Impact of Sterilization and Thickness of Casing Materials on Yield Attributes of Calocybe indica

Dibakar Panda and Mohan Kumar Biswas

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 03

Res Jr of Agril Sci (2021) 12: 1004–1011

CARAS

Res. Jr. of Agril. Sci. (May-Jun 2021) 12(3): 1004–1011
www.rjas.org

Full Length Research Article

Impact of Sterilization and Thickness of Casing Materials on Yield Attributes of *Calocybe indica*

Dibakar Panda*[1] and Mohan Kumar Biswas[2]

Received: 18 Apr 2021 | Revised accepted: 28 May 2021 | Published online: 12 Jun 2021

A B S T R A C T

Calocybe indica is an edible tropical mushroom which is famous for its good nutritive value and commercial cultivation. The present study is performed to produce *C. indica* and to obtain a suitable method of sterilization used for casing material and appropriate thickness of layer used while casing. Casing materials like FYM, soil and sand, were used with different sterilization methods like chemically sterilized with formalin and autoclave sterilization with different casing thickness like, 0.5 inch, 1.0 inch, 1.5 inch and 2.0 inch to appraise the yield of *C. indica*. The days required for pinhead initiation observed from casing material Sand and Soil sterilize in autoclave and thickness 0.5 inch A2B1 i.e., (17.00 days) while length of stalks observed from soil and sand sterilized with formalin and layer of thickness 0.5 inch A1B1 i.e., (14.70 cm) , and pileus diameter were found in sand and soil sterilized with formalin with layer of thickness 1.5 inch A4B3 i.e., (6.00 cm) yield on of first flush recorded from casing material sand and soil chemically sterilized with formalin with casing thickness 1.0 inch i.e., A4B2 (676.70gm) and second flush from sand and soil autoclaved and thickness 1.0 inch A4B2 (426.70 gm) total yield observed from casing material sand and soil autoclaved and thickness 1.0 inch A4B2 (1103.3 gm) yield with biological efficiency A4B2 (110.3%) was highest. Casing materials soil and sand with casing thickness one inch recorded highest biological efficiency during the production of milky mushroom.

***Key words*:** Biological efficiency, Casing material, Chemical sterilization, Milky mushroom, Pinhead, Yield

Calocybe indica is growing in the summer season farmers and consumers were attracted due to its healthy size, bearable yield, striking colour, flimsiness, high life span, and profitable market value. This mushroom is of Indian origin and rich source of Vitamin-B_2, E, A and C along with minerals such as, Phosphorous Potassium Calcium Zinc Iron and Selenium. Generally, wide range of lignocellulolytic substrates are used for mushroom farming because substrate is a major element for growth of mushrooms [1]. In Asia, paddy straw is commonly used for cultivation of different mushrooms like oyster mushroom, milky mushroom [2]. Paddy straw is considered as one of the best substrates which content high protein and results good yield [3].

The demand of milky mushroom is high in West Bengal so many private businesspersons are taking interest in its cultivation and sailing prospects of the milky mushroom in West Bengal is promising [4]. Milky mushroom needs a temperature of 30-35^{o}C and a relative humidity of 70-80 per cent for its farming and it is favorable to the climatic situation of West Bengal. In West Bengal, through the cultivation of different agricultural commodities a massive quantity of lignocellulosic residues (straws) is generated annually. About a million of tons of paddy straw as a residue is annually produced in the state of West Bengal, as the state has the maximum production of the rice [5]. This residue is suitable for cultivation of mushroom in the different seasons throughout the year [6]. Therefore, the present study is concerned with the determination of the best method of sterilization for casing materials and applicable thickness layer for the farming of milky mushroom commercially.

* Dibakar Panda
✉ dibakar097@gmail.com

[1-2] Department of Plant Pathology, Institute of Agriculture, Visva Bharati, Sriniketan - 731 236, West Bengal, India

MATERIALS AND METHODS

The experiment was performed in the experiential learning programme unit (Mushroom Laboratory) in the department of Plant Pathology, in a factorial completely randomized block design (CRD) setup with three replications. Casing materials were collected from agricultural farm of the institute and mushroom strain of *C. indica* was also collected from the Department of Plant Pathology, Institute of Agriculture, Visva-Bharati.

Table 1 List of treatment combinations

Treatment	Combination
A1B1	FYM and SOIL Chemically sterilized with formalin+0.5
A1B2	FYM and SOIL Chemically sterilized with formalin+1.00"

A1B3	FYM and SOIL Chemically sterilized with formalin+1.50"
A1B4	FYM and SOIL Chemically sterilized with formalin+2.00"
A2B1	SAND and SOIL Autoclave sterilization + 0.5"
A2B2	SAND and SOIL Autoclave sterilization + 1.00"
A2B3	SAND and SOIL Autoclave sterilization +1.50"
A2B4	SAND and SOIL Autoclave sterilization +2.00"
A3B1	FYM and SOIL Autoclave sterilization + 0.50"
A3B2	FYM and SOIL Autoclave sterilization +1.00"
A3B3	FYM and SOIL Autoclave sterilization +1.50"
A3B4	FYM and SOIL Autoclave sterilization +2.00"
A4B1	SAND and SOIL Chemically sterilized with formalin+0.50"
A4B2	SAND and SOIL Chemically sterilized with formalin+1.00"
A4B3	SAND and SOIL Chemically sterilized with formalin + 1.50"
A4B4	SAND and SOIL Chemically sterilized with formalin + 2.00"

We have used Casing material with sterilization (A) i.e., A1, A2, A3 and A4 in combination with Different casing thickness (B)., B1, B2, B3, B4 the experiment was performed in Factorial CRD setup with (3) replications. The treatments, here applied were combinations of both the experimental materials.

