TRICHOLOMA GIGANTEUM- A NEW TROPICAL EDIBLE MUSHROOM FOR COMMERCIAL CULTIVATION IN INDIA

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ABSTRACT

The varied agro climatic conditions of India offer greater scope for cultivation of a variety of mushrooms. During the year 2007, two milky mushroom (*Calocybe indica)* strains- Ci (P), Ci (N), and *Tricholoma giganteum* were collected from Coimbatore and Erode districts of Tamil Nadu. Trials conducted during 2008-10 showed that *Tricholoma* recorded significantly higher yield of 177 % bioefficiency compared with existing commercial isolate of *Calocybe indica*. The mushroom *Tricholoma* has been partially sequenced using ITS primers 1 and 2 and it shares 91 % homology with *Tricholoma giganteum* and is given with Gen bank accession number 120872. This mushroom can be best cultivated using paddy straw substrate through out the year in sunken blue poly houses (250 gauge thickness of siplaulin) with three feet depth to maintain a temperature range of 30-35°C and relative humidity of more than 70 % during the cropping period. This mushroom contains the essential macro, and micronutrients, carbohydrates, protein, fibre content and also possess antioxidant activity. Taste of the mushroom is excellent, with good odour and aroma and has shelf life of 3-4 days under room temperature and 5-6 days under refrigerated conditions. Six trials were conducted during the year 2010, where *Tricholoma giganteum* recorded significantly higher yields with bioefficiency of 164 to 174 %. Identification and domestication of wild mushroom strains for commercial production offers great scope for increasing the mushroom productivity of the country.

Key words: *Tricholoma*, bioefficiency, blue silpaulin polyhouse

INTRODUCTION

Mushroom production represents one of the most commercially important steps towards diversification of agriculture based on microbial technology for large-scale recycling of agro-wastes in an agricultural country like India. Commercial production of edible mushrooms represents unique exploitation of the microbial technology for the bioconversion of the agricultural, industrial, forestry and house-hold wastes into nutritious food. Out of about 2,000 edible fleshy fungi, 20 types are artificially cultivated and about 10 are being produced and marketed in sizeable quantities across the world [1]. In India, at present, four mushroom varieties viz., *Agaricus*

bisporus, Pleurotus spp., *Volvariella* spp. and *Calocybe indica* have been recommended for the year round cultivation. The Indian subcontinent is known worldwide for its varied agro climatic zones with a variety of habitats that favour rich mushroom biodiversity [2]. About two decades ago, *Calocybe indica* P. & C. was identified as a wild edible mushroom in India. . Only limited attempts were made for its cultivation until 1998 [3, 4, 5, 6]. However, complete commercial production techniques were evolved for the first time in Tamil Nadu [7].

Tricholoma giganteum Heim, a new edible mushroom pure white in colour resembling the morphology of *Calocybe indica,* was reported growing widely in summer in Indo-gangetic plains of Howrah district, Hooghly in India [8]. The possibility of commercial cultivation of *Tricholoma lobayense* was already explored in Tamil Nadu during 2002 [9]. The simple production techniques, substantial and sustainable yield, increased shelf life, attractive color, flavor and shape are the attractive features of this new edible mushroom. As a new introduction to the edible mushroom world, no doubt that our country has greater prospects and potentiality to exploit this mushroom. Development of mushroom strains well adapted to the hot climatic plains of India with suitable simpler cultivation technology, higher yield potential and prolonged shelf life are the present day needs of commercial cultivation. Keeping all these things in mind, the research work was initiated to find out the variations on the yield performance(*i.e.* bioefficiency) existing among the different strains of *C.indica* and *T. giganteum* under field conditions and to identify a better strain/species for commercial cultivation that would suit the hot climatic zones of our country. In order to identify a better strain well adapted to the hot climatic plains of India with suitable simpler cultivation technology, higher yield potential and prolonged shelf life, the present investigation was conducted.

MATERIALS AND METHODS

Collection of mushroom strains. During the year 2007, two milky (*Calocybe indica*) mushroom strains Ci (P) and Ci (N) and *Tricholoma* were collected from Erode and Coimbatore districts of Tamil Nadu. The mushrooms were pure cultured from the cap using tissue culture method and maintained on Potato Agar slants and used for further studies.

