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The use of liquid biofertilizers viz., *Azotobacter chroococcum* and *Bacillus megaterium* either alone or in combination were found significantly effective in improving growth and yield of button mushroom.Significantimprovement in number of fruits per bed, biological efficiencyand average weight of fruit body were observed by supplementation of bioferilizers. Biofertilizersinoculated treatments showed an increase of yield and biological efficiency in the range of 17.40 % to 64.19%and19.97% to 27.93 % respectively. Nitrogen content in *Azotobacter chroococcum* inoculated treatments and phosphorus content in *Bacillus megaterium*inoculated treatment were low as compared to control

Key words: *Azotobacter chroococcum*,*Bacillus megaterium*,*Agaricus bisporus*, yield

INTRODUCTION

Agaricus bisporus(J.E. Lange) Imbachis one of the most important edible mushrooms that is cultivated and consumed widely in the world (Toker *et al*. 2007). Itcontains high amounts of protein, minerals, vitamin B groups, D and K and low content of fat, calories, sodium and cholesterol (Saiqa *et al*. 2008).

Reproductive growth and fruit body production of mushrooms occur in casing layer, which in increases the yield (Noble *et al*, 2009). Most *A.bisporus* strains required casing layer. Mushroom growth promotion by casing layer is related to temperature modulation and carbon dioxide concentration as well as presence of saprotrophic bacteria like *Pseudomonas* sp (Noble *et al*. 2003).The microflora present in casing soil play an important role in mushroom fruit body initiation and development (Fermor, 2000).

Very limited study has been carried out on the aspect of role of microbial inoculants in mushroom cultivation in India. The present research presents the effect of liquid biofertilizer *Azotobacter chroococcum* and phosphate solublizing bacterium-*Bacillus megaterium* on casing and pasteurized compostand theireffect on button mushroom yield.

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MATERIALS AND METHODS

Organisms

The pure bacterial liquid cultures of *Azotobacter chroococcum* (AC) and phosphate solubalizing bacterium *Bacillus megaterium*(PSB-BM) were procured from Biological Nitrogen Fixation Scheme, College of Agriculture, Pune, India. These cultures were maintained aseptically and used as per the treatments followed during the experiment at the time of spawning and casing.

Spawn

Pure culture of *Agaricus bisporus* strain U-3 was procured from All India Coordinated Research Improvement Project on Mushroom (AICRIP on Mushroom) Pune center, College of Agriculture, Pune.

Compost

The compost was prepared by short method of

composting as per therecommended formula containing Wheat straw (1000kg) + Poultry manure (300kg) + Gypsum (100kg) and analyzed for its quality parameters (Vijay and Gupta, 1995).

Casing material

For casing purpose coir pith was mixed with gardensoil in 1:1 proportion. Before mixing coir pith was watered to increase its pH up to 6 to 6.5 and then mixture of soil and coir pith was pasteurized at 70^0C for 8 hours before use.

Biofertilizers

Fresh liquid based culture of AC, PSB-BM were mixed during spawning @ 25 ml/kg of compost and @ 25 ml/kg during casing separately and 12.5 ml/kg each in combination.

Nutrient content
Nitrogen content

The total nitrogen content in compost and casing before spawning and after harvesting was estimated Micro- Kjeldahl's method. (Nelson and Sommers, 1973).

Phosphorus content

Total Phosphorus content in compost and casing was determined as per the method described in A.O.A.C (Anonymous, 1980).

Experimental details

Seven treatments with four replications were laid out in CRD. While using the dose of AC, and PSB-BM in combination for treatment numbers T3 and T6, the volume of each liquid culture was maintained as 12.5 ml of *Azotobacter* and 12.5 ml PSB-BM to make total volume 25 ml, while in monoculture treatment it was 25 ml/kg

Mushroom cultivation technique

Standard polyethylene bag method (Shandilya, 1988) was used for *Agaricus bisporus* cultivation. Microbial inoculants were added on wet weight basis of compost @ 25 ml /kg at two different stages *viz.*at spawning and casing.