Preparation of spawn

Wheat grains are used as spawn substrate these substrates are boiled in hot water for 30 minutes then allow to dry after drying 0.2 per cent calcium carbonate ($CaCO_3$) was added to the spawn substrates and mixed properly. Spawn substrate (250g) was added to glass bottles, and the mouth of the bottles were plugged with non-absorbent cotton and the spawn bottles are put into autoclave for moist sterilization at 121°C at a pressure of 15 pounds per square inch (psi) for one hour then the bottle contains spawn substrates are allow to cool down and shaking the bottles to detach the grains from each other after which the spawn bottles were inoculated with some quantity of mother culture (*C. indica*) in laminar air flow. The spawn bottles are kept in a BOD for incubation at 25-30°C.

Casing materials and experimental conditions

Casing materials like Soil and sand are mixed in the ratio (3:1) and farm-yard manure and soil are also mixed in ratio (3:1). There are two types of sterilization techniques used for the sterilization of the casing materials. All the casing materials are sterilized in autoclave at 121°C for a duration of 2 hrs and chemically by using 2 per cent formalin solution for 72 hours. After a complete mycelial colonization in the spawn bag, the top most layer of bag was covered with a layer of (0.5, 1.0, 1.5 and 2.0 inch) of the prepared casing material. There are four different (0.5, 1.0, 1.5 and 2.0 inch) levels of thickness used while the casing of the same. Then the bags are shifted to the cultivation room which is a controlled environment in respect of temperature and relative humidity.

We have recorded data on the biological parameters viz., days required for pin head formation, stalk length and pileus diameter of sporophores, yield of fruiting bodies on different flushes, total average yield and biological efficiency and these observed data were analyzed to determine the best combination of sterilization technique and thickness of casing materials applied.

RESULTS AND DISCUSSION

As per the analysis is concerned all the treatment and interactions were highly significant (at 1% level of significance) that further suggests the post-hoc comparison of all of them to get the best treatment. To do so we have used least significant difference (LSD) Method of treatment comparison and obtained critical difference (CD) value separately for all the treatments and interactions of all the parameters [7]. By arranging treatments in their descending order and taking the pairwise difference of them, we have chosen the best treatments of A, B and AB separately with the help of CD for all the biological parameters [8]. The minimum number of days to pin head initiation after casing observed from A2B1 (17.00 days) sand and soil sterilized in Autoclave, thickness of casing 0.5 inch the same is evident from (Table 4). The maximum average number of stalk length Casing material with sterilization i.e., A1 FYM and soil Chemical sterilization with formalin with casing thickness i.e., B1 0.5″in the combination A1B1 (14.70 cm) shows maximum average stalk length the same is evident from (Table 7) [9]. The maximum average number of Pileus length Casing material with sterilization i.e., A4 Sand and soil Chemical sterilization with formalin with casing thickness i.e., B3 1.5″ in the combination of A4B3 (6.00 cm) gives the maximum average number of Pileus (d) width the same is evident from (Table 10). The maximum average number yield of first flush casing material sand and soil chemically sterilized with formalin with casing thickness 1.0″ i.e., A4B2 (676.70gm) gives the maximum average number of Yield the same is evident from (Table 13). The maximum average number yield of second flush casing material sand and soil chemically sterilized with formalin with casing thickness 1.0″ i.e., A4B2 (426.70 gm) gives the maximum average number of yield the same is evident from (Table 16) [10]. The maximum average number of total yield recorded from casing material sand and soil chemically sterilized with formalin with casing thickness 1.0″ i.e., A4B2 (1103.3 gm) gives the maximum average number of Yield the same is evident from (Table 19). The maximum average number of Biological Efficiency recorded from casing material sand and soil chemically sterilized with formalin with casing thickness 1.0″ i.e., A4B2 (110.3%) gives the maximum average number of biological efficiency the same is evident from (Table 22) [11].

Table 2 Analysis of Variance for days required for pin head formation

Source	DF	SS	MSS	F	*p*-value	Significance
A	3	8.50	2.83	5.23	0.005	Significant**
B	3	22.00	7.33	13.54	0.000	Significant **
AB	9	46.17	5.13	9.47	0.000	Significant **
Error	32	17.33	0.54			
Total	47	94.00				

Days required for pin head formation

As from (Table 2), all the treatment and interactions were highly significant (at 1% level of significance). It suggests the post-hoc comparison of all of them to get the best one treatment [12]. To do so we have used least significant difference (LSD) Method of treatment comparison and obtained critical difference (CD) value separately for all the treatments and interactions. It is given in (Table 3).

