

Bioconversion of Agricultural Wastes for Production of Milky Mushroom (*Calocybe indica*)

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Abstract

In order to study the effect of different substrates on the growth and yield of *C. indica*, an experiment was carried out with four different substrates viz., wheat straw, paddy straw, chopped leaf of sorghum and saw dust. The substrate of paddy straw was found significantly superior than other substrates by producing (955.66 gm/bag). The mixture of paddy straw + wheat straw was second best substrate where it produced (893.66 gm) mushroom/bag followed by wheat straw (793.67 gm), mixture of wheat straw + chopped leaves of sorghum (584.33 gm) and mixture of mixture of saw dust + wheat straw (535.33 gm). Spawn run period between (23- 27days), initiated of pinhead also recorded in the range of (45 – 52 days). Then the crop was harvested at the stage of complete mature fruit bodies. The 1st flush was harvested in the range (34-37days). Maximum No. of fruit bodies per bag were obtained from paddy straw substrate. Paddy straw provide better yield among them. Paddy straw and wheat straw was highly significant. Thus, paddy straw was best substrate for the cultivation of the milky mushroom (*Calocybe indica*) because it was given better yield and good quality than the other substrate.

Key words: *Calocybe indica*, substrates, growth and yield

Introduction

Huge quantities of agricultural wastes and other organic wastes are generated annually through the activities of agricultural, forest and food processing industries. These agricultural wastes and other organic wastes are abundantly available in our country. In India, during 1993-94 production of wheat straw and paddy straw was approximately 110.6 million tones and 153.6 million tones respectively (Mathew 1996). The lignocellulosic wastes obtained from the production edible mushrooms will used as an animal nutritive feed stock. Among the various physical, chemical and biological methods used for upgrading the digestibility and nutritive value of agricultural wastes, biodegradation by using white rot fungi including mushrooms have been found promising. The species of *Pleurotus* has ability to secrete hydrolyzing and oxidizing enzymes (Toyama & Ogawa, 1974; Daugulis and Bone, 1977; and Rajarathnam *et al.*, 1979) which enables them to grow and flourish over wide range of natural lignocellulosic waste materials.

Besides it is estimated that about 355 million tonnes of agricultural wastes are generated

annually and about half (170 millions) of this residue is left out for burning and incorporating soil in manure form. Recently about 385 million tones of agricultural wastes are available in India and half of these agricultural wastes unused. Even if one per cent of this crop residue is used to produce mushrooms, that will help in residues management and India will become a major mushroom producing country in the world (Tewari and Pandey, 2002). The lignocellulosic wastes obtained from the production edible mushrooms will used as an animal nutritive feed stock. Among the various physical, chemical and biological methods used for upgrading the digestibility and nutritive value of agricultural wastes, biodegradation by using white rot fungi including mushrooms have been found promising. The species of *Pleurotus* has ability to secrete hydrolyzing and oxidizing enzymes (Toyama & Ogawa, 1974; Daugulis and Bone, 1977; and Rajarathnam *et al.*, 1979) which enables them to grow and flourish over wide range of natural lignocellulosic waste materials. The major parts of these agricultural wastes are bunt after harvesting, resulting multifaceted hazards including oxygen defiant environment, respiratory diseases such as allergic, bronchitis, asthma, tuberculosis and poor visibility at night. The useful insects, bio-agents, earthworm and soil microbe are also reduced due to burning of agricultural wastes and its heating on soil surface. According to survey, in some places these agricultural wastes are spreads on the cultivated land but it is not well decomposed and they created the problems during farming. The main concerned of our planners to remove these air pollution and soil pollution for environmental protection. This can be done by scientific utilization of resources and bioconversion for mushroom production.

Around 800 million people living in 46 countries are malnourished, 40,000 die everyday of hunger and hunger related diseases (Swaminathan 1995). In this context the mushroom cultivation represent one of the economically viable processes for the bioconversion of agricultural and agro-industrial wastes in to protein rich food making it a potent weapon against malnutrition in developing countries like India which has lowest per capita consumption of protein in the world (Sohi, 1982, Wood, 1989, Chang & Miles 1989, Buswell & Chang, 1993).

