ORIGINAL ARTICLE

Influence of organic nitrogen supplementation on yield of paddy straw mushroom, *Volvariella volvacea* (Bull. Ex Fr.) Sing.

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Abstract

Aim: The present disquisition was intended to study the influence of organic nitrogen supplements on the yield of *Volvariella volvacea* strain CBE TNAU 1505. **Materials and Methods:** Horse gram seed powder, tamarind seed powder (obtained after removing the fruit pulp from tamarind fruit pod), and silkworm pupal waste powder were supplemented to paddy straw based mushroom beds. Beds are placed in poly house for cropping at 32-35°C and relative humidity of 80-85%. **Results and Discussion:** Supplementation of beds with horse gram and tamarind seed powder (1:1 w/w) at 2% level has increased the spawn run and yield (1226.3 g/5 kg of substrate recording 24.5% bio-efficiency). Horse gram + silkworm pupal powder (1:1) and tamarind seed powder + silkworm pupal powder (1:1) were found to be poor supplements. Beds supplemented with pupal powder favored the growth of *Coprinus* spp. Uptick in the yield on supplementation with tamarind seed and horse gram seed powder (1:1 ratio) is attributable to uniform spread of the combination of horse gram and tamarind seed powder as analogized to horse gram and tamarind seed powder alone. The mixture would have increased the proteinase activity due to the hydrolytic action of proteolytic enzymes. **Conclusion:** Amalgam of tamarind seed powder and horse gram seed powder escalated the yield of *V. volvacea* strain CBE TNAU 1505. Strain-specific response of *V. volvacea* to bed supplements needs future attention.

Key words: Eggs, enzymes, fungus, nitrate, substrate, tropical

INTRODUCTION

Composting of unacclimated raw organic wastes such as residues generated through agriculture, horticulture, forestry, food processing industries, livestock effluents, and urban wastes by mushroom fungi is one of the reliable strategies to eliminate polluting xenobiotic wastes.[1,2] World production and consumption of mushrooms has elevated substantially since the late 1990s.[3] *Volvariella volvacea* (Bull. Ex Fr.) Sing, belonging to family *Pluteacea* (Kolt and Pouz) of Phylum Basidiomycetes is eulogized as straw mushroom. It is one among the highly cherished mushrooms known for its distinct flavor, pleasant taste, and short cropping cycle.[4] *Volvariella* was held in such high regard that it was often presented as a tribute to Chinese royalty.[5,6] Being a fungus of tropics and subtropics, *V. volvacea* is suited for both, indoor and outdoor cultivation. Despite the fact that it has been cultivated for 300 years, multiple problems consorted with cultivation practices have greatly bounded the progression of paddy straw mushroom industry. Biological efficiency (conversion of the substrate into mushroom fruit bodies) is only 5.21% on banana leaves, 15% on straw-based substrates, and 30-40% on cotton-waste composts.[7,8] Albeit, *Volvariella* is an aggressive colonizer of any cellulosic substrate, poor shelf life, competitive saprophytic ability, hydrolytic enzyme potential, and productivity are major hindrances for this praiseworthy mushroom for not boosting commercial importance in India. Cellulases play a critical

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role during substrate colonization while; laccase dominates during sporophore development. Mannitol dehydrogenase, tyrosinase, and water activity were found to be expressed disparately with different morphogenic stages and strains of *V. volvacea*.[9,10] Supplementation with a variety of organic amendments not only upgraded the yield of *Volvariella* spp. but also formed fruiting bodies within 10-12 days of spawning when analogized to that of non-supplemented ones. In several efforts, researchers used a variety of supplements to paddy straw mushroom beds; most of them had used horse gram powder as the spawn supplement.[11-13] Perusal of available literature delineated the presence of reasonable amount of nitrogen in the seeds of tamarind and silkworm pupa which were disposed as waste.[14] Keeping the availability of horse gram in hark, the present delving is thus aimed to probe the influence of tamarind seed powder and silkworm pupal powder in ameliorating the yield of *V. volvacea*.

MATERIALS AND METHODS

All experiments were conducted at mushroom research and training center, Department of Plant Pathology, Coimbatore during 2014-15.

