QUALITY IMPROVEMENT OF CASING MATERIAL AND YIELD IN MILKY MUSHROOM (*CALOCYBE INDICA*) BY USING BIOFERTILIZERS AND DIFFERENT SUBSTRATES

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ABSTRACT

The study was undertaken to assess the effect of nitrogen fixing and phosphate solubilizing biofertilizers and different substrates for improvement of casing quality and fruit body yield in milky mushroom (*Calocybe indica*). The experiment was conducted in completely randomized design with eight treatments of biofertilizers viz., *Azotobacter* and Phosphate solubilizing bacteria (PSB) i.e. (*Bacillus megaterium* + *Pseudomonas striata*) and their combinations. The quality parameters of casing material for milky mushroom viz., mycelial growth, microbial count, C:N ratio and bulk density were analyzed in the laboratory and are interpreted.

The results revealed that inoculation of bacterial inoculants either in alone or in different combinations resulted in an increase in mycelial growth of *C. indica* compared to uninoculated control under *in vitro* conditions. Highest fresh (20.52 g) as well as dry weight (0.65g) of mycelia was obtained in consortium of *Azotobacter* + PSB i.e. (*B. megaterium* + *P. striata*), followed by treatment of PSB resulting in 20.08 g and 0.62 g fresh weight and dry weight of mycelia, respectively. The total nitrogen content of casing material increased significantly from 0.11 to 0.55% due to biofertilizers treatment of soil + FYM + coir pith based casing. However, organic carbon decreased from 1.46 to 0.55 per cent in same treatment. Further, the C:N ratio also decreased from 13.0 to 7.9 due to *Azotobacter* inoculation in casing soil upto crop harvesting. The microbial count of *Azotobacter* and PSB was also higher in the same treatment and it was recorded 21 x 10^5 and 23 x 10^5, respectively at casing, thereafter it decreased upto harvest of mushrooms with greater magnitude. The fruit body yield of milky mushroom increased from 12.89% to 79.81% due to inoculation of biofertilizers.

In another experiment, the wheat and soybean straws and their mixture were evaluated for optimization of fruit body yield of milky mushroom. The data revealed that the stipe length, pileus diameter and average fruit weight highest maximum in mixture of wheat and soybean straw (1:1). The highest fruit body yield/kg dry substrate was mixture of two straws followed by soybean and wheat straw alone and it ranged from 382 to 714 g/kg substrate and 38.2 to 71.4% respectively. The results revealed that N_2 fixing and phosphate solubilizing bacteria could increase the quality of casing material. Further, the mixture of wheat and soybean straw (1:1) as a substrate performed better than individual, reflected in higher fruit body yields of milky mushroom.

Keywords: milky mushroom, biofertilizers, substrate straw, yield

INTRODUCTION

A lot of bio-waste is being generated in rural areas and this can be utilized directly through mushroom cultivation. The cultivation of mushroom is unique in the sense that it is the most efficient and economically viable biotechnological process for the conversion of lignocellulosic waste material into high quality protein rich palatable food. Nowadays, there is a need to promote the cultivation of mushroom to meet the challenges of increasing world population as well as energy crisis. Production of food in a cheaper and simpler way carries more significance in developing countries like India. Considering the growth requirements, milky mushroom fits very well to this situation.

Calocybe indica, a popular mushroom was first time reported from India in 1976 [1]. It can be easily gown in the temperature range of 25 to 35 ^oC with 75 to 85% relative humidity. It has a good biological efficiency (60 to 70%) under optimal growth conditions. Its sporophores have long shelf life. The major advantage with this mushroom is that, it can be

best fitted in the relay cropping when no other mushroom can be grown mainly due to its higher temperature optima. It has quite good scope and can substitute other tropical mushrooms like *Pleurotus* and *Volvariella* [2]. *P. sajor-caju* and *C. indica* contains higher protein, dry matter, fat, carbohydrate and ash than *P. sajor-caju* [3]. This results in higher energy value for *Calocybe indica.*

Therefore, the present investigation "Quality improvement of casing material and yield in milky mushroom (*calocybe indica*) by using biofertilizers and different substrates" was planned with the objectives to assess the effect of biofertilizers on casing material quality and different substrate on fruit body yield of milky mushroom.