In this regard, Poppe (2000) have to considered the multilateral message of agricultural wastes conversion into mushrooms in the following categories:

1. Provision of food
2. Creation of jobs
3. Enhancement of family income
4. Control burning of waste and curb global warming
5. Lowering air pollution and CO₂ level
6. Cleaning the field, road sides and forest margins
7. Protection of natural mushrooms flora
8. Forest fire prevention
9. Spent mushroom substrates for garden compost bioremediation purposes

Numerous reasons for growing more mushrooms by agricultural residues and by-products as approved by Chang & Miles 1989; Stamets 1993 & Poppe 1995. -

1. Decreasing the mountain range of wastes.
2. Most mushrooms are fast productive and can be grown allover the year in temperate zones with heating in winter and in tropical zones with protection against dry hot seasons.

3. No fight for field is necessary because mushroom cultivation needs only small spaces and can even grow in vertical layers or on the floor in forests where they don't need one centimeter extra place.
4. Converting the wastes in to mushroom proteins and vitamins, while also mushrooms represent one of the world's greatest untapped sources of tasteful food in the future. And let us not forget the medicinal value of mushrooms species.
5. Mushroom cultivation is labour intensive but labours are readily available in most tropical regions.

The milky mushroom (*Calocybe indica*) was first reported from India by Purkayastha and Chandra in 1974. This mushroom may be successfully cultivated during summer months and therefore, designated as summer mushroom also. It has stout fleshy, milky white; large sporophores with long shelf life. Consumers are liked this mushroom due to its attractive shape, colour and delicious flavour. This mushroom normally grows on the humus rich soil under road side trees and in agricultural fields. In some places they called as “Kudhu” but, popularly known as “Dudhi Chhata”. In rainy season, wild forms of *C. indica* were sold in Calcutta and their edibility was confirmed by Purkaystha and Chandra (1974), Purkyastha (1979), Natural occurrence of *C. indica* in plains of Tamil Nadu and Rajasthan has also been reported by Krishnamoorthy, 1995 and Doshi *et al.*, 1989 respectively.

The nutritive value of *Calocybe indica* is comparable with other mushrooms. Dried sporophores of *C. indica* contains 17.69 per cent protein, 4.1 per cent fat, 3.4 per cent crude fibre and 64.26 per cent carbohydrate. Matured sporocarps contain 4 per cent soluble sugars, 2.9 per cent starch and 7.43 per cent ash. In addition to this, it has most of the mineral salts required by human body such as potassium, sodium, phosphorous, iron and calcium. Due to its alkaline and higher fibre content; it highly suitable for people with hyperacidity and constipation (Doshi *et al.*, 1988). In spite of sincere efforts made by different workers only limited success was achieved on the cultivation of this mushroom until 1998. Krishnamoorthy (1995) and Krishnamoorthy *et al.* (1997) identified a potential strain of *C. indica* occurring in a sugarcane fields near Coimbatore. Later it was released as a new variety called APK-2 from Tamil Nadu Agricultural University and started cultivation under artificial condition (Krishnamoorthy *et al.*, 1998). Cultivation of milky mushroom is becoming popular in Tamil Nadu, Andhra Pradesh, Karnataka, Rajasthan and West Bengal.

Food and Nutritional value of Mushrooms

Mushrooms were believed by the Greeks to give strength to the warriors while Romans regarded them as the 'Food of the Gods'. The Chinese prized mushrooms as health food, the 'elixir of life' etc. In developing countries malnutrition is one of the major problems because most of the population remains under the economic line. Mushrooms are considered to be healthy food because of their relatively high and qualitatively good protein content and because of their good vitamins, minerals and low fat content (Table-1). Mushrooms have been recommended by FAO as food that contributes to the protein nutrition of developing countries which depend largely on cereals.

Mushrooms are recognized all over the world as a good source of protein, vitamins and

minerals for human consumption. It has been used for their medicinal and tonic properties, they have proved beneficial for the patients suffering from hypertension, diabetes and had ailments. Mushrooms contain all the essential amino acids well as the most commonly occurring non-essential amino acid and amides. Mushrooms are good source of vitamins such as vitamin 'B₁' (Thiamine), vitamin 'B₂' (Riboflavin), niacin, biotin and vitamin 'C' (Ascorbic acid). Mushrooms are also good source of minerals such as potassium, phosphorus and sodium and contain low but available form of Iron, Potassium and Sodium ratio is very high which is desirable for patients of hypertension.