In Vitro Maintenance of Cultures

V. volvacea strain CBE TNAU 1505, collected from the germplasm repository of Mushroom Research Laboratory, Tamil Nadu Agricultural University, Coimbatore, India, was maintained on Potato Dextrose Agar medium in Petridishes. The media was sterilized at 121°C for 1.5 h in an autoclave. Each Petridish was inoculated with 9 mm mycelial discs of strains and incubated at 32 ± 2°C for 7 days. To maintain vigor, fresh isolations were made from the fruiting bodies every time after 2-3 subcultures. For this purpose, the strains were propagated in straw spawn and grown on paddy straw following the method suggested by Thomas *et al*.[15]

Supplementation to Beds

To identify the best nutritional supplement, for encouraging the yield of *V. volvacea*, organic supplements, namely, horse gram powder, tamarind seed powder (obtained after removing the fruit pulp from tamarind fruit pod), and silkworm pupal waste obtained from the Department of Sericulture, TNAU, Mettupalayam were used as bed supplements. Circular compact bed method was followed using paddy straw-based spawn of CBE TNAU 1505.[16] Steam sterilized paddy straw twists of 2.5 m length and 5-8 cm diameter; each twist weighing 1.25 kg was used for bed preparation. The twists were pre-soaked in cold water for about 24 h and steam sterilized at 1.46 kg/cm^2 for 1 h. Later, they were shade dried to get 65-75% moisture. The twists were compactly placed clockwise in a circular fashion as close as possible on a wooden plank to make the first layer and straw spawn was placed at the periphery of the first layer of the bed. 20 g of each pre-sterilized supplement was sprinkled in each layer over the spawn at 2% level (in dry weight basis). The second layer was formed over the first layer following the same procedure, but the twist was placed compactly in counterclockwise direction. Similarly, the third and fourth layers of bed were formed. The size of the bed measured 30 cm diameter and 20 cm height. Total weight of each bed was 5 kg on dry weight basis. The perfectly prepared bed was pressed tightly and placed in a poly house for cropping at 32-35°C and relative humidity of 80-85%. The growth parameters such as days taken for spawn run, pinhead formation, first harvest, and yield attributes were recorded periodically. Based on yield data, biological efficiency was calculated using following formula:

BE (%) = Fresh weight of mushroom yield in kg/quantity of dry substrate used × 100.

Statistical Analysis

To avoid experimental errors, the experiment has been carried out in 4 replicas and the results presented are mean values. Statistical software AGRES was used for the data analysis.

RESULTS AND DISCUSSION

Supplementation with horse gram powder, tamarind seed powder, and silkworm pupal waste powder in the beds of *V. volvacea* strain CBE TNAU 1505 proclaimed that, number of days taken for spawn run, pinhead formation, first harvest, egg weight assorted with different organic supplements added to the mushroom beds [Table 1 and Figure 1]. Amidst supplements, horse gram + tamarind seed powder supplementation at 2% level was found to be significantly superior regarding number of mushrooms harvested (50.9), yield (1226.3 g per 5 kg of substrate), and biological efficiency (24.5%). This was followed by horse gram and tamarind seed powder, when used separately at 2% level, which were found to be on par with particular to yield (1072.5 and 983.9 g/5 kg of substrate) and biological efficiency (21.4 and 19.6%), respectively. However, horse gram powder alone yielded more number of eggs (45.6) as compared to tamarind seed powder alone (43.9). Horse gram + silkworm pupal powder (1:1) and tamarind seed powder + silkworm pupal powder (1:1) were found to be poor supplements. Control beds with no supplements produced minimum number of eggs (26.0), total yield (464.1 g). and biological efficiency (9.2%).

