



Research Article

EVALUATION OF BACTERIAL ANTAGONISTS AGAINST *Mycogone perniciosa* CAUSING WET BUBBLE DISEASE OF WHITE BUTTON MUSHROOM (*Agaricus bisporus*) IN KASHMIR

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Abstract- Wet Bubble is a devastating disease in the crop production of mushrooms. Wet bubble disease causes extensive damage by bringing soft rot or decay of whole fruiting body. If not controlled well in time, the pathogen causes havoc damaging the entire crop. It causes serious crop losses in mushroom farms in India. The aim of the present study was to check the in vitro and in vivo efficacy of antagonists against Wet Bubble (*Mycogone perniciosa*) associated with the cultivation of *Agaricus bisporus*. Among bacterial antagonists evaluated in vitro, all the test antagonists, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Azotobacter* sp., exhibited stimulatory effects of varying degrees on *A. bisporus* mycelium. *Pseudomonas fluorescens*-103, *Bacillus subtilis*-116 and *Azotobacter* sp.-106 gave the highest mycelial growth inhibition of 100.0, 98.88 and 98.51 percent of the pathogen fungus, respectively. The incorporation of bacterial antagonists such as *P. fluorescens*, *B. subtilis* or *Azotobacter* sp. at different concentrations in pathogen-infested casing also yielded appreciable disease control with corresponding yield gains.

Keywords- Soft rot, fruiting body, antagonists, inhibition, pathogen

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Introduction

Mushroom production is one of the commercially important microbial technologies for large scale recycling of agro wastes. Mushroom industry has opened up new vistas of export earnings in the present scenario of economy. Present world production of Mushrooms is around 16 million tonnes. Among commercially cultivated mushrooms, *Agaricus bisporus* popularly known as white button mushroom or European mushroom is extensively cultivated throughout the world. Its large scale production is centered in Europe (mainly western part), North America (USA, Canada) and Southeast Asia (China, Korea, Indonesia, Taiwan and India). White button mushroom Production accounts for 35 to 45 percent of total world production. In India export oriented large units have been set up mainly in southern, western and northern regions, with production capacities between 2000-3000 tonnes per annum. A large number of small production units exist throughout India and function mostly during the autumn and winter months only. The annual production of mushrooms in India is estimated to be around 1,20,000 metric tonnes with 85 percent of this production being of button mushrooms [1]. Of late, much emphasis is being laid in production of mushrooms in the state of Jammu and Kashmir where 79,277 spawn bottles for laying about 150,000 trays/poly bags were distributed in 2009-2010 and about 5051.61 quintals mushroom harvested under Rashtriya Krishi Vikas Yojna (RKVY) alone [2]. The main problems in the speedy development of white button mushroom crop in the valley to the cottage level industry are mushroom diseases and pests. The crop is infested by a number of fungal, bacterial, and viral pathogens which result either in the partial or total failure of the crop or to the least deteriorates the quality of the crop. The cultivation of button mushrooms in the Jammu & Kashmir state is usually carried out in residential houses but in a few isolated cases specially built mushroom houses without any environmental control system and without the provision of compost pasteurization have also been used for the cultivation, thus

providing the conditions conducive for the growth and multiplication of pathogens and competitor moulds associated with mushroom culture. Although new farms with environmental control system and compost pasteurization facilities are coming up in the state, the number of such farms is very few [3]. By the end of 2010, the mushroom production of Jammu & Kashmir state has reached 950 metric tonnes per annum of which valley contributed 250 metric tonnes. The increase in the number of mushroom production units without the facilities of pasteurization coupled with the year round cultivation lead to the growth in the populations of a few fungal and bacterial pathogens, thus posing a serious threat in the profitable production of this crop. Major fungal diseases viz., dry bubble, wet bubble and cobweb are responsible for inflicting varying degree of crop losses in mushroom farms [4, 5]. Among these wet bubble disease (*M. perniciosa*) causes extensive damage by causing soft rot or decay of whole fruiting body. The pathogen causes damage to the entire crop. Wet bubble disease of *A. bisporus* poses problems to its speedy production. Hence, an attempt was made to evaluate different bioagents against the pathogen to manage this devastating disease.

