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Physiological and cultivation requirements of *Trametes versicolor*, a medicinal mushroom to diversify Indian mushroom industry

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ABSTRACT

Tramete versicolor (known as "turkey tail") is one of the most potent, and the best studied, of all medicinal mushrooms. This mushroom is known to possess anti cancer, anti viral properties and stimulates the immune system. It is native to tropical, subtropical and temperate zones and is highly adaptive. No attempt had been made in India to cultivate this precious mushroom. *T. versicolor* was collected from Sampaje forest area of Coorg, Karnataka (Western ghats), India. It occurred as a classic bracket fungus with multicolored, closely concentric zonations with slightly hairy surface, the stem was central and short. Pure culture was made on MEA and the cultural requirements were studied. The ambient temperature and pH for the mycelial growth was 25 °C and 4.5 to 6.0 respectively. The maximum mycelial growth was observed on MEA (14.93 mm/day), mycelium was pure white in color and moderately fluffy. The spawn was made on sorghum grain and the fructification was attempted on sawdust 90% + rice bran 10% substrate formulation. Spawn run was completed within 18–20 days at 25± 2 °C. Fructification was observed at 25 ± 2 °C and 80-85% R H. This is the first report of successful cultivation of *T. versicolor* from India. Successful cultivation of this mushroom will play a key role in the diversification of mushroom industry in India.

Key words: Characterization, Cultivation, India, *Tramete versicolor*, Western Ghats

Mushroom production in India started more than five decades ago. Although, the Indian mushroom industry has come a long way, its full potential is yet to be exploited. Until recent years mushrooms were considered only as a food material in India. Even as food, mushroom industry in India has only 4 mushrooms (button mushroom, oyster mushroom, paddy straw and milky mushroom) for commercialization. India is blessed with varied agro - ecological characteristics, abundance of agricultural wastes and manpower making it most suitable for the cultivation of all the types of mushroom (Tewari 2005). Cultivation of medicinal mushroom, *Ganoderma lucidum* has been reported from various labs in India (Rai 2003, Veena and Pandey 2006, Mishra and Singh 2006) and the medicinal mushrooms are gaining importance in the country. *Trametes versicolor* is one of the most potent and the best-studied medicinal mushroom (Stamets 2000). It has been renowned in Japan and China as medicine for thousands of years. The two immunologically active fractions are PSK, a protein bound polysaccharide and PSP, a polysaccharide peptide. High molecular-weight fractions from the mycelium have been studied in human clinical trials, especially polysaccharide Krestin (PSK), which is an approved drug paid for by national health care in Japan. PSK is given orally (often 3 grams/day), along with several chemotherapy protocols (Hobbs 2004). "PSK", accounted for 25.2% of the total national expenditure for anticancer agents in Japan. Studies conducted in human beings showed that cancer patients who received PSK daily had shown increased interferon production. Apart from these uses the mushrooms can be effectively utilized for bioremediation, enzyme production, aesthetic purposes and SMS utilization. With an objective of diversifying Indian mushroom industry, exploratory works are being conducted at IIHR, Bangalore, India. The mushroom, *T. versicolor* was collected from Coorg, Karnataka. The cultural conditions were studied and an attempt had been made to cultivate the mushroom.

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MATERIALS AND METHODS

The mushroom was collected from Sampaje forest area, Coorg, (Western Ghats), India during 2004–09. The isolation was done on Malt Extract Agar (MEA- HIMEDIA) medium and maintained on the same medium.

To find out the optimum temperature for mycelial growth

the culture was allowed to grow at 15, 20, 25, 30, 35 and 40º C. Five media, Malt Extract Agar, Raper's Complete Medium, Potato Dextrose Agar, Oatmeal Agar and Potato Malt Agar were tested for mycelial growth. Five mm discs of actively growing culture were cut and were kept in the center of the Petri-dishes. The linear mycelial growth was measured at 24h interval. The isolate was allowed to grow within a *p*H range 3.0 to 8.5 (at an interval of 0.5 *p*H). The broth of the best medium for mycelia growth was used for the study. The dry mycelial weight was taken and compared. The data was analyzed statistically.

