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Effect of pruned tea leaves on the yield and nutritional quality of two species of *Pleurotus* in North Bengal

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The study was conducted to analyse the efficiency of pruned tea leaves as a substrate for cultivation of *Pleurotus* species in North Bengal. Pruned tea leaf substrate was found to increase the production of *Pleurotus* species along with an increase in the nutritional components. Higher spawn run and yield was observed in pruned tea leaves single as well as in combination with paddy straw substrate. The biochemical constituents were also high in both the cases but higher when cultivated in pruned tea leaves. Fruitbody were dried and powdered and it was also observed that the powder was also contains significant good amount of total soluble protein, carbohydrates, total lipid and dietary fibre.

Key words: Pruned tea leaves, cultivation, *Pleurotus ostreatus*, *P. sajor-caju*, yield, dietary fibre

INTRODUCTION

Tea is one of the most important plantation crops in North East India. About 1233 million kg produced in the year 2015-2016, was the highest production in the last few years. Of this, about 329 million kg is from North Bengal (Tea Board of India, 2016). Oyster mushroom can be grown in a wide range of lignocellulosic substrateslike paddy straw, wheat straw singly or in combination in different ratio (Philippoussis *et al.* 2001;Das and Mukherjee, 2007). Sher *et al.* (2011)stated that using agro wastes in mushroom production helps in waste management. Patel and Trivedi (2015) also demonstrated that *Pleurotus* spp. can be grown on various agricultural wastes and in different agro climatic regions and thus it is becoming the most popular mushroom in India and many other countries. Pruning of tea leaves is commonly practiced in tea cultivation in North Bengal, which is an important step for production of new tea leaf twigs. This study is intended to evaluate the effect of pruned tea leaves as alternative substrates for cultivation of *Pleurotus* species in North Bengal and also to evaluate the nutritional components in fresh fruitbody as well as in sundried powdered fruitbody.

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MATERIALS AND METHODS

Mother culture

Mother culture of *Pleurotus ostreatus* and *P. sajor-caju* was procured from the Directorate of Mushroom Research (ICAR) Solan and maintained in Potato Dextrose Agar medium.

Preparation of Spawn

Wheat grains were used for the preparation of spawn. Wheat grains were boiled for 20 min., water drained and allowed to cool. To this, 0.5% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$ were added and mixed well. The grains (200 g) were filled in polypropylene bags and plugged with non-absorbent cotton and autoclaved at 121°C for 1h. After cooling,the grains were inoculated with actively growing mycelium of the *Pleurotus* spp. and incubated at 25-28°C for mycelial growth for 14 days.

Cultivation of mushroom
Substrate preparation

Pruned tea leaves were collected from the experimental tea garden of Immuno-Phytopathology laboratory, Department of Botany, University of

North Bengal and washed with tap water. Then the tea leaves were sun dried for 7-10 days. Fully dried tea leaves were soaked in water overnight. The substrate was washed again for 2-3 times and pasteurized at 55-65°C for 30 min. Then it was allowed to cool at room temperature.

Spawning

Layer spawning was done using 100g/ kg each of the cooled pasteurized substrates-paddy straw alone, pruned tea leaves alone and in combination with paddy straw (1:1 ratio) separately kept in bags and were tightly closed. Small holes were made in each bag for aeration and excess water drainage. The bags were then incubated at 20-30°C for 10-12 days. After incubation, the white mycelia covered the whole substrate. Then the plastic was removed and 80-90% moisture was maintained by spraying water on the substrate for 2-3 times in a day for pinhead initiation (Sarker *et al.* 2007).

Harvesting of fruit body

The pinhead appeared within 4-5 days of opening of the bags. Mature fruiting bodies can be harvested within a few days. The bags were kept with adequate moisture and humidity for subsequent fruiting.

Estimation of Moisture Content

100 grams of fresh mushroom was weighed and dried in hot air oven at 100-105°C and then cooled at room temperature. Moisture content of mushroom was estimated following Raghuramulu *et al.* (2003). Moisture content was calculated as
Moisture content (%) = (initial weight–final weight) × 100/weight of sample

Extraction and estimation of sugar

One gram of fresh mushroom tissue was crushed with 95% ethanol and the alcoholic fraction was evaporated in boiling water bath. Then the fraction was collected and the volume made up to 5 ml using distilled water. Then it was centrifuged at 10,000 rpm for 15 min and the supernatant was collected for estimation .

Total sugar

1 ml of extracted sample was taken and 4 ml of Anthrone's reagent was added and incubated in boiling water bath for 10 min. Then it was cooled in tap water and observed at 620 nm in colorimeter.