Materials and Methods

The present study was conducted during 2008 and 2009 at Mushroom Research and Training Centre, Division of Plant Pathology, SKUAST-Kashmir, Shalimar, Srinagar. A survey of mushroom farms in three districts viz., Srinagar, Budgam and Pulwama, of Kashmir Division was conducted in both spring and autumn crop seasons of 2008 and 2009, to ascertain the status of wet bubble disease (*Mycogone perniciosa*) of white button mushroom, *Agaricus bisporus* (Lange) Imbach. In vitro evaluation: The pure cultures *Pseudomonas fluorescens* isolates PS-103, PS-104 and PS-105, *Bacillus subtilis* isolates BS-101, BS-115 and BS-116 and *Azotobacter* sp. isolates Azt-106, Azt-108 and Azt-117 were obtained

Table-1 In vitro mycelial growth of *Agaricus bisporus* and *Mycogone perniciosa* in presence of bacterial antagonists

Bacterial isolate	Radial mycelial growth (mm) of <i>A. bisporus</i>	Percent growth stimulation	Radial mycelial growth (mm) of <i>M. Perniciosa</i>	Percent growth inhibition	Interaction	
					<i>M. perniciosa</i>	<i>A. bisporus</i>
<i>P. fluorescens</i> -103	53.15	(39.79)	0.50	(98.88)	A	MS
<i>P. fluorescens</i> -104	34.50	(07.07)	2.50	(94.44)	A	N
<i>P. fluorescens</i> -105	36.17	(11.49)	5.33	(88.14)	A	N
<i>B. subtilis</i> -101	34.0	(05.69)	11.16	(75.18)	N	N
<i>B. subtilis</i> -115	38.13	(26.55)	4.0	(91.11)	A	S
<i>B. subtilis</i> -116	41.13	(22.09)	0.0	(100)	A	S
<i>Azotobacter</i> -106	50.47	(36.50)	0.67	(98.51)	A	MS
<i>Azotobacter</i> -108	38.18	(26.45)	5.5	(87.77)	A	S
<i>Azotobacter</i> -117	40.50	(28.08)	11.5	(74.44)	N	S
Control	32.0	-	45.0	-		

S = Stimulatory, MS = More stimulatory, N = Neutralistic, A = Antagonistic – with clear inhibition zone

from the Division of Environmental Sciences, SKUAST-K Shalimar, the isolates were maintained and mass multiplied by subculturing on nutrient Agar (NA)/as well as on King's –B medium, incubating the culture at 25±2°C for 48 hours. The antagonistic potentialities of these bacterial isolates were tested against the growth of *A. bisporus* and *M. perniciosa* using dual culture technique [6]. The PDA was prepared, autoclaved and poured in petri plates and the bacterial strains were separately streaked on PDA in the centre of the petri plate. After streaking, 5mm discs of both *M. perniciosa* (7-day old culture) and *Agaricus bisporus* (10-day old culture) were equidistantly placed on two sides of the bacterial streak and incubated at 23± 2°C for three days. The petri plates with pathogen *M. perniciosa* and host *A. bisporus* only served as controls. Each treatment was replicated thrice. The petri plates were incubated at 23± 2°C. Observations on colony diameter of both *M. perniciosa* and *A. bisporus* in treated plates was recorded and the percent inhibition over control calculated according to the formula given by Vincent [7].

$$\text{Percent mycelial growth inhibition} = \frac{(C - T)}{C} \times 100$$

Where C = Radial mycelial growth (mm) in check

T = Radial mycelial growth in the treatment (mm)

Based on the growth of the host and the pathogen, the bacterial isolates were grouped into various categories as proposed by Ahlawat and Rai (1997) with slight modifications [8] as I – Antagonistic, II. Neutralistic, III. Stimulatory, IV. More stimulatory.

In vivo evaluation

The test bio-control agents, which have shown maximum in vitro antagonism against *M. perniciosa* and no in vitro, inhibition against *A. bisporus*, were evaluated in vivo against wet bubble disease. The antagonists (1×10^8 cfu/g⁻¹ soil) were admixed separately with casing mixture at the rate of 0.5, 1 and 2 percent before inoculation of the pathogen. The pathogen was inoculated and the antagonist admixed casing mixture spread over spawn-run compost, filled in 10 kg polythene bags. For each treatment three replications were run, each replication comprising a single bag. Treatments without inoculation of pathogen and/or bio-control agents served as controls. The percent disease intensity and yield were calculated for one month cropping period, whereas other quality characters were recorded only during first flush.