Basidiomycete fungi utilize substrate protein by the production of extracellular proteinases.[17] Nitrogen level and enzymatic activity of substrate are known to be increased by organic supplementation, which affect the yield of *Volvariella* spp.[11,18,19] Nitrogen is prime

Table 1: Influence of organic nitrogen as bed supplements on the growth and yield of *V. volvacea* strain CBE TNAU 1505

Supplement	DFSR*	DFPF*	DFFH*	Number of eggs harvested*	Average weight (g)/egg*	Yield (g)/ five kg of paddy straw*	BE%*
Horse gram seed powder	4.5^{a}	6.3^{a}	10.8^{a}	45.6^{b}	23.5^{a}	1072.5^{b}	21.4^{b} (27.55)
Tamarind seed powder	5.6^{c}	6.8^{bc}	11.6^{abc}	43.9^{c}	22.4^{b}	983.9^{b}	19.6^{b} (26.27)
Pupal powder	6.0^{de}	7.5^{e}	13.5^{cd}	35.9^{e}	20.4^{c}	734.6^{c}	14.7^{c} (22.54)
Horsegram+tamarind powder (50:50)	5.3^{b}	6.5^{ab}	11.3^{ab}	51.0^{a}	24.0^{a}	1226.3^{a}	24.5^{a} (29.66)
Horse gram+pupal powder (50:50)	5.6^{c}	7.1^{cd}	12.1^{abc}	37.6^{d}	22.1^{b}	833.2^{c}	16.6^{c} (24.04)
Tamarind+pupal powder (50:50)	5.8^{cd}	7.3^{de}	12.8^{bcd}	38.8^{d}	20.4^{c}	795.4^{c}	15.9^{c} (23.49)
Control	6.1^{e}	8.0^{f}	14.1^{d}	26.0^{f}	17.8^{d}	464.1^{d}	9.2^{d} (17.65)
CD (*P*=0.05)	0.2	0.3	1.8	1.4	0.6	105.9	2.4

DFSR: Days taken for spawn run, DFPF: Days taken for pinhead formation, DFFH: Days taken for first harvest, BE: Biological efficiency, *V. volvacea*: *Volvariella volvacea*. *Values are mean of four replications. Means followed by a common letter are not significantly different at *P*=0.05 by one-way ANOVA. The data in parenthesis are arcsine transformed values

constituent of amino acids which make up proteins and polysaccharide chitin, a cell wall component of many fungi.[20] Normally, some fungus prefers nitrate form of nitrogen while others prefer ammonical form still, others can utilize neither nitrate nor ammonia and may require an organic nitrogen-containing compound. This requirement for organic nitrogen may actually be a demand for a specific amino acid. A fungus that can utilize nitrate will also harness ammonium and organic nitrogen compounds for its nitrogen requisite.[21] Darlington and Scazzocchio[22] chronicled that, utilization of one particular nitrogen by fungus for a long time would rise the pH of medium. Thereafter, Sprent[23] first reported the association between pH and nitrogen metabolism. In accordance to Rajarathnam *et al.*[24] the nature and amount of nitrogen present in amendment had influenced the yield of mushrooms. In the current study, organic amendments such as a combination of horse gram and tamarind seed powder at 1:1 ratio when supplemented at 2% level increased the yield of *V. volvacea* strain CBE TNAU 1505. Comparable results were obtained by Prabhu.[25] The reason for uptick in yield of mushrooms due to the addition of gram powders might be ascribed to increase of protein content in the growing medium with concomitant proteolytic enzyme activity by mushroom fungus.[26,27]

Nevertheless, weed molds especially, *Coprinus* spp. were observed in mushroom beds supplemented with silkworm pupal powder which contains high protein (60-65%).[28] These results perspicuously indicate that silkworm pupal powder with more than sufficient levels of nitrogen would have increased the bed temperature thereupon, encouraging harmful organisms. Besides, the nature of nitrogen might have led to reduced form of ammonia favoring the growth of caprogenic *Coprinus* spp.[29] According to Jang *et al.*[30] and Ahlawat and Tewari[31] *Coprinus* requires acidic (5-6) pH and increased bed temperature (40°C). They also betokened that *Coprinus* requires almost 4 times more nitrogen than that of Volvariella. The addition of amendments like soybean meal favored weed molds, especially *Coprinus* spp. and reduced the yield of *Pleurotus* spp.[32] Gupta and Vijay[33] discerned that supplementation of spawn indeed resulted in escalated bed temperature that favored the growth of weed molds in *Agaricus bisporus* beds resulting in yield abatement. Banik and Nandi[34] ascertained the highest biological efficiency in *V. volvacea* (48.2%) when the paddy straw beds were supplemented with biogas slurry manure at 1:1 ratio. However, this trial was stewarded at an ambient temperature range of 30-32°C at NIRJAFT, Calcutta. Henceforth, supplements were used to increase the bed temperature that favors the growth of *V. volvacea*. Yield accretion on supplementation with tamarind seed and horse gram seed powder (1:1 ratio) is attributable to uniform spread of the combination of horse gram and tamarind seed powder as equated to horse gram and tamarind seed powder alone. The amalgam would have increased the proteinase activity due to the hydrolytic action of proteolytic enzymes as scrupulously indicated by Kalisz *et al.*[17] The protein content in tamarind seed powder needs to be estimated to draw future conclusions in this respect.