Table 1: Proximate composition (Per cent fresh weight) of the cultivated mushrooms

Mushrooms /Vegetable	Moisture	Protein	Fat	Carbohydrate	Fibre	Ash	Calorie
<i>Agaricus bisporus</i>	90.1	2.9	0.3	5.0	0.9	0.8	36
<i>Pleurotus sajor-caju</i>	90.2	2.5	0.2	5.2	1.3	0.6	35
<i>Volvariella volvacea</i>	90.1	2.1	1.0	4.7	1.1	1.0	36
Cabbage	91.9	1.8	0.1	4.6	1.0	0.6	27
Cauliflower	90.8	2.6	0.4	4.0	1.2	1.0	30
Potato	74.7	1.6	0.1	22.6	0.4	0.6	97

Source: Rai and Sohi, 1988.

The success of any agricultural nation depends upon the ability of their people to sustainably convert the natural resources into economic wealth with judicious application of schemes and technologies without endangering the environment. The only solution to feed the coming generation without harming the environment is by organic farming cultivation. White button mushroom (*Agaricus bisporus*) and milky mushroom (*Calocybe indica*) is also growing seasonally by small and marginal farmers in rural area with good results. Today mushroom growing in Tarai region of Uttar Pradesh, Himanchal Pradesh and Haryana has taken the shape of a cottage industry and tones of mushroom reach Delhi from these areas at price reasonable enough for a common man to consumption. Milky mushroom (*Calocybe indica*) provides the good profit for this cultivation. Oyster mushroom and paddy straw mushroom can not grow in the month of April to July due to higher temperature in the hotter regions. Therefore milky mushroom (*Calocybe indica*) can be fitted for cultivation in higher temperature. Milky mushroom (*Calocybe indica*) provides much benefit to the small farmers by this cultivation.

Now the farming system has been changed during last 21st century and farmer taking an interest in latest cultivation techniques so as to get the maximum per capita income. The cost of cultivation is increasing day by day due to high cost of fertilizers, fungicides, insecticides, seeds, labour and irrigation. Fertile land under cultivation of crops is reducing due to urbanization, globalization and industrialization. Due to which many of the farmers becoming landless laborers'. Those farmers don't have land unable to purchase it for crop cultivation because of huge

increment in the cost of fertile land. Climate change and environmental degradation too are playing a substantial role. The world is losing between 5 and 10 million hectares of agricultural land annually due to severe degradation. About 592,000 sq.km of India's land has already deteriorated and this is likely to affect 177 million people.

Marginal land holding farmers unable to purchase all the agricultural and essential inputs because of their higher cost and try to find out an alternative over these problems by getting loans from either by government or private sectors. Due to unfavorable environmental conditions crop failure occurs this leads to suicides of farmers, malnutrition, poverty, hunger, unemployment. India can make rapid progress in mushroom industry by cultivating and commercializing of temperate and tropical mushrooms. But, they are still cultivated on small scale in some pockets on a specific substrates and yield potential is not satisfied due to specific substrates materials. There are need to evaluate various substrates and different casing materials for enhancing better growth behaviour and yield potential of mushrooms. Therefore, present investigation was on effect of substrates on growth behaviour and yield potential of milky mushroom (*Calocybe indica*).

Now these days many scientists gave a new dimension for mushroom production in India. Purkayastha and Chandra in 1974, Purkayastha and Nayak 1979, Krishnamoorthy *et al.* 1997, Kumar *et al.* 2001, Theradimani *et al.* 2001 Sherin *et al.* 2004, Amle *et al.* 2006, Usha 2007, Amle *et al.* 2007, Biswas and Singh 2009.

Materials And Methods

Mushroom culture: Culture of *Calocybe indica* (P.& C.) was obtained from G.B. Pant University of Agriculture and Technology, Udham Singh Nagar (Uttarakhand). This culture was sub-culture and maintained on PDA medium in a BOD incubator at $25 \pm 2^\circ\text{C}$ temperature.