Growth and yield performance studies of wild mushroom strains. The Growth and yield performance studies of wild mushroom strains was conducted at the Mushroom Research and Training Centre, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. A milky mushroom variety- *Calocybe indica* var.APK 2 was identified and released as a commercially cultivated species from Tamil Nadu Agricultural University, Coimbatore during 1998. It was used for comparison. The cultures of different strains of wild *C.indica* Ci (P) and Ci (N) collected from different places and *Tricholoma giganteum* were inoculated onto sorghum grains for spawn production. Using the sorghum-based spawn, cylindrical beds were prepared using paddy straw as substrate (1 kg of paddy straw/bed) .Four to six holes were made on the sides of the beds for aeration. The beds were incubated at room temperature of 28$\pm$2˚C for spawn running. After complete spawn run, the beds were cut into two equal halves and steamed casing soil (garden soil) was applied uniformly over the spawn run beds. The moisture level was maintained by regular water spraying on beds. The cased beds were incubated in partially sunken poly houses roofed with blue coloured high density polythene sheet, where a temperature of 30-35˚C with 75-80% relative humidity was maintained. The observations on Days For Spawn Run (DFSR), Days

For Pin head Formation (DFPF), Days For First Harvest (DFFH), Days to complete three harvests (total cropping period), total yield, and bio efficiency was recorded or calculated.

$$\text{Bio efficiency (\%)} : \frac{\text{Total weight of harvested mushrooms}}{\text{Dry weight of the substrate used}} \times 100$$

Molecular characterization

Isolation of DNA from *Tricholoma.* The mushroom was grown in malt extract broth and the mycelial mat was collected and ground with lysis buffer. After maceration the tube was kept in room temperature for 30 min and 150 µl of potassium acetate was added, vortexed for 2-3 seconds and kept in freezer for 30 min. The tubes were centrifuged at 15, 000 rpm for 5 min. The supernatant was transferred to another tube and equal volume of isopropyl alcohol was added. The tube was mixed by inversion and centrifuged at 15,000 rpm for 2 min. and the supernatant was discarded. The DNA pellet was washed in 300 µl of 70 % ethanol and centrifuged at 10,000 *g* for one min. and the supernatant was discarded. The pellet was air dried and dissolved in 50 µl of 1X TE buffer and used as genomic DNA for PCR reaction [10].

Sequencing of *Tricholoma* . The genomic DNA extracted from the pure culture of *Tricholoma* was used for PCR studies. The Polymerase Chain Reaction primers, ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') were used to amplify the ITS of ribosomal DNA which encompasses the 5.8 sRNA gene and both ITS -1 and ITS-2 regions [11]. The PCR reaction mixture (50 µl containing 1 U of Taq DNA polymerase, 5 µl of 10X PCR buffer,160 µM each of dATP, dCTP, dGTP, dTTP (MBI Fermentas), 50 pM each of ITS-1 and ITS-4 primers, 2 µl of 5 % glycerol and 40 ng of genomic DNA) were performed in a mater cycler with PCR conditions consisting 34 cycles of 1 min denaturation at 95°C, 30 s annealing at 50˚C, 1 min 20s elongation at 72˚C and ending by 10 min final elongation step at 72˚C with lid heating option at 110˚C [10]. Amplified products were run on 2 % agarose gel, stained with ethidium bromide and visualized under UV illumination. The sequencing was done using ITS-1 (forward) and ITS-4 (reverse primer) and the nucleotide sequence comparisons were performed using Blast Multiple Alignment Tool (BLAST) network sequences against the National Centre for Biotechnology Information (NCBI) database.