Polyethylene bag of 18× 24 cm size (150 gauge) were filled with 10 kg compost per bag and spawned with strain of *Agaricus bisporus* U-3 @ 0.75% of wet weight of compost. The bags were tied and 15 to 20 pinholes were made all over the surface of bag and kept for incubation at 25-26^0C temperature. After complete spawn run the bags were opened and 4 cm thick layer of casing was applied.The bags were then kept for spawn run and watered with spray pump once or twice a daily depending upon the moisture requirement. Necessary precautions were taken to maintain a relative humidity of 80 - 90% and a temperature of $25^0 \pm 2^0$ C at spawn run and $16^0 \pm 2^0$ C at the time of fruiting in growing room by using Air Handling Unit. A total of four flushes were harvested in the course of investigation.

RESULTS AND DISCUSSION

Compost quality

The quality parameters of compost like pH, EC, bulk density per centnitrogen etc, were analyzed before spawning to know the feasibility of compost for button mushroom cultivation**.** Resultswerein agreement with the standards (Vijay and Gupta, 1995).Analysis of prepared compost revealed that total nitrogen and phosphorus content during spawning was 2.3% and 1.02% respectively (Table 2).

Effect on days required for pinhead formation:

Supplementing the compost and casing material with liquid biofertilizers have profound implication in *Agaricus bisporus* cultivation (Table 3).Early pinning and button formation was observed in the liquid biofertilizer treated bags over the unsupplemented control. A substantial decrease of 2-5 days was observed in pinhead initiation over the control. A similar result was also observed in the case of button formation. These results are in general agreement with earlier similar studies(Ahlawat and Rai, 1997). Wange and Gitay (2008) found that a combination of *Azotobacter* and PSB application spawningand casing showed early pinning which can be correlated with the present result .

Effect on days required for button formation

As in the case of pinning, the button formation was reduced by 2 to 5 days over absolute control. Best

Table 1 : Treatment details

Treatments	Details (combinations)
T1	Inoculation of AC@ 25 ml/kg during spawning
T2	Inoculation of PSB-BM @25 ml/kg during spawning
T3	Inoculation of AC+ PSB-BM @ 25 ml/kg during spawning
T4	Inoculation of AC@ 25 ml/kg during casing
T5	Inoculation of PSB-BM @ 25 ml/kg during casing
T6	Inoculation of AC+ PSB-BM @ 25 ml/kg during casing
T7	Control. (No biofertilizer)

Table 2 : Parameters of compost during spawning

Parameters of compost	Contents of compost
Nitrogen at spawning	2.3 %
Phosphorus at spawning	1.02%
pH	7.3
Moisture content	67 %
Bulk density	0.55 g/ml
Colour	Dark brown
C:N ratio	17:1
EC	1.09 dsm^{-1}

followed by T3 and T2. AC+ PSB-BM @ 25 ml/kg of casing combination than AC + PSB-BM @ 25 ml/kg of compost i.e.(180.0).

In monoculture treatment, a similar result was observed. The PSB-BM inoculant was more effective as compared to AC when applied singly in casing and in compost.

Effect on average weight of fruit body

The data on fruit body weight (Table 3) showed a similar trend in which maximum weight was recorded in the combination of AC + PSB-BM @ 25 ml/kg of casing, followed by AC + PSB-BM @ 25 ml/kg of compost (14.31g).Lowest average weight (10.84 g) was obtained in absolute control (Table 3, Fig.3). Improvement in average fruit weight of button mushroomwas due to the use of biofertilizers as compared to un-inoculated treatment (Gitay and Wange, 2008), and the present study confirms the same.