Spawn preparation: The spawns of *Calocybe indica* was prepared on wheat grains (*Triticum aestivum* Linn.). Well cleaned wheat grains were water soaked for over night and boiled until grains become soft. After boiling excess water was drained off and the grains were cooled in plastic tray. These cooled grains were mixed with 2% chalk (calcium carbonate) and 2% gypsum (calcium sulphate). Gypsum and chalk were added to avoid clumping of grains. This mixture (250 g/ bottle) was filled in the cleaned 500 ml saline bottles and sterilized in autoclave at 121°C temperature (15 lb) for 1hr. These bottles were allowed to cool at room temperature and then inoculated with mycelial bits of 7 days old cultures of *Calocybe indica* respectively. These inoculated bottles were incubated at $25 \pm 2^\circ\text{C}$ temperature in a B.O.D. incubator for mycelial growth and development. The bottles were shaken at 4 days interval to spread the mycelium among the grains. The mycelium completely impregnated the grains within 2 weeks.

Substrates preparation: In order to study the effect of different substrates on the growth and yield of *C. indica*, an experiment was carried out with four different substrates viz., wheat straw, paddy straw, chopped leaf of sorghum, saw dust. These selected straw and saw dust were soaked in water for 15 hours and then excess water was drained off from substrates. These moist substrate were sterilized by autoclave at 10lb pressure for $\frac{1}{2}$ hour.

Spawning: Well prepared paddy straw, wheat straw, chopped leaf + wheat straw (1:1), wheat straw + paddy straw (1:1) and saw dust + wheat straw (1:1) were spawned with spawn of *Calocybe indica* @ 3% moist weight basis. These spawned substrates were filled in polythene

bag, (50 x 55 cm). The upper surface of substrate was covered with paper sheet within the polythene bags. These mushroom bags were placed vertically in growing chamber where temperature ranges between 28-35°C. Each bags contain 4 kg moist spawned substrate. Mushroom bags were completely colonized by mushroom mycelium within 15-20 days.

Casing: Milky mushroom needs casing for fruit body initiation. After completion of spawn run or mycelial growth in the beds, the 3 cm thick casing layer spread on the surface of mushroom bed. These casing materials were prepared by sterilized clay loam soil and two years old farm yard manure (1:1) ratio.

Cropping: Mushroom beds were sprayed regularly with water to maintain sufficient moisture level in the casing surface. Pinheads appear ranged between 44 to 52 days after spawning and they were ready for harvest within another one week. After the first harvest, the casing medium is gently ruffled, slightly compacted blank, sprayed daily with water.

Observation and measurement: Growth behaviour such as spawn run period, initiation of pinhead and harvesting of fruit bodies were observed in days. The morphological parameters of fruit bodies and yield of mushroom were also measured during investigation.

- A. Time taken in days: Spawn run period, initiation of pinhead and harvesting of flushes
- B. Morphological parameters of fruit bodies: Cap diameter (cm), stalk length (cm), weight of sporophore (gm), total no. of fruiting bodies per bag (gm), full pinhead (gm), premature fruit body (gm), half mature fruit body (gm), mature fruit body (gm), over mature fruit body (gm)
- C. Yield in gm: Yield of different flushes and total yield

Statistical analysis of data: Each treatment was kept for three replications and data were analyzed statistically following standard procedure to draw the conclusion (Panse and Sukhatme, 1967).

Results and Discussion

Effect of substrates on growth behaviour of milky mushroom (*Calocybe indica*)

Spawn run period: The data presented in the (Table 4) indicated that spawn run period was very fast on Paddy straw substrate, where it took in (23 days) followed by mixture of Wheat straw and Paddy straw substrate (25 days). Mixture of Saw dust and Wheat straw was taken maximum time for spawn run period. Wheat straw and mixture of wheat straw + chapped leaf of sorghum were colonized by mushroom mycelium in (26 days). Paddy straw substrate was better among themselves because that requires minimum time spawn run period (i.e. 23 days). Paddy straw and Saw dust + Wheat straw have much significant differences.

Initiation of pin head: The mushroom pin head were first initiated (44 days) from Paddy straw substrate followed by mixture of paddy straw + wheat straw (47 days), wheat straw (49 days), mixture of wheat straw + chapped leaf of sorghum (51 days) and mixture of saw dust + wheat straw (52 day). Paddy straw, wheat straw + paddy straw and Saw dust +wheat straw have significant difference between each other.