Table 3 Post-hoc comparison of the treatments

Treatment	SE(m)	SE(D)	CD (5%)
A	0.2125	0.3005	0.612
B	0.2125	0.3005	0.612
AB	0.4249	0.6009	1.224

By arranging treatments in their descending order and taking the difference of them, we have chosen the best treatments of A, B and AB separately it is given in (Table 4). From (Table 4) we have found interaction of A and B i.e. A2B1 required minimum time for pin head initiation.

Table 4 Days required for pin head formation

Name	CD (5%)	Treatments	Difference	Significance
A	1.074	A3-A4 (22.25-21.08)*	1.17	Sign
		A4-A1 (21.08-20.75)	0.33	NS
		A1-A2 (20.75-20.58)	0.17	NS
B	1.074	B4-B3 (22.58-21.83)	0.75	NS
		B3-B2 (21.83-20.50)*	1.33	Sign
		B2-B1 (20.50-19.75)	0.75	NS
AB	2.147	A3B4-A3B3 (23.30-23.00)	0.30	NS
		A3B3-A2B4 (23.00-23.00)	0.00	NS
		A2B4-A1B4 (23.00-22.30)	0.70	NS
		A1B4-A2B3 (22.30-22.00)	0.30	NS
		A2B3-A1B3 (22.00-21.70)	0.30	NS
		A1B3-A3B1 (21.70-21.70)	0.00	NS
		A3B1-A4B4 (21.70-21.70)	0.00	NS
		A4B4-A4B2 (21.70-21.30)	0.40	NS
		A4B2-A3B2 (21.30-21.00)	0.30	NS
		A3B2-A4B1 (21.00-20.70)	0.30	NS
		A4B1-A4B3 (20.70-20.70)	0.00	NS
		A4B3-A2B2 (20.70-20.30)	0.40	NS
		A2B2-A1B1 (20.30-19.70)	0.60	NS
		A1B1-A1B2 (19.70-19.30)	0.40	NS
		A1B2-A2B1 (19.30-17.00)*	2.30	Sign

Figures in the parentheses are the average number of days require to pin head initiation after casing for treatment respective combinations

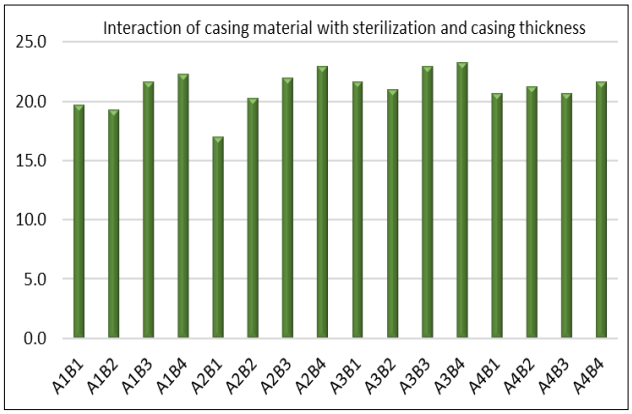

Fig 1 Average number of days required for pin head formation

Stalk length of fruiting bodies

Table 5 Analysis of variance for stalk length of fruiting bodies

Source	DF	SS	MSS	F	*p*-value	Significance
A	1	20.53	6.84	6.19	0.002	Significant**
B	3	11.60	3.87	3.49	0.027	Significant **
AB	3	54.82	6.09	5.51	0.000	Significant **
Error	16	35.39	1.11			
Total	23	237.25				

Table 6 Post-hoc comparison of the treatments

Treatment	SE(m)	SE(D)	CD (5%)
A	0.3036	0.4293	0.875
B	0.3036	0.4293	0.875
AB	0.6072	0.8587	1.749

From (Table 7) we have found that interaction of A and B i.e. A1B1 shows highest stalk length in centimeter.

Table 7 Stalk length of fruiting bodies

Name	CD (5%)	Treatments	Difference	Significance
A	0.875	A1-A4 (11.67-11.53)	0.14	NS
		A4-A3 (11.53-11.00)	0.53	NS
		A3-A2 (11.00-10.00)*	1.00	Sign
B	0.875	B1-B3 (11.83-11.12)	0.71	NS
		B3-B2 (11.12-10.63)	0.49	NS
		B2-B4 (10.63-10.63)	0.00	NS
AB	1.749	A1B1-A4B3 (14.70-12.30)*	2.40	Sign
		A4B3-A4B4	0.20	NS