Table 3 : Effect of biofertizers on days required for pinhead initiation, button formation, number of fruits per bag and average fruit body weight of *Agaricus bisporus*

No.	Treatments	pinhead formation (Days)	Button formation (Days)	Number of fruits/bag	Average fruit body weight
T1	AC@ 25 ml/kg of compost during spawning.	14.00	18.00	165.75	12.03
T2	PSB-BM @25 ml/kg of compost during spawning.	12.25	16.25	175.50	12.64
T3	AC+ PSB-BM @ 25 ml/kg of compost during spawning.	12.75	17.00	180.00	14.31
T4	AC@ 25 ml/kg of casing material.	15.50	19.50	170.00	12.10
T5	PSB-BM @ 25 ml/kg of casing material.	14.75	18.75	174.00	12.67
T6	AC+ PSB-BM @ 25 ml/kg of casing material	14.50	17.50	185.75	15.02
T7	Control	17.00	21.00	156.50	10.84
	S. E ±	0.34	0.40	3.93	0.41
	C.D at 5%	1.03	1.18	11.66	1.23

result was obtained in the T2 combination whereby a substantial reduction in fruiting was observed (Table 3). It was followed by treatments T3 and T6. Earlier similar studies (Ahlawat and Rai, 1997; Hamid-Reza *et al*. 2013; Vijay and Gupta, 1992; Fermor, 2000) had also confirmed that microflora of casing soil play important role in fruitbody initiation and development.

Effect on number of fruits per bed

A significant increase in average number of buttons formed per bed was observed in all the biofertilizer treated bags over the unsupplemented controls. The maximum number of fruits per bed (185.75) was recorded in T6 combination. It was

Effect of liquid biofertilizer on yield of Agaricus bisporus

The data on yield on button mushroom (Table 4) showed significant increase in yield at all concentrations and combinations tested over the control. The biological efficiency also showed a similar trend. All the treatments of biofertilizers showed improvement in button mushroom yield by 17.40 % to 64.19 % over control (Table 4, Fig. 4).

The highest biological efficiency of 27.93% was obtained in T6 and was at par with T3 Among the monoculture treatments maximum per centof biological efficiency*viz.,*22.32 % and 22.14 % was obtained in T2 and T5 respectively (Ahawat and

Table 4 : Effect of liquid biofertilizers on yield of *Agaricus bisporus*

No.	Treatments	Yield/bag (kg/10kg compost)	Yield (kg/q dry substrate)	Per cent increase over control	Biological efficiency (%)
T1	AC@ 25 ml/kg of compost during spawning	1.99	19.97	17.40	19.97
T2	PSB-BM @ 25 ml/kg of compost during spawning	2.23	22.32	31.21	22.32
T3	AC+ PSB-BM @ 25 ml/kg of compost during spawning	2.52	25.25	48.44	25.25
T4	AC@ 25 ml/kg of casing material	2.06	20.60	21.10	20.60
T5	PSB-BM @ 25 ml/kg of casing material	2.21	22.14	30.15	22.14
T6	AC + PSB-BM @ 25 ml/kg of casing material	2.79	27.93	64.19	27.93
T7	Control	1.70	17.01	-	17.01
	S. E ±	0.12	0.12	-	-
	C.D at 5%	0.35	0.35	-	-

Table 5 : Effect of liquid biofertilizers on nitrogen and phosphorus content of spent compost and casing

No.	Treatment	Total nitrogen content in compost (%)	Total nitrogen content in casing (%)	Total phosphorus content in compost (%)	Total phosphorus content in casing (%)
T1	AC@ 25 ml/kg of compost during spawning.	1.85	0.09	0.72	0.20
T2	PSB-BM @ 25 ml/kg of compost during spawning.	1.31	0.09	0.51	0.19
T3	AC + PSB-BM @ 25 ml/kg of compost during spawning.	1.95	0.10	0.61	0.23
T4	AC@ 25 ml/kg of casing material.	1.21	0.13	0.67	0.20
T5	PSB-BM @ 25 ml/kg of casing material.	1.19	0.09	0.64	0.13
T6	AC + PSB-BM @ 25 ml/kg casing material.	1.31	0.17	0.67	0.18
T7	Control	1.10	0.09	0.81	0.22
	S. E ±	0.02	0.007	0.009	0.005
	C.D at 5%	0.07	0.02	0.02	0.01