Harvesting of flushes: The first flush from paddy straw substrate was harvested in (50 days) and it was followed by mixture of paddy straw +wheat straw where it harvested in (52 days). The wheat straw was taken much time (56 days) for flush harvesting. The result was significantly difference between paddy straw and mixture of saw dust + wheat straw. The second flush was first

harvested (66 days) from mixture of wheat straw + chapped leaf of sorghum followed by paddy straw (67 days). The maximum cropping period was recorded as (72 days) from mixture of wheat straw + saw dust. These finding confirmative with results of Tandan and Sharma (2006) and Sherin *et al.* (2004). They have sown spawn run period, flush harvesting and yield of milky mushroom on various substrates in their respective experiments.

Effect of substrates on weight of growth stages of milky mushroom (*Calocybe indica*)

The observations were recorded for weight of different growth stages such as initiated pinhead, full pinhead, pre mature, half-mature, mature and over-mature fruit body and the result are presented in the (Table 4) The paddy straw was best substrate for all above growth stages and it was produced (38.67 gm) mature fruit body for harvesting. The mixture of wheat straw + paddy straw was also superior than other substrates and these were weighted (240 mg, 7.33 gm, 15.33 gm, 18.67 gm, 36.67 gm and 123.67 gm) for initiated pinhead, full pinhead, pre-mature, half mature, mature and over mature fruit body respectively.

Effect of substrates on growth parameters and yield of milky mushroom (*Calocybe indica*)

Cap diameter: The growth parameters like cap diameter, stalk length, number of fruit bodies and yield of *Calocybe indica* on various substrate presented in the Table 2. The cap diameter of *Calocybe indica* ranged from (5.33 cm to 7.13 cm) according to utilized various substrates. The maximum cap diameter (7.13 cm) was measured from paddy straw followed by mixture of wheat straw + paddy straw (6.83 cm), wheat straw + chapped leaf of sorghum (5.62 cm) and saw dust + wheat straw (5.33 cm). The paddy straw substrate and wheat straw + chapped leaf of sorghum have significant difference in both the substrates.

Stalk length: The maximum stalk length was measured from paddy straw substrate (8.67 cm) and wheat straw + paddy straw substrate (7.83 cm). Saw dust + wheat straw substrate was produced small stalk length (i.e. 7 cm). The result of mixture of saw dust + wheat straw and paddy straw have significantly difference.

Total number of fruit bodies: The maximum number of (14) fruit bodies were obtained from paddy straw substrates and minimum fruit bodies (7.67) obtained from saw dust + wheat straw and result was significant differences. The wheat straw + chapped leaves of sorghum and paddy straw was also highly significant difference among themselves. This finding is conformity with the result of Chakravorty and Sarkar (1978) reported that the performance of composed substrate was best in all the respects like spawn run period, number of buds and number of fructifications but the yield buds of mushroom on non composted substrates was quite satisfactory.

Yield of first and second flushes: Maximum yield of first flush was obtained from paddy straw (523.33 gm) than other substrates. The mixture of wheat straw + paddy straw was also better than wheat straw, whereas yield obtained (483.33 gm and 407.67 gm) respectively. The mixture of saw dust + wheat straw was yielded less (288.67 gm). The yield of second flush was reduced than yield was first flush among all substrates. The yield performances of all substrates were similar as like yield performance the fist flush.

Total yield: It is evident from the data (Table 2) on yield performance with various utilized substrates. The substrate of paddy straw was found significantly superior than other substrates by

producing (955.66 gm/bag). The mixture of paddy straw + wheat straw was second best substrate where it produced (893.66 gm) mushroom/bag followed by wheat straw (793.67 gm), mixture of wheat straw + chapped leaves of sorghum (584.33 gm) and mixture of mixture of saw dust + wheat straw (535.33 gm). Thus, paddy straw was best substrate for the cultivation of the milky mushroom (*Calocybe indica*) because its was given better yield and good quality than the other substrate.

This result is in conformity with the finding of Krishnomoorth et al. (2000) and Esmaran and Thomos (2003) reported paddy straw to be the best substrate for cultivation of *Calocybe indica*. Krishnamoorthy *et al.* (1997) also reported that *Calocybe indica* can be cultivated on a wide range of cellulosic substrate namely, paddy straw, maize stalk, sorghum stalk, vetiver grass, sugarcane bagasse, soybean hay and ground nut haulms. However, paddy straw and maize straw stalk found most suitable substrate for commercial production of milky mushroom. Amle et al. (2006) also studied the different agricultural wastes wheat straw, cotton stalk, soybean, sugarcane bagasse and its mixture for cultivation of *Calocybe indica*.