Mother spawn was made on sorghum grain. The substrate consisting of sawdust and rice bran (90:10) was used for the fructification. Bag system was adopted and moisture level of the substrate was maintained at 65%. Substrate (1 kg wet substrate) was filled in polypropylene bags (40 × 20 cm and 150 gauges) and plugged with non-absorbent cotton after putting a plastic (PVC) ring at the neck. The substrate was sterilized at 15 psi for 2 h. The sterilized bags were inoculated (aseptically) with sorghum grain spawn (@6%) and incubated in dark at 25 ±2 °C. After complete colonization, the bags were removed and kept in cropping rooms. A temperature of 25±2°C and RH of 80–85% was maintained in cropping room. Light was provided for 12 hr/day and to facilitate ventilation in rooms, doors were kept open for 30 min and three times in a day. Observations on spawn run period, days required for primordial initiation, number of fruit bodies, biological efficiency, fruit body characters like pileal width, thickness and stalk length were recorded.

RESULTS AND DISCUSSION

Habitat

Mushroom was located on dead hardwoods and they occurred in groups. Classic bracket fungus with close concentric zonations, slightly hairy surface with a wavy outer margin (Fig 1). Polyporous mushroom with short stalk and pileus had alternate creamish white and brown zones. Spore print was white in color and spores were cylindrical/ elliptical shape. Spores were smooth and slightly curved. The spores had an average dimension of 4.69 µ (3.46 – 6. 05 µ) × 2.54µ (1.9–2.7 µ). This mushroom is described as mushroom of highly variable in color with tones of grays and browns, often with bluish, greenish, reddish or whitish zones (Hobbs 1995).

Cultural characteristics

The mushroom was cultured on MEA medium using tissue culture technique. Mycelium was whitish, medium fluffy, sheet like and leathery. The cultural requirements of the isolate were studied (Figs 2, 3, 4). The optimum temperature was 25 ºC (14.93 mm/day), followed by 30º C (12.73 mm/day). The *p*H of the medium did not have much influence on the mycelial growth. It grew within a *p*H range

Fig 1 Natural occurrence of *T. Versicolor*

of 3–8.5, however the mushroom preferred a *p*H of 4.5 to 6.0. The highest growth rate was noticed on MEA (14.93 mm/day), followed by RC medium (13.13 mm/day). Information about the cultural requirements of the species is essential for the maintenance of the culture and cultivation.

Cultivation

Spawn was made on sorghum (*Sorghum bicolor*) grain amended with chalk powder 4% ($CaCO_3$). The substrate used for cultivation was sawdust and rice bran (90:10). The