Table 6 : Effect of liquid biofertilizers on disease incidence

No.	Treatments	Number of bags contaminated out of eight bags	Disease incidence (%)
T1	AC @ 25 ml/kg of compost during spawning.	-	-
T2	PSB-BM @ 25 ml/kg of compost during spawning.	2	25
T3	AC + PSB-BM @ 25 ml/kg of compost during spawning.	-	-
T4	AC@ 25 ml/kg of casing material.	-	-
T5	PSB-BM @ 25 ml/kg of casing material.	4	50
T6	AC + PSB-BM @ 25 ml/kg of casing material.	-	-
T7	Control	5	62.5

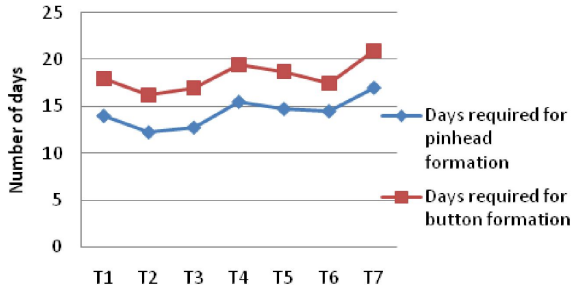

Fig. 1 : Days required for pinhead formation and button formation

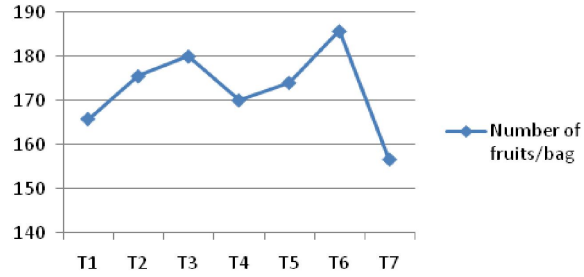

Fig. 2 : Number of fruits/bag

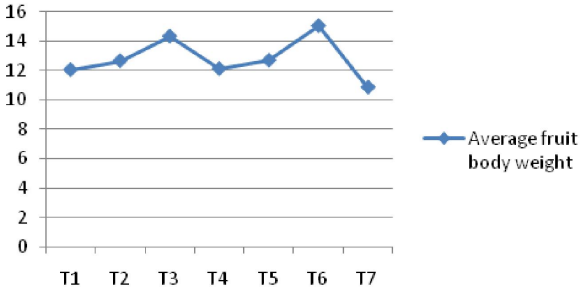

Fig. 3 : Average fruit body weight

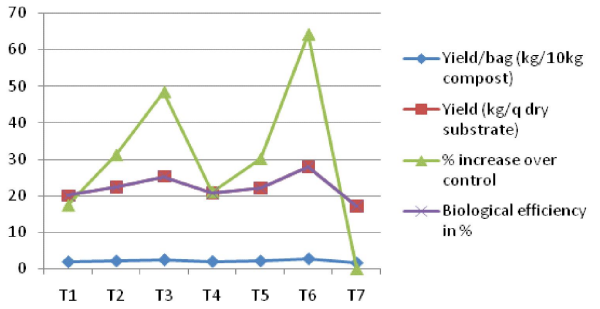

Fig. 4 : Effect of liquid biofertilizer on yield

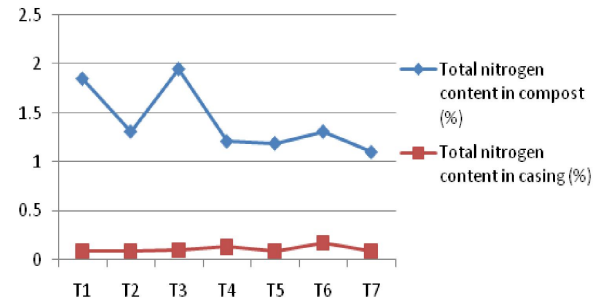

Fig. 5 : Nitrogen content in spent compost and casing

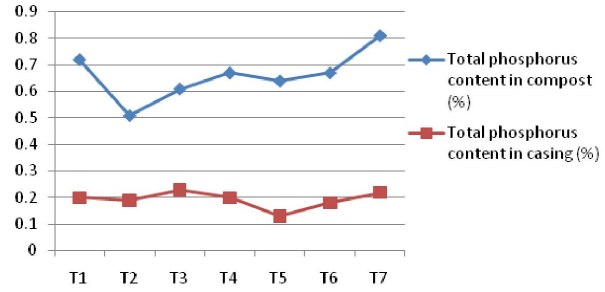

Fig. 6 : Phosphorus content in spent compost and casing

Fig. 7 : Effect of liquid biofertilizers on number of fruits per bed. Treatment T6 showing maximum number of fruit/bed as compare to Treatment T7

Fig. 8 : Effect of liquid biofertilizers on button mushroom yield. Treatment T6 showing maximum yield as compare to treatment T7

Fig. 9 : Maximum average weight of fruit body was observed in treatment. T6 and lowest in absolute control treatment T7

Rai, 1997) observed mixing of PSB gave significant higher yield i.e. 20 to 25 % of *A. bisporus* than in un-inoculated treatment. Similarly inoculation of AC in casing soil @ 1.0 % w/w gave higher mushroom yield of *A. bisporus*. Further, higher yield of

A. bisporus and *A. bitorquis* due to inoculation of casing soil with *A.faecalis, B. megaterium, B.circulans-II* and *B. thuringiensis* was observed (Ahlawat and Rai, 2000; Ahlawat *et al.* 2002).

Effect of biofertilizers applied at time of spawning and casing on quality of spent compost and casing

Effect on nitrogen content in spent compost and casing