	(12.30-12.10)		
	A4B4-A1B2	0.30	NS
	(12.10-11.80)		
	A1B2-A3B1	0.00	NS
	(11.80-11.80)		
	A3B1-A1B3	0.30	NS
	(11.80-11.50)		
	A1B3-A4B1	0.30	NS
	(11.50-11.20)		
	A4B1-A3B4	0.20	NS
	(11.20-10.90)		
	A3B4-A2B4	0.20	NS
	(10.90-10.70)		
	A2B4-A3B3	0.00	NS
	(10.70-10.70)		
	A3B3-A3B2	0.10	NS
	(10.70-10.60)		
	A3B2-A4B2	0.10	NS
	(10.60-10.50)		
	A4B2-A2B3	0.50	NS
	(10.50-10.00)		
	A2B3-A2B2	0.30	NS
	(10.00-9.70)		
	A2B2-A2B1	0.10	NS
	(9.70-9.60)		
	A2B1-A1B4	0.90	NS
	(9.60-8.70)		

Figures in the parentheses are the average number of Stalk length of fruiting bodies for treatment respective combinations

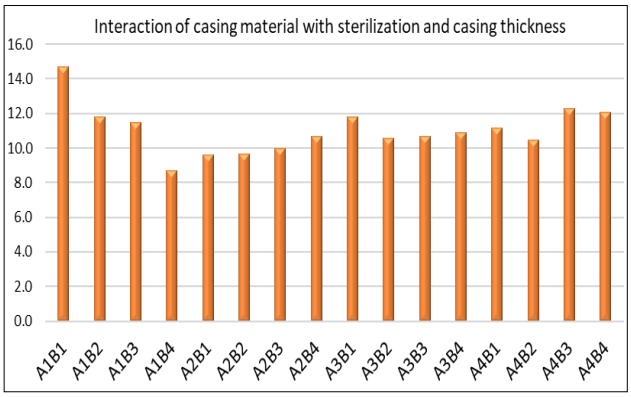

Fig 2 Average stalk length of fruiting bodies (cm)

Pileus diameter of fruiting bodies

Table 8 Analysis of variance for pileus diameter of fruiting bodies

Source	DF	SS	MSS	F	*p*-value	Significance
A	1	106.34	35.45	157.95	0.000	Significant**
B	3	2.44	0.81	3.63	0.023	Significant **
AB	3	13.02	1.45	6.45	0.000	Significant **
Error	16	7.18	0.22			
Total						

Table 9 Post-hoc comparison of the treatments

Treatment	SE(m)	SE(D)	CD (5%)
A	0.1368	0.1934	0.394
B	0.1368	0.1934	0.394
AB	0.2735	0.3868	0.788

From (Table 10) we have found the interaction of A and B i.e. A4B3 shows maximum pileus diameter in centimeter.

Table 10 Pileus diameter of fruiting bodies

Name	CD (5%)	Treatments	Difference	Significance
A	0.394	A4-A3	1.20	Sign
		(6.79-5.59)*		
		A3-A1	2.24	Sign
		(5.59-3.35)		
		A1-A2	0.03	NS
		(3.35-3.32)		
B	0.394	B3-B2	0.28	NS
		(5.08-4.80)*		
		B2-B1	0.06	NS
		(4.80-4.74)		
		B1-B4	0.29	NS
		(4.74-4.45)		
B	0.788	A4B1-A4B4	0.20	NS
		(7.30-7.10)*		
		A4B4-A4B2	0.30	NS
		(7.10-6.80)		
		A4B2-A3B3	0.10	NS
		(6.80-6.70)		
		A3B3-A3B2	0.60	NS
		(6.70-6.10)		
		A3B2-A4B3	0.10	NS
		(6.10-6.00)		
		A4B3-A3B4	1.00	Sign
		(6.00-5.00)*		
		A3B4-A3B1	0.40	NS
		(5.00-4.60)		
		A3B1-A2B3	0.70	NS
		(4.60-3.90)		
		A2B3-A1B1	0.20	NS
		(3.90-3.70)		
		A1B1-A1B3	0.00	NS
		(3.70-3.70)		
		A1B3-A2B1	0.30	NS
		(3.70-3.40)		
		A2B1-A2B2	0.00	NS
		(3.40-3.40)		
		A2B2-A1B4	0.40	NS
		(3.40-3.00)		
		A1B4-A1B2	0.10	NS
		(3.00-2.90)		
		A1B2-A2B4	0.20	NS
		(2.90-2.70)		

Figures in the parentheses are the average number of Pileus length of fruiting bodies for treatment respective combinations

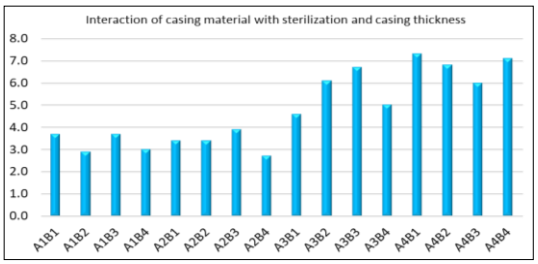

Fig 3 Average pileus length of fruiting bodies (cm)

Yield of fruiting bodies on first flush

Table 11 Analysis of variance for yield of fruiting bodies on first flush

Source	DF	SS	MSS	F	*p*-value	Significance
A	3	127043.23	42347.74	40.43	0.000	Significant**
B	3	55993.23	18664.41	17.82	0.000	Significant**
AB	9	86879.69	9653.30	9.22	0.000	Significant**
Error	32	33516.67	1047.40			
Total	47	303432.81				

Table 12 Post-hoc comparison of the treatments

Treatment	SE(m)	SE(D)	CD (5%)
A	9.3425	13.2123	26.913
B	9.3425	13.2123	26.913
AB	18.6851	26.4247	53.825

From (Table 13) we have found the interaction of A and B i.e. A4B2 shows maximum average yield on first flush in gram.