Statistical analysis

The data collected were subjected to statistical analysis wherever needed. The differences exhibited by the treatments in various experiments were tested for their significance as per the methods suggested by Gomez and Gomez [9]. The 'Minitab' computer software was used for data analysis.

Results

Use of bacterial antagonists

In vitro evaluation

Perusal of the data [Table-1] reveals that none of the test isolates of the three bacterial genera exhibited any antagonism to the growth of *A. bisporus* mycelium. While *P. fluorescens* isolate PS-103 and *Azotobacter* sp. Isolate, Azt-106 were more stimulatory, *Azotobacter* sp. isolates Azt-108 and Azt-117 showed

stimulatory effect on in vitro growth *A. bisporus* [Fig-1]. The *P. fluorescens* isolates PS-104, PS-105 and *B. subtilis* isolate BS-101 exhibited neutralistic effects on *A. bisporus* growth. On the contrary, all the test isolates, except *B. subtilis* isolate BS-101 and *Azotobacter* sp. isolate Azt-117, exhibited antagonism of varying degrees against *M. perniciosa*. The isolate BS-101 and Azt-117 showed only neutralistic effects on the growth of the pathogen *M. perniciosa*.

In vivo evaluation

Effect on disease development

The data [Table-2] reveals that all the bacterial antagonists reduced the percent disease intensity as compared to pathogen infested-untreated check-I. Compared to a wet bubble intensity of 16.23 percent obtained in pathogen infested-untreated check-I, the disease was reduced to 1.85 percent, with the disease control of 88.60 percent in treatment receiving *P. fluorescens* at 2.0%, followed by disease intensity of 4.81-5.18, with the disease control of 68.08-70.36 percent in the treatments receiving *P. fluorescens* at 1% or *B. subtilis* at 2% concentrations. *P. fluorescens* at 5% and *Azotobacter* sp. at 2% concentrations were the next best treatments exhibiting wet bubble intensity of 7.04-7.41 percent, with the disease control of 54.34-56.62 percent. Out of all the bacterial antagonist treatments, *Azotobacter* sp. at 0.5% concentration was least effective treatment showing disease intensity of 13.33 percent, with the disease control of 37.76 percent.

Effect on yield and yield components

The incorporation of bacterial antagonists in pathogen-infested casing was found to show significant effects on yield and yield components such as number and weight of fruit bodies. It is evident from the [Table-3] that minimum number (82.83 - 83.00) of fruit bodies per kg mushroom was recorded in treatments which received *B. subtilis*-116 or *P. fluorescens*-103 each at 2% concentration as compared to that (92.83) in uninfested-untreated check. *B. subtilis*-116 and *P. fluorescens*-103 each at 1% concentration and *Azotobacter* sp.-106 at 2% concentration were the next best treatments providing 84.33 - 84.50 fruit bodies per kg mushroom, compared to 94.16 obtained in untreated check. The application of bacterial antagonist treatments in pathogen-infested casing significantly affected the average weight of fruit-bodies. The average weight of fruit-body (10.68 g) as obtained in untreated check was found to improve significantly (12.01 - 12.14g) in treatments receiving, *P. fluorescens*-103 at 1 and 2% concentration or *B. subtilis*-116 at 2% concentrations. *Azotobacter*-106 at 2% concentration and *B. subtilis*-116 at 1% concentration were the next best treatments providing the fruit-body weight of 11.74 - 11.93 g. The average weight of fruit-bodies in next treatments ranged from 11.05 - 11.38 g compared to 10.93 g in un-infested-untreated check. The button yields also improved significantly with the application of bacterial antagonist treatments. *P. fluorescens*-103 at 2% concentration exhibited maximum yield (12.42 kg/quintal) as good as that obtained in uninfested-untreated check (12.84 kg/quintal compost). The same antagonist @ 1.0% concentration or *B. subtilis*-116 at 2.0% concentration were the next best treatments with the average yield of 10.09 - 10.99kg/quintal compost. Applications of *Azotobacter* sp at 0.5-1.0% concentration was the least effective antagonist providing button yield of 5.14-6.70 kg / q compost similar to that obtained in infested-untreated check (6.18 kg/q compost).