Studies on the nutritive value of *Tricholoma giganteum.* Moisture contents of mushrooms were estimated by drying 50 g of fresh mushrooms in an oven at 80^{o}C for three consecutive days. It was later cooled in a desiccator and reweighed. The moisture content was arrived from the differences in the weight [12]. The crude protein content of the mushroom was estimated by Micro Kjeldahl method [13]. The total carbohydrate content was determined by following anthrone method [14]. Estimation were made of ascorbic acid [15], crude fat [12], total phenolic content using the Folin-Ciocalteu method [16], crude fibre [17], total ash content (18), total nitrogen analyzed by Diacid extract method-semiautomatic Kjeldahl distillation, total phosphorous by Triacid extract method- vanodamolybdate calorimetric method, total potassium and total calcium [19]. Antioxidant activity was measured using Ferric reducing antioxidant power (FRAP) assay [20] as described previously. All nutrients and ingredients were analyzed on dry weight basis. The micro nutrients were expressed in mg /kg. The experiments on the

estimation of nutritive values were performed at the Post harvest Technology Centre at Tamil Nadu Agricultural University, Coimbatore-3.

Performance testing of *T. giganteum* in Farmer's field at different locations. Different strains of *C. indica* and *T.giganteum* were subjected to growth and yield performance trials at three commercial mushroom farms *viz.,* (Farm 1 -Sujiie Mushroom Farm, Farm 2 -Sun Mushroom Farm, Farm 3- Kongu Mushroom Farm of Erode district) as per the method described earlier. The cropping room conditions of 30-35˚C with 75-80 % relative humidity were uniformly maintained in all the farms. The performances of the mushrooms were tested in different commercial farms at different locations so as to test/judge the acceptance of the consumers. The observations on days for spawn run (DFSR), days for pin head formation (DFPF), days for first harvest (DFFH), days to complete three harvests (total cropping period), total yield, and bioefficiency were recorded. Harvested mushrooms were cleaned and packed (250 g) separately in perforated polythene bags and placed under natural conditions (room temperature).Under refrigerated condition they were placed in non-perforated polythene bags and observations on keeping quality were recorded.

All the experiments conducted during the study were laid out based on completely randomized block design (CRD). Statistical software (AGRES) was used for the analysis of the data.

RESULTS AND DISCUSSION

Collection of mushroom strains and their morphology. The *Calocybe indica* strains *viz.,* Ci (P), Ci (N) had long stipe measuring 3-6.7 cm in diameter with centrally attached stipe similar to the commercially cultivated *Calocybe indica* var.APK 2. The stipe of *Tricholoma* is sub globose, short, 3.9- 4.2 cm with small hair like white outgrowths on the stipe initially and in matured mushrooms the stipe looked smooth at the time of harvest (approximately five days after pin head formation) (Fig. 1).

Figure 1. Morphology of *Tricholoma giganteum*

Yield performance studies of wild mushroom strains. The mushroom was cultivated in partially sunken polyhouses roofed with blue coloured high-density polythene sheet, where a temperature of 30-35˚C with 75-80 percent relative humidity was maintained (Figure 2.) A maximum mean yield of 884.9 g/bed with a biological efficiency of 177% was observed for various strains of *T. giganteum* and is compared to *Calocybe indica* var. APK2 (Table 1).

Figure 2. Cultivation of *T. giganteum*

Table 1. Performance of wild *C. indica* isolates and *T. giganteum* at the Mushroom Research Laboratory, TNAU, Coimbatore.

Strain	DFSR	DFPF	DFFH	Yield (g/500g dry paddy straw)	Bio efficiency (%)
C. i(APK2)	14.6	8.3	13.3	727.0	145.3
C. i(N)	16.6	10.3	15.3	662.3	132.4
C. i(P)	14.6	7.3	12.3	861.2	172.4
T. giganteum	14.3	7.6	12.6	884.8	176.9
CD at (0.05)				12.28	
SEd				5.92	

Pooled mean of two trials, SEd: Standard Deviation

Molecular characterization. Amplification of the ITS regions of *Tricholoma* with IT -1 and ITS-4 primers showed 91 % homology with *T.gignateum.* The sequences were submitted to NCBI and given Gen Bank accession number 120872.

Performance testing of *T. giganteum* at farmer's location. This study was conducted in different farms at different locations under controlled conditions (with temperature range of 30-

35˚C with 75-80 % relative humidity) so as to test the yield performance and the consumers acceptability of the mushroom species.