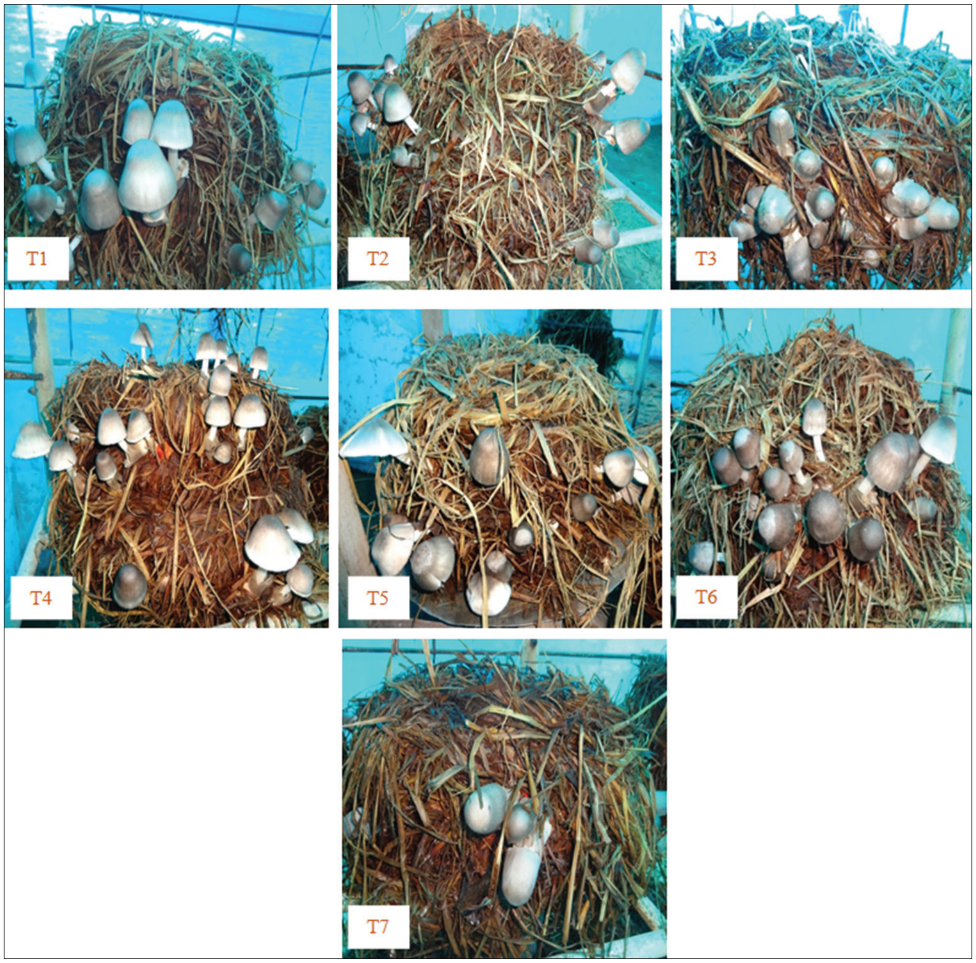

Figure 1: *Volvariella volvacea* strain CBE TNAU 1505 beds supplemented with various organic nitrogen supplements. T1: Horse gram powder, T2: Tamarind seed powder, T3: Silkworm pupal powder, T4: Horse gram + Tamarind seed powder, T5: Horse gram + Silkworm pupal powder, T6: Tamarind seed + Silkworm Pupal powder, T7: Control (untreated)

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