Reducing sugar

Ethanol (80%) extract was used for estimating the reducing sugar according to Nelson-Somogy's methodology described by Plummer 1973. Extract (2 ml) was mixed with 2 ml of alkaline copper tartrate and boiling was done. Determination of reducing sugar using Arsenomolybdate was carried out at 620 nm.

Estimation of soluble protein content

1gm tissue was crushed in sodium phosphate buffer (pH 7.2) at 4°C and centrifuged at 10000 rpm for 15 min. Supernatant was collected and estimated following the method as described by Lowry *et al.* (1951). To 1ml of protein sample 5ml of alkaline reagent (1ml of 1% $CuSO_4$ and 1ml of 2% sodium potassium tartarate, added to 100 ml of 2% Na_2CO_3 in 0.1 NaOH) was added. This was incubated for 15 min. at room temperature and then 0.5ml of 1N Folin Ciocalteu reagent was added and again incubated for further 15 min. following which optical density was measured at 720 nm.

Determination of total lipid content

Total lipid was determined following the methodology described by Folch *et al.* (1957). 5gm of grinded mushroom was suspended in 50 ml of chloroform: methanol (2:1v/v) mixture then mixed thoroughly and kept for 72 hours. The solution was filtrated and centrifuged at 10000 rpm for 15 min. The upper layer of methanol was removed by pasteur pipette and chloroform was evaporated by heating. The remaining was the crudelipid.

Determination of dietary fibre

Dietary fibre content was estimated from the Arbro Pharmaceuticals, New Delhi, following the methodology of Association of Official Analytical Chemists (1995). 10 gm mushroom powder was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered and washed with hot water until the total acid removed from the residue. Then 200 ml of

boiling 0.313 N NaOH was added and boiled for 30 minutes and again the mixture was filtered and washed with hot water till free from alkali. It was then dried overnight at 80-100°C and weighed (We) in an electric balance. Then the sample was heated in a muffle furnace at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represented the weight of crude fibre.

Crude fibre (g/100 g sample) = [100 – (moisture + fat)] × (We-Wa)/Wt of sample

RESULTS AND DISCUSSION

In the present investigation using pruned tea leaves as alternative substrates for cultivation of oyster mushroom it was observed that the mycelia colonize rapidly over the tea leaves. However, the growth rate was higher in case of paddy straw alone substrate and in combined substrates with pruned tea leaves and paddy straw (1:1 ratio). Upadhyay *et al.* (1996) reported that the tea leaves along with the straw helps in rapid mycelial growth of *Pleurotus* sp. Development of fruiting body was rapid in case of tea leaves alone substrate as well as in combined substrates (Fig. 1). Production of *P. ostreatus* was more in case of combined substrate than that of paddy straw or pruned tea leaves as alone (Table 1). *P. sajor-caju* was also cultivated using the pruned tea leaves as substrate. Using pruned dry tea leaves as substrate, rapid growth of mycelium and higher yield of *P. sajor-caju* was noted (Fig. 2). Further, the duration for complete mycelial growth become reduced and an increase in pin head formation was observed in pruned tea leaves substrate over mixed substrate. Gulser and Peksen (2003) also reported that using of tea waste as a supplement helps in rapid mycelial growth as well as high yield. Chukowry *et al.* (2009) suggested that the use of tea waste at 25% ratio showed better growth and yield. Yang *et al.* (2015) reported that the use of tea wastes in combination with other substrates in the ratio of 60-40% for the cultivation of *P. ostreatus* shows better yield.

Moisture content of the fruiting body at different stages were evaluated. Mature pileus grown in pruned tea leaves substrates showed highest moisture content. Results (Fig. 3) revealed that the

Table 1 : Cultivation of *P. ostreatus* and *P. sajor-caju* using pruned tea leaves as alternative substrate in comparison with paddy straw alone and in combination

Substrate	*Pleurotus ostreatus*					*Pleurotus sajor-caju*				
	Yield (gms)			Total production (gm/kg substrate)	Total Harvest (kg)[a]	Yield (gms)			Total production (gm/kg substrate)	Total Harvest (kg)
	1st flush	2nd flush	3rd flush			1st flush	2nd flush	3rd flush		
Tea Leaves	280	135	65	480	2.660	210	90	45	345	2.135
	390	145	80	615		185	80	50	315	
	300	160	95	555		240	110	45	395	
	265	105	55	425		275	90	25	390	
	130	75	20	225		235	75	20	330	
	210	105	45	360		270	60	30	360	
Paddy straw	290	180	75	545	2.935	310	190	70	570	2.855
	320	130	90	540		320	140	55	515	
	280	140	50	470		290	100	60	450	
	275	100	70	445		250	120	45	415	
	250	120	65	435		275	100	55	430	
	310	130	60	500		290	130	55	475	
Tea leaves + Paddy straw (1:1)	200	120	90	410	3.230	290	150	90	530	3.165
	350	120	50	520		300	220	45	565	
	500	210	115	825		270	190	40	500	
	350	100	60	510		310	100	60	470	
	250	100	45	395		280	275	95	645	
	375	120	70	570		275	100	80	455	