Table-2 Effect of bacterial antagonists incorporated in *Mycogone perniciosa*-infested casing on percent intensity of wet bubble disease of white button mushroom (*Agaricus bisporus*) during spring 2009 and 2010

Treatment		Spring 2009	Spring 2010	Mean	Diseases control (%)
<i>P. fluorescens</i> -103	0.50%	7.41 (15.57)	6.67 (14.96)	7.04 (15.36) ^d	56.62
	1.00%	5.18 (13.09)	4.44 (12.16)	4.81 (12.62) ^c	70.36
	2.00%	2.22 (8.56)	1.48 (5.96)	1.85 (7.26) ^b	88.6
<i>B. Subtilis</i> - 116	0.50%	9.63 (18.05)	10.37 (18.76)	10.0 (18.40) ^e	38.38
	1.00%	6.67 (14.82)	8.15 (16.54)	7.41 (15.68) ^d	54.34
	2.00%	5.18 (13.09)	5.18 (13.09)	5.18 (13.09) ^c	68.08
<i>Azotobacter</i> -106	0.50%	15.56 (23.23)	11.11 (19.47)	13.33 (21.35) ^f	37.76
	1.00%	10.37 (18.76)	9.63 (18.05)	10.0 (18.40) ^e	38.38
	2.00%	8.15 (16.54)	6.67 (14.96)	7.41 (15.75) ^d	54.34
Check I (infested - untreated)		16.23 (23.75)	16.23 (23.75)	16.23 (23.75) ^g	-
Check II (uninfested - untreated)		0.0 (2.86)	0.0 (2.86)	0.0 (2.86) ^a	-
S.E±		-1.16	-0.9	-0.73	
CD (p=0.05)		-2.37	-1.83	-1.47	

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

Table-3 Effect of bacterial antagonists incorporated in *M. perniciosa*-infested casing on the number and weight of fruit bodies and button yield during spring 2009-2010 (pooled over years)

Bact. antagonists		No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield kgs/q compost
<i>P. flourescens</i> -103	0.5%	88.16 ^c	11.38 ^c	8.78 ^d
	1.0 %	84.50 ^d	12.04 ^a	10.99 ^b
	2.0%	83.00 ^e	12.14 ^a	12.42 ^a
<i>B. subtilis</i> -116	0.5%	88.66 ^c	11.33 ^c	7.83 ^f
	1.0%	84.33 ^{de}	11.93 ^{ab}	9.58 ^{cd}
	2.0%	82.83 ^f	12.01 ^a	10.09 ^{bc}
<i>Azotobacter</i> -106	0.5%	90.50 ^b	11.05 ^d	5.14 ^g
	1.0%	88.50 ^c	11.33 ^c	6.70 ^g
	2.0%	84.66 ^d	11.74 ^b	7.94 ^{ef}
Control I (infested-untreated)		94.16 ^a	10.68 ^e	6.18 ^g
Control II (uninfested-untreated)		92.83 ^a	10.93 ^d	12.84 ^a
SE±		0.69	0.10	0.45
CD (p=0.05)		1.38	0.21	0.90

Means of three replications; means followed by similar letter(s) are statistically identical.

Table-4 Effect of bacterial antagonists incorporated in *M. perniciosa*-infested casing on quality parameters of white button mushroom (*Agaricus bisporus*) during spring 2009-2010 (pooled over years)

Bacterial antagonist		Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
<i>P. flourescens</i> - 103	0.5%	5.34 ^b	3.29 ^d	4.66 ^{cd}	1.25 ^c
	1 %	5.38 ^b	3.35 ^c	4.69 ^{bc}	1.28 ^{bc}
	2%	6.11 ^a	3.48 ^{ab}	4.80 ^a	1.31 ^a
<i>B. subtilis</i> -116	0.5%	5.29 ^b	3.24 ^{de}	4.60 ^e	1.27 ^c
	1%	5.35 ^b	3.31 ^d	4.61 ^d	1.31 ^b
	2%	5.51 ^b	3.45 ^b	4.72 ^b	1.32 ^a
<i>Azotobacter</i> - 106	0.5%	5.29 ^b	3.21 ^e	4.63 ^d	1.24 ^d
	1%	5.35 ^b	3.26 ^d	4.69 ^{bc}	1.27 ^c
	2%	5.47 ^b	3.33 ^{cd}	4.83 ^a	1.31 ^a
Control (untreated)		5.32 ^b	3.42 ^{bc}	4.29 ^f	1.23 ^d
Control II (uninfested- untreated)		6.26 ^a	3.56 ^a	4.26 ^f	1.25 ^{cd}
SE±		0.16	0.05	0.02	0.01
CD (p=0.05)		0.32	0.10	0.05	0.03

Means of three replications; means followed by similar letter(s) are statistically identical.