Nitrogen content in spent compost after completion of trial showed decrease over initial nitrogen content from 2.3% to 1.1 %. Lowest nitrogen content (1.1%) in compost was found in absolute control treatment (Table 5, Fig. 5). Nitrogen reduction from spent mushroom compost was previously reported from AC inoculated treatment by Wange *et al*. 2008.

Effect on phosphorus content in spent compost and casing

Phosphorus content in spent compost showed a declining trend as compared to initial phosphorus level (Table 5, Fig. 6). The reduction in phosphorus content in spent compost may be because of utilization of phosphoruswhich was made available by the microbes and subsequently used by the fungus for its growth and development (Wange *et al*. 2008).

Effect on number of bags contaminated

Contamination of *Trichoderma* was observed at the time of 3rd harvest, on few beds. More number of bags were contaminated (62.5%)in control treatment where no biofertilizers were used. In treatments AC + PSB-BM @ 25 ml/kg of casing and AC @ 25 ml/kg of casing contamination was not noticed (Table 6). This may be because *Azotobacter* inoculants showed antagonism to *Trichoderma viride.*Pers(Ahlawat and Rai, 2000).

All the treatments of biofertilizers (AC and PSB-BM) either alone or in combination were found significantly effective in improving the growth and yield of *A. bisporus.* Monoculture of AC @ 25 ml/kg in casing performed better when it was inoculated in compost.From the present investigation it can be concluded that the treatment of AC + PSB-BM @25 ml/kg of casing was found to be effective because it showed, reduction in days required for pinhead initiation and days required for button formation as compared to control. Significant increase in number of fruits (185) per bed, yield per 10 kg of compost (2792 g), improvement in fruit weight (15.01 g), higher biological efficiency (27.92%), higher nitrogen content in casing and reduction in number of bags contaminated in *A. biosporus* over un-inoculated control treatment, hints at a technology development towards higher production of button mushroom (Figs. 7,8,9).

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