[a]Total production from 6 replicate bags harvested during 3 flushes in each substrates

moisture content was higher in pruned tea leaves as well as paddy straw substrate alone in comparison with combined substrates. Mature pileus possess higher sugar content in compare to other stages of *P. ostreatus* and *P. sajor-caju.* However, total sugar content was higher in *P. ostreatus* than *P. sajor-caju* grown in pruned tea leaves (Fig.4 A,B).

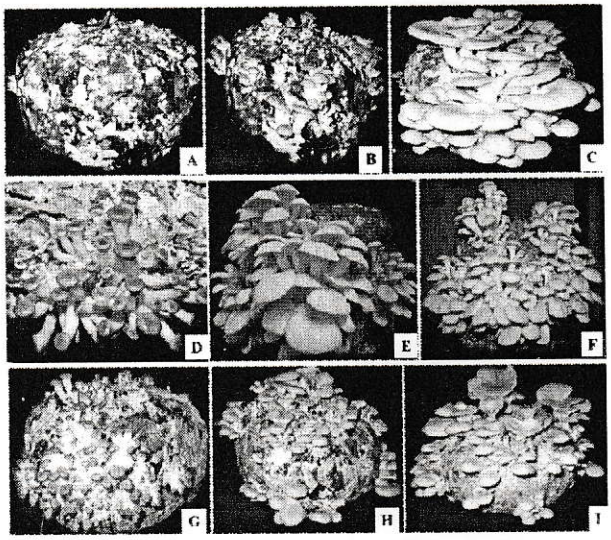

Fig. 1 : Cultivation of *P.ostreatus* using pruned tea leaves substrate(A-C); Paddy straw substrate (D-F) and Pruned tea leaves in combination with paddy straw (G-I)

Patil *et al.* (2008) have shown the effect of different substrate on productivity and proximate composition of *P. florida*. Roy *et al.* (2015) also demonstrated that total and reducing sugar content was higher in mature pileus of *P. djamor*. Protein content of the mature pileus grown on pruned tea leaf substrate was significantly higher in comparison to paddy straw substrate and combined substrates (Fig. 4C). Similar observation was also made by Breene (1990) and reported that the total soluble protein content of oyster mushroom ranges from 190-300mg/gm tissue.

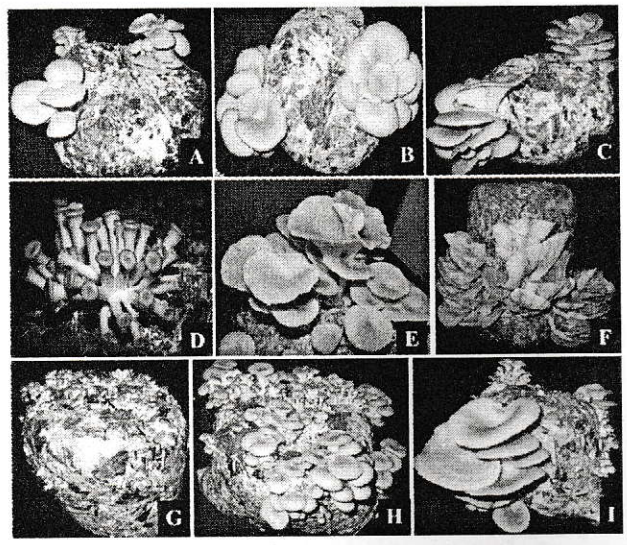

Fig. 2 : Cultivation of *Pleurotus sajor-caju* using pruned tea leaf substrate (A-C), paddy straw alone (D-F) and in combination with paddy straw and pruned tea leaf substrate (G-I)

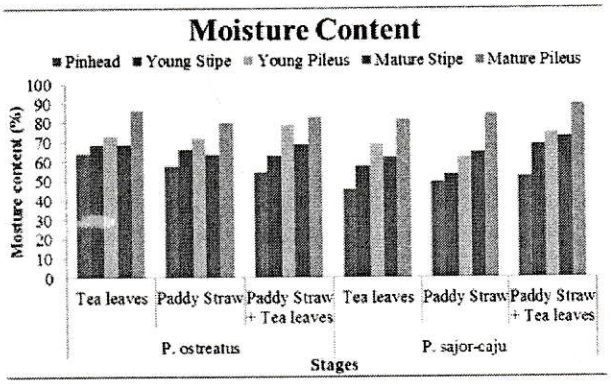

Fig. 3 : Moisture content of various stages of *P.ostreatus* and *P.sajor-caju* cultivated on different substrates

Lipid content was also found to be higher in fruitbodies grown in combined substrates than single substrate. (Fig.5A).