MATERIALS AND METHODS

The study was carried out at All India Coordinated Research Project on Mushroom, College of Agriculture, Pune-5. Wheat straw was used as substrate for mushroom cultivation. It was chopped into 4-5 cm pieces. The chopped straw was filled in gunny bags and soaked in fresh water for overnight. Then the excess water was allowed to drain off. This substrate was then pasteurized by steam at 80 °C for 1 h and allowed to cool. After cooling, the substrate was used for filling the polythene bags. Before filling, the polythene bags were disinfected with 5% formalin. The 20 days old spawn was used for spawning and it was done with 4% grain spawn in uniform layers [2, 4]. After inoculation the polythene bags were tied, labeled and perforated at 20 to 25 points with the help of sterile pin for aeration. The above procedure was carried out aseptically in sterile room. First of all, two years old sieved FYM, sandy loam soil about neutral pH (7.3-7.8) and coir pith were drenched with 5% formalin separately and kept in airtight condition for 24 hrs. Before application of casing material on spawn run bed, complete removal of residual formalin was ensured by smelling. Various casing mixtures were prepared by uniform mixing of sterilized sandy loam soil, FYM, coir pith and carrier based biofertizers as per the treatments.

T_1 Soil + FYM (1:1), T_2 Soil + FYM + Coir pith (1:1:1), T_3 Soil + FYM + *Azotobacter* (1:1 + 1% *Azotobacter*), T_4 Soil + FYM + PSB (1:1 + 1% PSB), T_5 Soil + FYM + Coir pith + *Azotobacter* (1:1:1 + 1% *Azotobacter*), T_6 Soil + FYM + Coir pith + PSB (1:1:1 + 1% PSB), T_7 Soil + FYM + *Azotobacter* + PSB (1:1 + 1% *Azotobacter* + 1% PSB) and T_8 Soil + FYM + Coir pith + *Azotobacter* + PSB (1:1:1 + 1% *Azotobacter* + 1% PSB).

A uniform layer of casing material was applied over the surface of spawn run bed. The thickness of casing was kept 3 cm [2]. After casing, the beds were left for case run at ambient temperature. The casing layer was kept moist by spraying water regularly. Application of 0.01% Bavistin and 0.02% Nuvan was undertaken immediately after casing to avoid any incidence of pests and diseases during case run. The samples of casing materials were drawn from all treatments at the time of casing and at last harvest of milky mushroom. These samples were analyzed in the laboratory by adopting standard analytical procedures for organic carbon, total nitrogen, bulk density and microbial population in casing material.

Another experiment was laid out to assess the effect of different substrates on growth and fruit body yield of milky mushroom. The wheat, soybean and their mixture were used as substrate for cultivation of milky mushroom. The experiment comprised three treatments, each with seven replications in completely randomized design. Data was recorded for days required for spawn run, case run and fruiting, stipe length, pileus diameter, fruit body weight and total yield.

RESULTS AND DISCUSSION

The effect of different bacterial cultures alone and in combinations, on growth of *C. indica* was assessed in liquid culture under *in-vitro* conditions by measuring mycelial growth. Highest fresh weight (20.52 g) as well as dry weight (0.65 g) of mycelia was obtained in treatment of *Azotobacter* + PSB (*B. megaterium* + *P. striata*) followed by PSB alone resulting in 20.08 g and 0.62 g fresh and dry weight of mycelia respectively. Among the individual bacterial inoculant treatments, PSB resulted in production of highest fresh weight (17.71 g) as well as dry weight (0.56 g) of mycelia growth. The least fresh weight (8.63 g) as well as dry weight (0.19 g) of mycelium was recorded in uninoculated treatment (Table 1).

The data clearly indicated that inoculation of bacterial inoculants either alone or in different combinations resulted in an increase in mycelia growth of *C. indica* as compared to uninoculated control.

While studying the effect of microbial inoculants on mycelial growth of white button mushroom under *in-vitro* conditions, similar trend of results have been reported [5]. Significantly higher fresh weight (23.83g) and dry weight (0.71g) of mycelia of *P. sajor-caju* due to the treatment of mixed bacterial culture viz., *Azotobacter chroococcum, B. megaterium* and *P. striata* in malt extract medium were obtained in *in vitro* condition [6].

Table 1. Effect of enrichment of casing material with *Azotobacter* and PSB on mycelial weight and bulk density of casing material.

Treatments	**Mycelial weight on PDA broth**		**Bulk density (g/cm^3)**		**Percent reduction in bulk density**
	Fresh (g)	**Dry (g)**	**At casing**	**At harvest**	
Soil + FYM	8.63	0.19	1.42	1.41	1.16
Soil + FYM + Coir pith	13.15	0.26	1.42	1.40	1.66
Soil + FYM + *Azotobacter*	14.54	0.31	1.40	1.36	4.12
Soil + FYM + PSB	17.71	0.56	1.41	1.37	3.96
Soil + FYM + Coir pith + *Azotobacter*	15.18	0.48	1.36	1.30	5.85
Soil + FYM + Coir pith + PSB	16.55	0.51	1.37	1.32	5.00
Soil + FYM + *Azotobacter* + PSB	20.08	0.62	1.39	1.33	6.24
Soil + FYM + Coir pith + *Azotobacter* + PSB	20.52	0.65	1.34	1.24	9.98
S.E. ±	-	-	0.003	0.001	-
C.D. at 5%	-	-	0.010	0.003	-