Table 13 Yield of fruiting bodies on first flush

Name	CD (5%)	Treatments	Difference	Significance
A	26.913	A4-A3 (560.83-547.92)	12.91	NS
		A3-A2 (547.92-67.50)*	80.42	Sign
		A2-A1 (467.50-40.00)*	27.50	Sign
B	26.913	B2-B4 (552.50-15.00)*	37.50	Sign
		B4-B1 (515.00-489.17)	25.83	NS
		B1-B3 (489.17-59.58)*	29.59	Sign
AB	53.825	A4B2-A3B4 (676.70-10.00)*	66.70	Sign
		A3B4-A4B1 (610.00-600.00)	10.00	NS
		A4B1-A3B2 (600.00-590.00)	10.00	NS
		A3B2-A3B3 (590.00-28.30)*	61.70	Sign
		A3B3-A2B4 (528.30-503.30)	25.00	NS
		A2B4-A4B4 (503.30-503.30)	0.00	NS
		A4B4-A2B2 (503.30-486.70)	16.60	NS
		A2B2-A1B1 (486.70-466.70)	20.00	NS
		A1B1-A3B1 (466.70-463.30)	3.40	NS
		A3B1-A4B3 (463.30-463.30)	0.00	NS
		A4B3-A1B2 (463.30-456.70)	6.60	NS
		A1B2-A2B3 (456.70-453.30)	3.40	NS
		A2B3-A1B4 (453.30-443.30)	10.00	NS
		A1B4-A2B1 (443.30-426.70)	16.60	NS
		A2B1-A1B3 (426.70-393.30)	33.40	NS

Figures in the parentheses are the average number of yield of first flush for treatment respective combinations

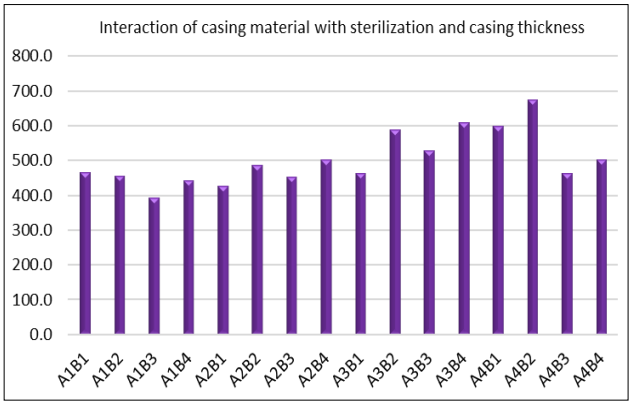

Fig 4 Average number of yield on first flush (gm)

Yield of fruiting bodies on second flush

Table 14 Analysis of variance for yield of fruiting bodies on second flush

Source	DF	SS	MSS	F	*p*-value	Significance
A	3	19015.06	6338.35	3.85	0.018	Significant*
B	3	18901.73	6300.58	3.83	0.019	Significant *
AB	9	38436.85	4270.76	2.59	0.023	Significant *
Error	32	52670.67	1645.96			
Total	47	129024.31				

Table 15 Post-hoc comparison of the treatments

Treatment	SE(m)	SE(D)	CD (5%)
A	11.7117	16.5628	33.737
B	11.7117	16.5628	33.737
AB	23.4233	33.1256	67.475

From (Table 16) we have found the interaction of A and B i.e. A4B2 shows maximum yield of fruiting bodies in gram.

Table 16 Yield of fruiting bodies on second flush

Name	CD (5%)	Treatments	Difference	Significance
A	33.737	A3-A4 (340.83-340.00)	0.83	NS
		A4-A2 (340.00-333.33)	6.67	NS
		A2-A1 (333.33-92.58)*	40.75	Sign
B	33.737	B2-B4	21.25	NS

		(351.25-330.00)		
		B4-B1	0.33	NS
		(330.00-329.67)		
		B1-B3	33.83	Sign
		(329.67-95.83)*		
AB	67.475	A4B2-A3B3	70.00	Sign
		(426.7-356.7)*		
		A3B3-A4B1	0.00	NS
		(356.7-356.7)		
		A4B1-A3B4	3.40	NS
		(356.7-353.3)		
		A3B4-A2B4	3.30	NS
		(353.30-350)		
		A2B4-A2B2	0.70	NS
		(350-343.3)		
		A2B2-A3B3	3.30	NS
		(343.30-340)		
		A3B3-A2B1	3.3	NS
		(340-336.7)		
		A2B1-A4B4	10.00	NS
		(336.7-326.7)		
		A4B4-A3B1	13.40	NS
		(326.70-313.30)		
		A3B1-A1B1	1.30	NS
		(313.30-312.00)		
		A1B1-A2B3	8.70	NS
		(312.00-303.3)		
		A2B3-A1B3	13.30	NS
		(303.3-290)		
		A1B3-A1B4	0.00	NS
		(290-290)		
		A1B4-A1B2	11.70	NS
		(290-278.3)		
		A1B2-A4B3	28.30	NS
		(278.30-250)		