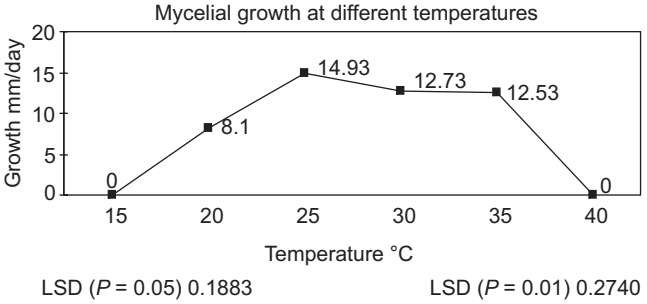

Fig 2 Mycelial growth of *T. versicolor* at various temperatures

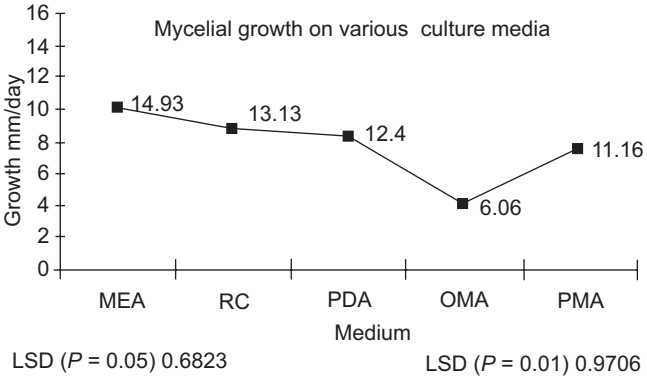

Fig 3 Mycelial growth of *T. versicolor* on various medium

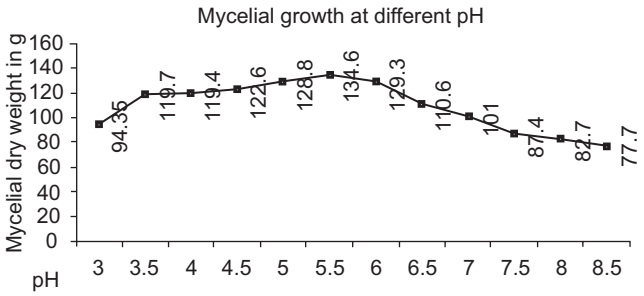

Fig 4 Mycelial growth of *T. versicolor* at different pH levels

moisture level was maintained at 65% and sterilized at 121°C and 15lb for 90 min. Spawning was done at 6% of wet weight basis and incubated at 25±2 °C. The spawn run was completed within 18 – 20 days (Table 1). The spawn run bags were opened and kept at 24 ± 2 °C and 80-85% R.H and primordial initiation was observed within 14–20 days of opening (Fig 5). It took 30–45 days for the full development of the fruiting body (Fig 6). This mushroom is considered as one of the mushroom, which can decompose most hardwoods (Stamets 2000) and recommended oak, alder, poplar, elm, eucalyptus woods for the cultivation. However, in India availability of these woods are limited and in present attempt, successful cultivation was done on sawdust mixture which was collected from local sawmills.

The fruiting bodies were classical bracketed ones with concentric zonations as occurred in the nature and often formed overlapping of fruit bodies. The fruiting bodies were

Table 1 Details of fruit body formation in *T. versicolor*

Bag no.	Spawn run (days)	Primordial initiation (days)	Yield (g)	No of fruit bodies	BE (%)
1	18	14	66.3	12	18.94
2	19	19	64.4	18	18.40
3	20	15	71.3	17	20.37
4	18	16	68.3	26	19.51
5	18	19	66	22	18.86
6	18	20	68.3	24	19.51
7	19	17	67.2	23	19.20
8	20	18	70	25	20.00
9	20	17	74.2	16	21.20
10	18	19	71.2	24	20.34
11	19	20	65	19	18.57
12	18	15	69.2	10	19.77
13	19	19	66.8	36	19.09
14	18	17	73.6	12	21.03
15	19	20	72.6	30	20.74
16	18	19	67.5	23	19.29
17	19	20	65.9	28	18.83
18	18	16	68.4	17	19.54
Average	18.66	17.77	68.67	21.22	19.62

Fig 5 Primordial initiation of *T. versicolor* (cultivation at IIHR, Bangalore)

Fig 6 Fructification of *T. versicolor* (cultivation at IIHR, Bangalore)

whitish with light to dark brown zonations. Undersurface covered with whitish pores. On maturity, pore area changed to buff color. The Biological Efficiency (B.E) ranged from 18.4 – 21.2% with an average fruit weight of 2.7g (0.5 – 11.2g) and 60 - 65% yield was obtained during first flush and 35 – 40% from second flush. The number of fruit bodies obtained from 1 kg wet substrate varied from 10 – 36. The average pileus width was 51.0mm (17.0 – 100 mm) and it was very thin (1.6 mm) as the name indicates ("*Trametes*" means one that is thin). Average stalk length had a measurement of 9.0 mm (2.0 – 20.0 mm).

Of all the mushrooms used today for their medicinal qualities, more research has been performed on this species than any other, including shiitake (*Lentinula edodes*), or reishi (*Ganoderma lucidum*). PSK, the pharmacologically important constituent from the species has been used both orally and intravenously as an immune adjuvant in clinical

medicine (Hobbs 1995). As a super oxide and hydroxyl radical scavenger, against many human cancers, against nasopharyngeal carcinoma are some among the long list of pharmacological properties. In addition, its bright colour and classical bracket nature makes it suitable for aesthetic purpose (Pandey and Veena 2007).

Trametes species are abundant in Western Ghats region of India. They are highly adaptive and easy to culture and cultivate. This mushroom is circumpolar and widely distributed throughout the temperate, subtropical and tropical regions of the world (Stamets 2000). The growth requirements of the species are highly suitable for Indian condition. With all favorable conditions required for cultivation of the species, we had not yet started to cultivate the mushroom. Considering the enormous uses of the species and alarming rate of increase of cancer patients in the country, successful cultivation of indigenous isolate of this mushroom will of great use. In addition to its excellent medicinal property, it also has potential for bioremediation, enzyme production, aesthetic purposes and SMS utilization. Thus the cultivation of the mushroom will play a key role in the diversification of mushroom industry in India. However, collection of more species and domestication, correct identification of the species and refinement of cultivation technology is required to tap exact potential of the mushroom.

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