The bulk density of casing materials was determined at the time of casing and at the harvest of mushroom crop. The data revealed that the bulk density of casing materials decreased significantly in the treatments of biofertilizers compared to uninoculated treatments. The treatments of Soil + FYM and Soil + FYM + Coir pith showed significantly lower magnitude of decrease i.e. by 0.01g/cm^3 and 0.02 g/cm^3. The treatment of Soil + FYM + Coir pith + *Azotobacter* + PSB showed significantly highest magnitude of decline (by 0.10g/cm^3) among all the treatments (Table 1). Per cent reduction in bulk density (Table 1) was higher in casing mixtures inoculated with biofertilizers as compared to uninoculated treatments. The treatment of Soil + FYM + Coir pith + *Azotobacter* + PSB showed highest reduction (9.98%) in bulk density among all the treatments followed by treatment of Soil + FYM + *Azotobacter* + PSB (6.24%).

The effect of enrichment of casing material with *Azotobacter* and PSB on C:N ratio, organic carbon and total nitrogen contents of casing materials were determined at the time of casing and after harvest of mushroom crop. The data revealed that the total nitrogen content of casing materials increased significantly with greater magnitude in treatments inoculated with biofertilizers as compared to uninoculated treatments (Table 2). However, the treatment of Soil + FYM + Coir pith + *Azotobacter* + PSB showed significantly higher total nitrogen (0.55%) contents as compared to rest of treatments. The organic carbon content decreased from casing stage to harvesting stage and highest decrease was in soil + coir pith + FYM with all biofertilizers (1.46 to 0.55%). The C:N ratio of casing materials narrowed at harvesting stage contributed by an increase in total nitrogen content in increased and decreased organic carbon of casing materials at crop harvest stage (Table 2).

C:N ratio of casing mixture declined with greater magnitude in treatments inoculated with biofertilizers as compared to uninoculated treatments. The decline in C:N ratio was highest (13.0 :1 to 7.9 :1) in the treatment of Soil + FYM + Coir pith + *Azotobacter*.

Initial *Azotobacter* and PSB count in the treatments of Soil + FYM and Soil + FYM + coir pith was zero since we used sterilized soil, FYM and coir pith during casing. The *Azotobacter* and PSB count for same treatments at casing and harvest were 1 x 10^5/g and 0.5 x 10^5/g and 1.5 x 10^5/g and 1 x 10^5/g of casing material respectively (Table 3). Highest count of *Azotobacter* and PSB was estimated in the treatment of Soil + FYM + Coir pith + *Azotobacter* + PSB followed by treatment Soil + FYM + *Azotobacter* + PSB i.e. 21 and 14 and 23 and 14.5 x 10^5/g of casing material, respectively. Population of *Azotobacter* and PSB of casing materials declined at harvest of mushroom crop (Table 3). A decline in microbial population of *Azotobacter* and PSB at crop harvest of white button mushroom has also been reported by earlier workers [5].

Table 2. Effect of enrichment of casing material with *Azotobacter* and PSB on organic carbon, total N content and C:N ratio of casing material.

Treatments	**Organic carbon content (%)**		**Total nitrogen content (%)**		**C : N ratio**	
	At casing	**At harvest**	**At casing**	**At harvest**	**At casing**	**At harvest**
Soil +FYM	0.71	0.229	0.05	0.20	13.6:1	11.6:1
Soil + FYM + Coir pith	0.80	0.301	0.06	0.27	14.1:1	11.3:1
Soil + FYM + *Azotobacter*	1.18	0.389	0.09	0.48	13.2:1	8.0:1
Soil + FYM + PSB	0.98	0.419	0.07	0.39	13.5:1	10.9:1
Soil + FYM + Coir pith + *Azotobacter*	1.22	0.386	0.09	0.49	13.0:1	7.9:1
Soil + FYM + Coir pith + PSB	1.02	0.424	0.08	0.39	13.3:1	10.8:1
Soil + FYM + *Azotobacter* + PSB	1.26	0.484	0.10	0.47	12.9:1	10.4:1
Soil + FYM + Coir pith + *Azotobacter* + PSB	1.46	0.555	0.11	0.55	12.8:1	10.2:1
S.E. ±	0.002	0.001	0.004	0.001	0.35	0.025
C.D. at 5%	0.006	0.004	0.011	0.004	1.02	0.072

The yield data (Table 3) as affected by different biofertilizers revealed that significantly higher average fresh fruit weight (61.46 g) was recorded in the treatment of Soil + FYM + Coir pith in conjunction with biofertilizers followed by the treatment of Soil + FYM + biofertilizers (60.87g). More or less similar trend of the results were also observed in respect of total fresh yield and it ranged from 216.38 to 389.06 g/kg substrate. The results indicated that the yield of milky mushroom increased from 12.89 to 79.81% due to inoculation of biofertilizers and highest increase was observed in the treatment of soil + FYM + coir pith with all biofertilizers. Similar trend of results have also been reported by earlier workers in *Pleurotus* sp. [6, 7] and in button mushroom [8].