Figures in the parentheses are the average number of yield of second flush for treatment respective combinations

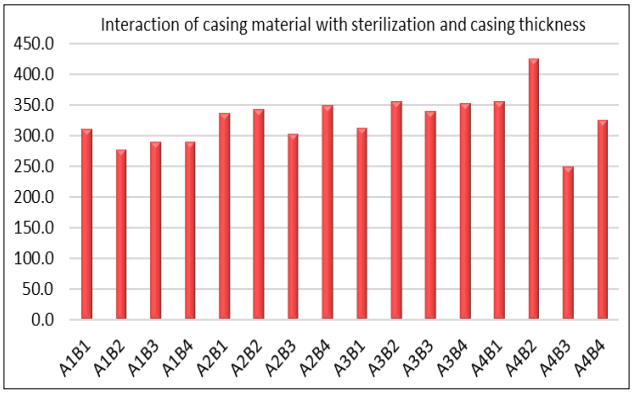

Fig 5 Average number of yield on second flush (gm)

Total average yield

Table 17 Analysis of variance for total average yield

Source	DF	SS	MSS	F	*p*-value	Significance
A	3	205509.75	68503.25	14.49	0.000	Significant**
B	3	134419.75	44806.58	9.48	0.000	Significant**
AB	3	199795.08	22199.45	4.70	0.001	Significant**
Error	32	151300.67	4728.15			
Total	47	691025.25				

Table 18 Post-hoc comparison of the treatments

Treatment	SE(m)	SE(D)	CD (5%)
A	19.8497	28.0718	57.180
B	19.8497	28.0718	57.180
AB	39.6995	56.1435	114.361

From (Table 19) we have found that interaction of A and B i.e.A4B2 shows maximum average yield in gram.

Table 19 Total average yield

Name	CD (5%)	Treatments	Difference	Significance
A	57.180	A4-A3	28.75	NS
		(900.83-872.08)		
		A3-A2	72.08	Sign
		(872.08-00.00)*		
		A2-A1	67.42	Sign
		(800-732.58)*		
B	57.180	B2-B4	75.42	Sign
		(903.75-28.33)*		
		B4-B1	9.50	NS
		(828.33-818.83)		
		B1-B3	64.25	Sign
		(818.83-54.58)*		
AB	114.361	A4B2-A4B1	146.6	Sign
		(1103.3-956.7)*		
		A4B1-A3B2	10.00	NS
		(956.7-946.7)		
		A3B2-A3B4	50.00	NS
		(946.7-896.7)		
		A3B4-A3B3	28.40	NS
		(896.7-868.3)		
		A3B3-A2B4	5.00	NS
		(868.3-853.3)		
		A2B4-A2B2	23.30	NS
		(853.3-830)		
		A2B2-A4B4	0.00	NS
		(830-830)		
		A4B4-A1B1	51.30	NS
		(830-778.7)		
		A1B1-A3B1	2.00	NS
		(778.7-776.7)		
		A3B1-A2B1	13.4	NS
		(776.7-763.3)		
		A2B1-A2B3	10.00	NS
		(763.3-753.3)		
		A2B3-A1B2	18.3	NS
		(753.3-735)		
		A1B2-A1B4	1.70	NS
		(735-733.3)		
		A1B4-A4B3	20.00	NS
		(733.33-713.30)		
		A4B3-A1B3	30.00	NS
		(713.3-683.3)		

Figures in the parentheses are the total average Yield for treatment respective combinations