Table 2: Effect of substrates on growth parameters of milky mushroom (*Calocybe indica*)

Treatment	Cap diameter(cm)	Stalk length (cm)	No. of fruiting bodies per bag	Yield of 1st flush (gm)	Yield of 2nd flush (gm)	Total yield (gm)
Paddy Straw	7.13	8.67	14.00	523.33	432.33	955.66
Wheat Straw + Paddy Straw (1:1)	6.83	7.83	10.83	483.33	410.33	893.66
Wheat Straw	6.50	7.33	10.67	407.67	386.00	793.67
Wheat Straw + Chapped leaves of sorghum(1:1)	5.62	7.60	9.00	314.33	275.00	589.33
Saw dust + wheat straw (1:1)	5.33	7.00	7.67	288.67	246.67	535.33
SEm _e	0.41	0.52	1.19	20.67	26.65	-
CD(P=0.05)	1.31	1.62	3.75	65.13	83.96	-

Table 3: Effect of substrates on weight of growth stages of milky mushroom (*Calocybe indica*)

Treatment	Initiated of pin head(mg)	Full pin head(gm)	Premature fruit body(gm)	Half mature fruit body(gm)	Mature fruit body(gm)	Over mature fruit body(gm)
Paddy straw	277.33	9.00	16.00	19.67	38.67	125.00
Wheat straw + Paddy straw (1:1)	240.00	7.33	15.33	18.67	36.67	123.67
Wheat straw	237.33	6.67	14.00	17.67	35.33	121.67
Wheat straw + Chapped leaves of sorghum (1:1)	235.33	7.00	13.67	15.67	33.33	120.60
Saw dust + wheat straw (1:1)	231.33	6.00	13.33	14.67	32.00	118.00
SEM _e	13.36	0.56	0.52	0.84	2.33	2.44
CD(P=0.05)	42.10	1.75	1.63	2.66	7.34	7.70

Table 4: Effect of substrates on growth behaviour of milky mushroom (*Calocybe indica*)

Time taken in (days)				
Treatments	Spawn run period	Initiation of pin head	Harvesting of flushes	
			1 st flush	II nd flush
Paddy straw	23	45	50	67
Wheat straw + Paddy straw (1:1)	25	47	52	69
Wheat straw	26	49	56	68
Wheat straw + Chapped leaves of sorghum(1:1)	26	51	53	66
Saw dust + wheat straw(1:1)	27	52	55	72
SEM _e	0.49	0.80	1.66	3.44
CD (P=0.05)	1.55	2.53	5.23	10.82

Summary and Conclusion

Mushroom cultivation is the most suitable technology for creating wealth and health out of wastes from plants, animals and industries which are abundantly available on earth. Huge

quantities of agricultural wastes and other organic wastes are generated annually through the activities of agricultural, forest and food processing industries. These agricultural wastes and other organic wastes are abundantly available in our country. The major parts of these agricultural wastes are bunt after harvesting, resulting multifaceted hazards including oxygen deficient environment, respiratory diseases such as allergic, bronchitis, asthma, tuberculosis and poor visibility at night. The useful insects, bio-agents, earthworm and soil microbe are also reduced due to burning of agricultural wastes and its heating on soil surface. According to survey, in some places these agricultural wastes are spread on the cultivated land but it is not well decomposed and they created the problems during farming. The main concern of our planners to remove these air pollution and soil pollution for environmental protection. This can be done by scientific utilization of resources and bioconversion for mushroom production.

Mushroom production represented an attractive method of improving the nutritional quality of lignocellulosic wastes for use as an animal feed stock. Among the various physical, chemical and biological methods used for upgrading the digestibility and nutritive value of agricultural wastes, biodegradation by using white rot fungi including mushrooms have been found promising. There is a need to evaluate various substrates for enhancing better growth behaviour and yield potential of mushrooms. Therefore, present investigation was on effect of substrates on growth behaviour and yield potential of milky mushroom. These studies will help to mushroom growers for selection of suitable substrates, for better growth behaviour and yield potential of milky mushroom.

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Fig. I: Effect of substrates on growth parameters of milky mushroom (*Calocybe indica*)