The effect of different substrates on growth and yield (Table 4) of milky mushroom revealed that lowest time (days) i.e. 16.42, 8.14 and 18.00 for spawn run, case run and fruiting respectively were recorded in the treatment of wheat and soybean straw (1:1) as compared to individual substrates. The total no. of fruits/bed/kg substrate, stipe length, pileus diameter of fruit and average fruit weight were highest in the mixture of wheat and soybean straws i.e. 17.28, 13.64 cm 11.32 cm and 41.85 g, respectively followed by soybean and wheat straw alone.

The yield and biological efficiency (Table 4) of milky mushroom was highest in the substrate of wheat and soybean straw mixture (1:1), followed by soybean straw and wheat straw alone and ranged from 382 to 714 g/kg substrate and 38.2 to 71.4% respectively. The enhanced growth and yield of milky mushroom on mixture of soybean and wheat straw has also been reported by earlier workers [9-10].

Table 3. Effect of enrichment of casing material with *Azotobacter* and PSB on microbial population (*cfu* x 10^5/g) and yield of milky mushroom.

Treatments	Microbial population (*cfu* x 10^5/g) of casing material				Average fresh fruit body weight (g)	Total fresh yield (g)/kg substrate	Percent increase of yield
	Azotobacter		PSB				
	At casing	At harvest	At casing	At harvest			
Soil +FYM	-	1.0	-	0.5	46.33	216.38	-
Soil + FYM + Coir pith	-	1.5	-	1.0	48.85	244.27	12.89
Soil + FYM + *Azotobacter*	13.5	6.0	-	-	56.63	301.86	39.50
Soil + FYM + PSB	-	-	17.0	8.5	57.42	344.52	59.22
Soil + FYM + Coir pith + *Azotobacter*	17.0	10.0	-	-	56.76	321.81	48.73
Soil + FYM + Coir pith + PSB	-	-	19.5	12.0	57.75	365.54	68.93
Soil + FYM + *Azotobacter* + PSB	18.5	10.5	21.0	13.0	60.87	365.22	68.79
Soil + FYM + Coir pith + *Azotobacter* + PSB	21.0	14.0	23.0	14.5	61.46	389.06	79.81
S.E. ±	-	-	-	-	0.93	5.25	-
C.D. at 5%	-	-	-	-	2.73	15.38	-

Table 4. Effect of different substrates on days required for spawn run, case run, fruiting, in *Calocybe indica*.

Treatments	Days for			Mean number of Fruits/ bed/kg substrate	Stipe lingth (cm)	Pileus diameter (cm)	Av. Fruit weight (g)	Yieldg/ kg substrate	Biological Efficiency (B.E.) %
	Spawn run	Case run	Fruiting						
Wheat straw	20.00	10.00	21.0	11.85	8.35	6.3	32.42	382	38.2
Soybean straw	18.42	9.00	19.57	14.42	11.11	8.9	36.0	510	51.0
Wheat straw + Soybean straw	16.42	8.14	18.00	17.28	13.64	11.32	41.85	714	71.4
S.E. ±	0.125	0.14	0.07	0.20	0.096	0.078	0.28	5.47	0.57
C.D. at 5%	0.38	0.45	0.21	0.61	0.29	0.24	0.86	17.70	1.77

CONCLUSION

The conclusions drawn out from present investigation are elaborated as follows:

- *In vitro* studies revealed that the highest fresh weight (20.52 g) and dry weight (0.65 g) of mycelial growth of *C. indica* was in liquid culture inoculated with *Azotobacter* + PSB.
- Percent of reduction in bulk density was higher in casing mixtures inoculated with biofertilizers as compared to uninoculated treatments.
- The C:N ratio of casing materials narrowed towards harvesting stage as total nitrogen content of casing materials increased and decreased organic carbon decreased with greater magnitude.
- Decline in the population of *Azotobacter* and PSB was noticed in casing materials at harvest of mushroom crop. However, the higher count of *Azotobacter* and PSB at casing and harvest of the crop was observed in the treatment of Soil + FYM + Coir pith + *Azotobacter* + PSB.
- The results in general indicated that the quality of casing material improved due to inoculation of N_2 fixing and phosphate solubilizing bacteria in casing materials of milky mushroom as reflected in higher yields.
- The mixture of wheat and soybean straw (1:1) as a substrate was found suitable for maximization of fruit body yield in milky mushroom followed by soybean straw and wheat straw alone.

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