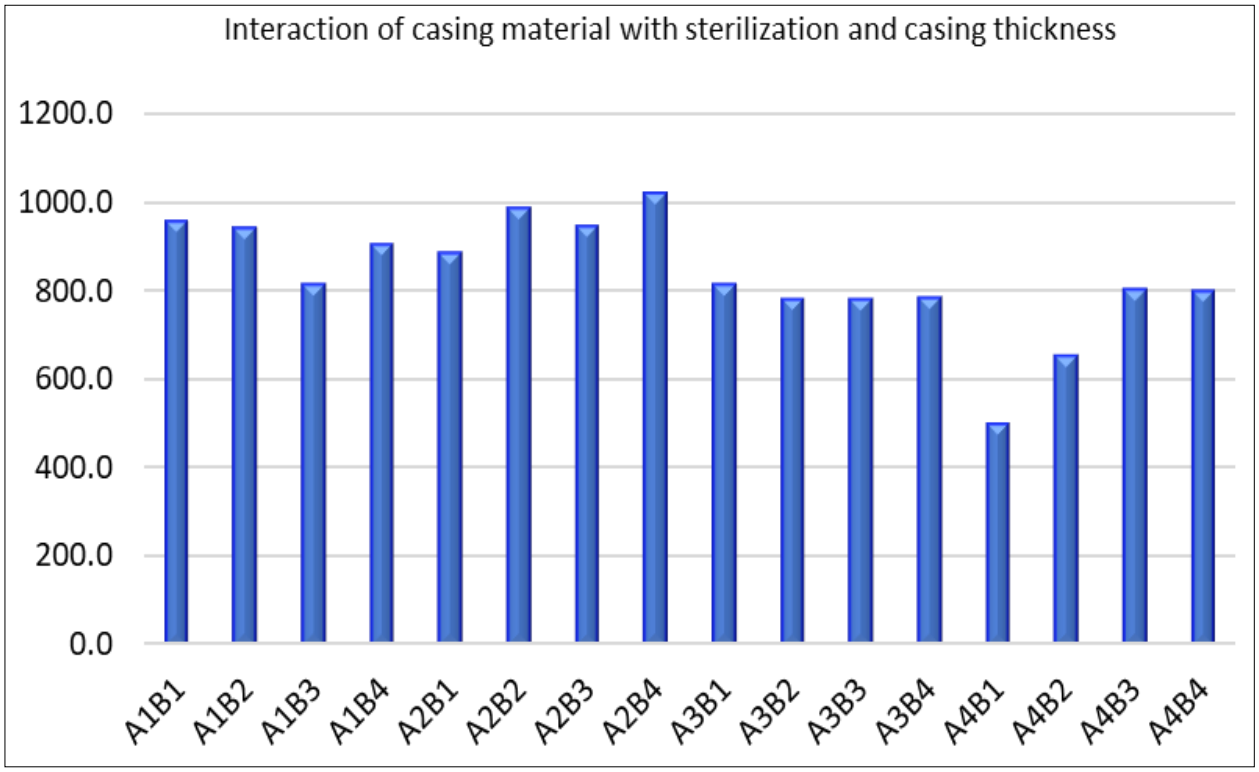

Fig 6 Average number of total yield (gm)

Biological efficiency

Table 20 Analysis of variance for biological efficiency

Source	DF	SS	MSS	F	*p*-value	Significance
A	3	2055.09	685.03	14.49	0.000	Significant**
B	3	1344.19	448.06	9.48	0.000	Significant **
AB	3	1997.95	221.99	4.70	0.001	Significant **
Error	32	1513.00	47.28			
Total	47	6910.25				

Table 21 Post-hoc comparison of the treatments

Treatment	SE(m)	SE(D)	CD (5%)
A	1.98497	2.80718	5.7180
B	1.98497	2.80718	5.7180
AB	3.96995	5.61435	11.4361

From (Table 22) we have found the interaction of A and B i.e. A4B2 gives highest biological efficiency in percentage.

Table 22 Biological efficiency

Name	CD (5%)	Treatments	Difference	Significance
A	5.7180	A4-A3 (90.08-87.20)	2.87	NS
		A3-A2 (87.20-80.00)*	7.20	Sign
		A2-A1 (80.0-73.25)*	6.75	Sign
B	5.7180	B2-B4 (90.37-82.83)*	7.54	Sign
		B4-B1 (82.83-81.88)	0.95	NS
		B1-B3 (81.88-75.45)*	6.42	Sign
AB	11.4361	A4B2-A4B1 (110.33-95.67)*	14.66	Sign
		A4B1-A3B2 (95.67-94.67)	1.00	NS
		A3B2-A3B4 (94.67-89.67)	5.00	NS
		A3B4-A3B3 (89.67-86.83)	2.84	NS
		A3B3-A2B4 (86.83-85.33)	0.50	NS
		A2B4-A2B2 (85.33-83.0)	2.33	NS
		A2B2-A4B4 (83.0-83.0)	0.00	NS
		A4B4-A1B1 (83.0-77.87)	5.13	NS
		A1B1-A3B1 (77.87-77.67)	0.20	NS
		A3B1-A2B1 (77.67-76.33)	1.34	NS
		A2B1-A2B3 (76.33-75.33)	1.00	NS
		A2B3-A1B2 (75.33-73.5)	1.83	NS
		A1B2-A1B4 (73.55-73.33)	1.70	NS
		A1B4-A4B3 (73.333-71.33)	2.00	NS
		A4B3-A1B3 (71.33-68.33)	3.00	NS

Figures in the parentheses are the total average Yield for treatment respective combinations

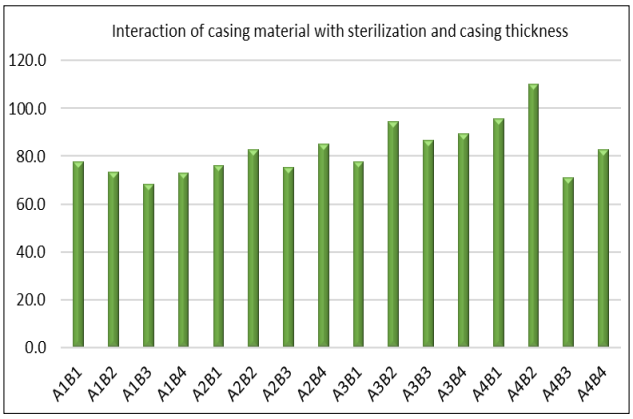

Fig 7 Average biological efficiency (%)