Fig-1 In vitro evaluation of bacterial antagonists

Effect on quality parameters of sporophores

The incorporation of bacterial antagonists in pathogen-infested casing significantly affected the sporophore quality parameters such as pileus weight, pileus diameter, stipe weight and stipe diameter [Table-4].

- **Pileus Weight:** The maximum pileus weight (6.11 g) among the different bacterial antagonist treatments was exhibited by *P. fluorescens*-103 at 2% concentration followed by pileus weight by that (5.38-5.51 g) in the treatments receiving *P. fluorescens* at 1.0%, *Azotobacter* sp.-106 and *B.subtilis*-116 at 2.0% concentration, compared to the pileus weight (5.32 g) obtained in untreated check.
- **Pileus dia:** The maximum pileus dia (3.45 - 3.48 cm) among the treatments was exhibited by *B.subtilis*-106 and *P. flourescens*-103 both at 2.0% concentration statistically similar to that obtained in uninfested -untreated check (3.56 cm). The next best pileus dia of 3.29 - 3.35 cm was obtained in the treatment receiving *P. flourescens*-103 at 0.5 - 1.0% concentrations.
- **Stipe weight:** The stipe weight of 4.29 g was obtained in untreated check was found to significantly improve to 4.80 - 4.83 g in treatments receiving *P.flourescens*-103 or *Azotobacter* sp-106 each at 2.0% concentration. *P.flourescens*-103 and *Azotobacter* sp-106 each at 1.0% concentration were in the next best treatments showing stipe wt of 4.69-4.72g. The average weight of stipe in uninfested-untreated check was (4.26 g).
- **Stipe dia:** The maximum dia of stipe 1.35 cm was exhibited in treatment receiving *B. subtilis*-116 at 2.0% concentration as compared to that (1.23 cm) obtained in untreated check. *P. flourescens*-103 and *Azotobacter*-106 each at 2.0% concentration were the next best treatments providing the stipe dia of 1.31 cm compared to stipe dia of 1.25 cm obtained in uninfested-untreated check.

Discussion

Wet bubble disease of white button mushroom has been reported as one of the serious diseases in India. The effective management of wet bubble disease in mushroom production houses requires preferably the use of components other than chemicals (fungicides) such as botanicals and bio-control agents. The bacterial antagonists, evaluated under the present investigation, revealed that all the test antagonists – *Pseudomonas* spp., *B. subtilis* and *Azotobacter* spp. – exhibited no adverse effects in vitro on *A.bisporus* with simultaneous inhibitory effects of varying degrees on *M. perniciosa* except for *B. subtilis* isolate BS-101 and *Azotobacter* isolate Azt-117 which were neutral towards the pathogen. Attempts have been made by several workers to control *M. perniciosa* and other pathogens of the *A. bisporus* under in vitro conditions and almost all of them have reported similar findings [10,11,12,13]. The usefulness of *P.flourescens* and *B. subtilis* in the control of moulds/diseases observed in the present investigations are in conformity with those of [14, 15]. The antagonistic behaviour of fluorescent pseudomonads against mushroom pathogens with increase in mushroom yields has been reported by many other workers [10,13,16,17] also claimed good success of siderophore-producing isolate (C116) of fluorescent pseudomonads against *M. perniciosa*.

Conclusion

Among spore cultures of bacterial antagonists evaluated in vitro, revealed that all the test antagonists *Pseudomonas flourescens*, *Bacillus subtilis* and *Azotobacter* sp. exhibited stimulatory effects of varying degree on *Agaricus bisporus* mycelium with antagonistic effects on *M. perniciosa* mycelium, *Bacillus subtilis*-116, *Pseudomonas flourescens*-103 and *Azotobacter*-106 gave the highest mycelial growth inhibition of 100.0, 98.88 and 98.51 percent of pathogen fungus, respectively. The incorporation of bacterial antagonists such as *P. flourescens*, *B. subtilis* or *Azotobacter* sp. at different concentrations in pathogen-infested casing also yielded appreciable disease control with corresponding yield gains.

Application of research: The present work demonstrated that application of bacterial antagonists against *Mycogone perniciosa* is considered as an applicable, safe and cost effective method for controlling Wet Bubble disease of button mushroom.

Research Category: Biofertilizers

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