The results of yield trials conducted at Farm 1 indicated *T. giganteum* as the best performer based on maximum mean yield (820 g /bed) and bio efficiency (164%) compared to *C. indica* var. APK2 with 172% bioefficiency (Table 2). Also, at Farm 2, *T.gignateum* performed with significantly higher yield of 847 g/ bed and bioefficiency of 169% compared to *C. indica* var. APK 2 (827 g/ bed; 165% bioefficiency) (Table 3). At Farm 3, T. *gignateum* recorded a yield (870 g/ bed; 174% bioefficiency) on par with *C. indica* var. APK 2 (Table 4). In all three trials, there was no variation in DFSR, DFPF and DFFH. The nutritive values analyzed for *P. djamor* showed the presence of all essential nutrients with a calorific value of 19.8 Kcal/100 g of fresh mushrooms (Table 5).

No incidence of pest and disease was recorded. The mushrooms could be stored under room temperature for two days and under refrigerated storage for 6 days without any microbial spoilage and liquefaction. The mushroom *T. giganteum* resembles milky mushroom in morphology. However, the stipe is sub-globose in *T. giganteum* where as in milky mushroom the stipe is elongated. The possibility of commercial cultivation of a new edible mushroom, *Tricholoma lobayense* closely resembling *C. indica* was reported [21].

Table 2. Yield performance of wild *C. indica* isolates and *T.giganteum* at Farm 1**.**

Strain	DFSR	DFPF	DFFH	Yield (g/500g dry paddy straw)	Bio efficiency (%)
C. i (APK2)	15.3	8.6	13.3	804.0	160.8
C. i (N)	16.3	10.3	15.3	682.6	136.5
C. i (P)	14.6	7.6	12.00	730.49	146.0
T. giganteum	14.3	7.3	11.3	820.0	164.0
CD at (0.05)				14.25	
SEd				6.80	

Mean of four replications , SEd: Standard Deviation

Table 3. Yield performance of wild *C. indica* isolates and *T.giganteum* at Farm 2.

Strain	DFSR	DFPF	DFFH	Yield(g/500g dry paddy straw)	Bio efficiency (%)
C. i (APK2)	14.0	8.6	13.00	827.0	165.4
C. i (N)	16.0	10.3	15.3	693.8	138.8
C. I (P)	14.3	8.3	13.3	740.7	148.1
T. giganteum	13.6	7.3	12.3	847.0	169.4
CD at (0.05)				12.33	
SEd				5.23	

Mean of four replications, SEd: Standard Deviation

Table 4. Yield performance of wild *C. indica* isolates and *T.giganteum* at Farm 3.

Strain	DFSR	DFPF	DFFH	Yield(g/500g dry paddy straw)	Bio efficiency (ù)
C. i (APK2)	14.6	8.6	13.6	866.0	171.2
C. i (N)	17.0	10.6	15.3	617.0	123.5
C. I (P)	14.6	7.6	12.0	771.0	154.2
T. giganteum	14.3	7.3	11.6	870.0	174.0
CD at (0.05)				9.91	
SEd				4.68	

Mean of four replications, SEd: Standard Deviation

Table 5. Nutritive value of *Tricholoma giganteum*

Parameter	Nutritive values on dry weight basis
Moisture$	86.20
Crude protein#	32.9
Carbohydrate#	11.8
Crude Fat#	0.91
Crude fibre#	20.71
Ash#	8.32
Iron*	5.60
Manganese*	1.18
Zinc*	1.38
Copper*	1.10

$ - Presented in fresh weight basis
# - % dry weight basis
* - Presented in mg/kg (dry weight basis)

CONCLUSION

Development of mushroom strains well adapted to the hot climatic plains of India with suitable simpler cultivation technology, higher yield potential and prolonged shelf life are the present day needs of commercial cultivation. Being a tropical mushroom, *T. giganteum* has greater scope for commercial exploitation throughout the globe. The simple production techniques with sustainable yield, increased shelf life, attractive color, flavor and shape are the special features of this new edible mushroom.

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