CONCLUSION

Yield contributing factors of *C indica* were evaluated on the basic of casing materials used viz., loamy soil, loamy soil with sand, and farm yard manure (FYM) and layer of thickness applied viz., 0.5inch, 1.0 inch, 1.5 inch, 2.0 inch. Maximum biological efficiency was recorded in sand and soil (110.3%) with thickness 1.0 inch followed by the (95.67%) Primordia initiation occurred earlier in sand and soil sterilized in autoclave i.e., A2B1 (17days) with thickness 0.5 inch followed by farm yard manure and soil chemically sterilized with formalin i.e., A1B2 (19days). The greatest stalk length observed with casing material FYM and soil chemically sterilized with formalin with casing thickness 0.5″ i.e., A1B1 (14.70cm) followed by (12.30cm) from casing material sand and soil chemically sterilized with formalin with casing thickness 1.0″ i.e., A4B3. Pileus thickness with maximum diameter were found with sand and soil chemically sterilized with formalin at casing thickness 1.5″ (6.0 cm) i.e., A4B3 followed by FYM and soil autoclaved and thickness of casing is 2.0″ i.e., A3B4. Yield contributing factors of *C indica* were evaluated from the effects of four different thicknesses of loamy soil, sand, FYM casing materials were presented in above tables. The casing layer is an essential component for the artificial farming of *C. indica* the finding shows that 0.5 inch to 1inch layer thicknesses of casing materials shows positive results on the number of days required for primordia initiation, days required to harvest, the number of fruiting bodies, stalk length, and diameter of pileus, biological and economic yield. The highest and lowest biological efficiencies were recorded from 1 inch thickness layer (110.3%) and 1.5inch thickness layer (68.33%), respectively. If the layer of casing is not loose enough then the primordia cannot come to the top casing layer. In this present study the most efficient layer of thickness found i.e., 1 inch layer of thickness. The combination of casing materials sand and soil with 1 inch casing thickness proved as good casing material and appropriate layer of thickness which likely played a role in stimulating the initiation of the fruiting body. Thus, the layer of one inch thickness of sand with loamy soil was the best casing material and the rice straw was the best substrate for the commercial cultivation of *C. indica*.

LITERATURE CITED

1. Amin R, Khair A, Alam N, Lee TS. 2010. Effect of different substrates and casing materials on the growth and yield of *Calocybe indica*. *Mycobiology* 38(2): 97-101.
2. Kalha CS, Gupta V, Singh SK, Tandon G. 2011. Effect of different supplements and bio-fertilizers on the yield of *Calocybe indica* P and C. *Environment and Ecology* 1: 34-36.
3. Chakravarty DK, Sarkar BB, Kundu BM. 1981. Cultivation of *Calocybe indica*, a tropical edible mushroom. *Current Science* 50: 550.
4. Doshi A, Sindana N, Chakravarti BP. 1989. Cultivation of summer mushroom *Calocybe indica* Purkayastha and Chandra in Rajasthan. *Mush Science* 12: 395-399.
5. Purkayastha RP, Chandra A. 1976. A new technique for in vitro production of *Calocybe indica*: an edible mushroom of India. *Mushroom Journal* 40: 112-113.
6. Hayes WA. 1985. Interaction between compost and casing soil substrates in the culture of *Agaricus bisporus*. *Mushroom Journal* 151: 281-288.
7. Eswaran A, Thomas S. 2003. Effect of various substrates and additives on sporophores yield of *Calocybe indica* and *Pleurotus* species. *Indian Journal of Mushroom* 21: 8-10.
8. Chakraborty U, Sikdar SR. 2010. Intergeneric protoplast fusion between *Calocybe indica* (milky mushroom) and *Pleurotus florida* aids in the qualitative and quantitative improvement of sporophore of the milky mushroom. *World Journal of Microbiol Biotechnology* 26: 213-225.
9. Krishnamoorthy AS, Muthuswamy MT, Nakkeeran S. 2000. Technique for commercial production of milky mushroom *Calocybe indica* P&C. *Indian Journal of Mushroom* 18: 19-23.
10. Tandon G, Sharma VP. 2006. Yield performance of *Calocybe indica* on various substrates and supplements. *Mushroom Journal* 15: 33-37.
11. Sassine YN, Ghora Y, Kharrat M, Bohme M, Abdel-Mawgoud AMR. 2005. Waste paper as an alternative for casing soil in mushroom (*Agaricus bisporus*) production. *Journal of Applied Science Research* 1: 277-284.
12. Pandey M, Singh K, Shukla HP. 2004. The effect of different casing materials on yield of button mushroom (*Agaricus bisporus*). *Progress Agriculture* 4: 71-72.