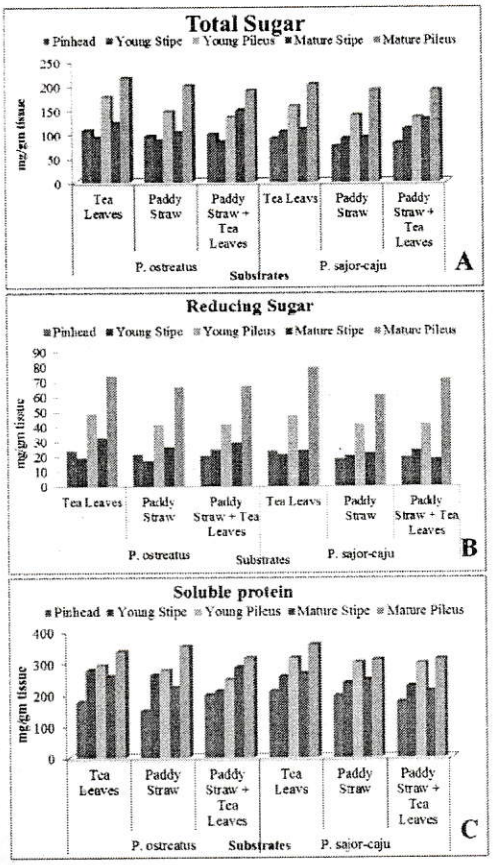

Fig. 4 : Total sugar (A), reducing sugar (B) and total soluble protein(C) content of *P.ostreatus* and *P.sajor-caju* cultivated on different substrates

Dietary fibre was also estimated and it was found that the fibre content of fruiting body of pruned tea leaves substrate was higher in *P. sajor-caju* than *P. ostreatus*. Mattila *et al.* (2000) explained that

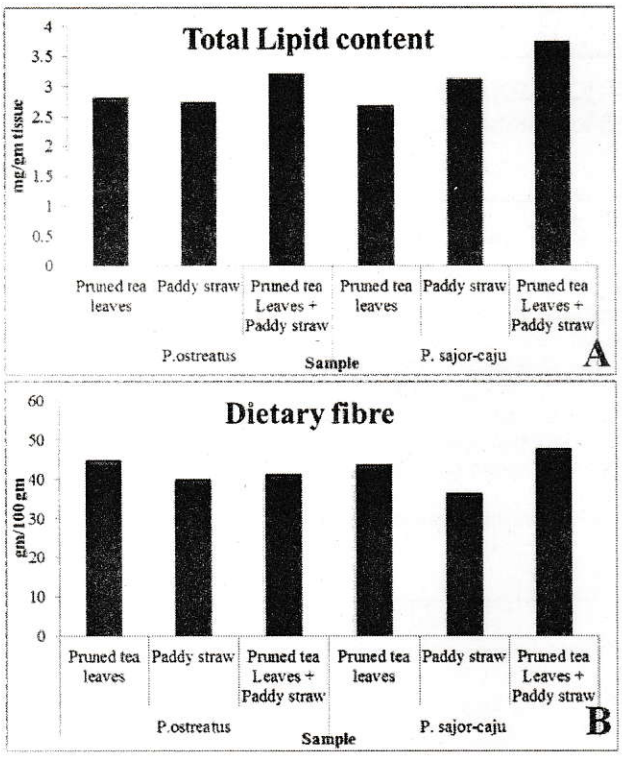

Fig. 5 : Total lipid content (A) and dietary fibre (B) of *P.ostreatus* and *P. sajor-caju* cultivated on different substrates

dried mushrooms contain about 22% protein, which includes most of the essential amino acids, 5% fat, mostly in the form of linoleic acid, 63% carbohydrates including fibre and 10% minerals. Manzi *et al.* (2004) reported that dietary fibre content of *P. ostreatus* was about 47.3% and *P. eryngii* contains about 34.6% dry weight basis.(Fig.5B).

Tea is one of the major economic crops in North Bengal. The present study intends to analyse the feasibility of utilising the pruned tea leaves for *Pleurotus* cultivation in the regions of North Bengal. The results of the study indicate that the pruned tea leaves can be utilized as an effective alternative substrate for cultivation of *Pleurotus* species. Further, the study paves way to better utilization of this underutilised substrate.

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