-67/8h	11.38	6.70
55		9.10	8.45
60		12.19	8.74
65		12.63	11.70
67		12.99	12.09
70		13.10	12.80
50	67-70/6h	12.55	7.42
55		10.70	7.26
60		12.94	9.30
65		11.93	10.80
67		12.60	12.20
70		12.71	12.06
CD (0.5)		0.38	0.43

Mycogone management through micro-organisms

Spraying of two bacterial spp (*Bacillus* sp and *Alcaligenes* sp) and an actinomycete *Streptomyces* sp @one ml bacterial culture containing 10^6-10^7cfu/ml diluted in 50ml of water on each bag (0.1m^2area) after two days of casing application revealed that *Bacillus* and *Alcaligenes* sp are capable of controlling wet bubble disease. In control-II which was inoculated with *Mycogone* and was not sprayed with any microbial solution yielded 6.75 kg/dt compost whereas after spraying with *Bacillus s*p and *Alcaligenes* sp the yield increased to 14.12 and 17.75 kg/dt compost, respectively (Table 5).

Management of wet bubble through chemicals

Evaluation of different fungicides (carbendazim, chlorothalonil, sporgon, Indofil Z-78, Indofil M-45) against *Mycogone perniciosa* and *A. bisporus* revealed that carbendazim and sporgon gave 100 per cent inhibition of *M. perniciosa* at all the concentrations tried 20-200 ppm whereas it resulted just 17 and 10% inhibition of *A. bisporus*, respectively (Table 6). Chlorothalonil also resulted in 100 per cent inhibition at 100 and 200ppm of *M. perniciosa* and it gave 31 % inhibition of *A. bisporus* at this concentration. Both Indofil Z-78, Indofil M-45 were more toxic to *A.bisporus* at 500ppm as compared to *M. perniciosa*.

Table 5. *Mycogone* management through micro-organisms

	Treatments	Inoculated / uninoculated with *Mycogone*	Yield kg dt^{-1}	No. of No. of Fruit bodies of *Agaricus*	Sclerodermoid	
					Small	Large
1.	B9	Inoculated	14.12	1307	74	0
2.	B9	uninoculated	16.87	1075	0	0
3.	B18	Inoculated	17.75	1623	43	13
4.	B18	uninoculated	18.62	1455	0	0
5.	B20	Inoculated	9.30	604	32	17
6.	B20	uninoculated	14.52	1355	0	0
7.	Control-I (No *Mycogone)*	Uninoculated	18.77	1375	0	0
8.	Control-II (with *Mycogone*)	Inoculated	6.75	507	77	32
CD (0.05)			0.87			

B9=*Bacillus* sp; B18=*Alcaligenes;*B20= *Streptomyces*

Table 6. *In vitro* efficacy of some fungicides against *Mycogone perniciosa*

Fungicide	Concentration	Growth of *Agaricus bisporus* (mm)	Growth of of *Mycogone perniciosa* (mm)
Carbendazim	20	24 (17)	0 (100)
	50	22 (24)	0 (100)
	100	18 (37)	0 (100)
	200	18 (37)	0 (100)
Chlorothalonil	20	22 (24)	11 (82)
	50	22 (24)	10 (84)
	100	20 (31)	0 (100)
	200	20 (31)	0 (100)
Sporgon	20	26 (10)	0 (100)
	50	26 (10)	0 (100)
	100	9 (69)	0 (100)
	200	9 (69)	0 (100)
Indofil Z-78	50	29 (0)	32 (50)
	100	26 (10)	26 (59)
	200	8 (72)	16 (75)
	500	0 (100)	12 (81)
Indofil M-45	50	22 (24)	26 (49)
	100	16 (44)	22 (65)
	200	10 (65)	16 (75)
	500	0 (100)	10 (84)
Control		29	63

Figures in parentheses represent % inhibition

Jhune *et al*. [6] screened 12 isolates of bacteria and 71 isolates of actinomyectes isolated from mushroom compost and casing mixture and observed AJ-117, AJ-136 and AJ-139 as promising bioagents. Though, almost negligible attempts

have been made to control *M. perniciosa* through botanicals but the inhibition of fungal growth by plant extracts is not uncommon and has been reported earlier by a number of workers [7,8]. Gandy [9] made an interesting observation that *Acremonium strictum* produces a heat stable antibiotic compound possibly a cephalosporin, which is inhibitory to *M. perniciosa* but no attempts have been made to explore this approach as both fungi are pathogenic to mushrooms. Bhatt *et al.* [10] claimed good success with a siderophore producing volatile (C116) of fluroscent pseudomonas against *M. perniciosa.*

Out of five fungicides (carbendazim, mancozeb, formalin, chlorothalonil, sporgon) and two other chemicals (K_2HPO_4, $CaCl_2$) tried, carbendazim proved most effective in managing wet bubble disease among all the fungicides/ chemical tested at two concentration (Table 7). Sporgon also proved equally good at all the three concentrations tried mancozeb was as effective as the two other chemicals K_2HPO_4 and $CaCl_2$. In control the yield was as low as 2.03 kg per quintal of compost.

Table 7. Management of wet bubble through chemicals

Fungicide	**Concentration**	**Yield ($kgdt^{-1}$)**	
		Inoculated	**Uninoculated**
Carbendazim	0.1	10.87	12.35
	0.15	10.75	11.04
	0.2	8.91	10.59
Mancozeb	0.1	8.70	7.35
	0.15	6.33	6.85
	0.2	6.52	5.90
Formalin	0.1	7.63	10.24
	0.15	9.70	9.39
	0.2	9.39	9.93
Chlorothalonil	0.1	9.82	10.77
	0.15	9.03	9.63
	0.2	9.92	11.80
Sporgon	0.1	10.54	11.95
	0.15	10.37	11.36
	0.2	10.90	11.89
K_2HPO_4	0.1	8.36	12.60
	0.15	5.37	12.62
	0.2	6.39	12.60
$Cacl_2$	0.1	8.24	12.62
	0.15	7.54	11.84
	0.2	7.43	10.11
Control		2.03	11.66
	CD (0.05)	0.93	1.01

Bubble diseases infect the reproductive mycelia and are unable to infect the vegetative mycelia. The infection takes place through specific and a specific interaction between the surface molecules of both the fungi. Fungal hydrophobins self assemble at hydrophobic and hydrophilic interfaces into surface active amphipathic membranes. The pathogen and host attach to each other by hydrobic interactions between the hydrobhobin layers. After the initial attachment *Mycogone* can grow inter and intra cellular in *A. bisporus* fruiting body hyphae. Since bubble disease infect the reproductive hyphae only, it is logically appropriate that effective concentration of fungicide should available at that point of time in the casing to inhibit the infection. *M. perniciosa* appeared to be quite sensitive against sporgon, carbendazim and chlorothalonil. All these fungicides resulted in effective control of the pathogen. Application of carbendazim, benomyl, chlorothalonil, TBZ, prochloraz manganese complex (Sportak 50 WP) into casing mixture have been reported very effective for the management of wet bubble by several workers [6,10-18].

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