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Evaluation of Different Methods of Substrate Preparation for Cultivation of *Calocybe indica*

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ABSTRACT: Now a days mushroom has become an important alternative to non-veg food. Indian climate especially climate of West Bengal is favourable for cultivation of mushroom by using agricultural wastes. Choosing the best substrate method for bed preparation is of great importance in the efficient cultivation of mushrooms. Thus, the present study has been designed to evaluate the suitable method of bed preparation of straw substrate for generating better yield of milky mushroom. It was observed that the substrate, chopped paddy straw sterilized with hot water resulted into the maximum yield (1193.30g) followed by chopped paddy straw treated with steam sterilization (1060.00g) and chopped paddy straw treated with chemicals, Formalin and Bavistin (1028.30g). Also, the maximum biological efficiency was observed on the substrate chopped paddy straw treated with hot water (119.3%) followed by chopped paddy straw treated with steam sterilization (106.0%) and chopped paddy straw treated with chemicals chemical, Formalin and Bavistin (102.8%). So, the efficient cultivation of milky mushroom can be done by using substrate, chopped paddy straw and sterilizing it by using hot water.

Keywords: Biological Efficiency, Formalin, Hot Water, Milky Mushroom, Sterilization.

INTRODUCTION

Milky mushroom is a tropical mushroom and generally cultivated in summer season, for the growth of mushrooms straw substrates play an important role (Purkayastha and Chandra, 1974). Different types of agricultural waste products like wheat straw, paddy straw etc. can be used for the cultivation of milky mushroom. The climatic condition of West Bengal favours for production of milky mushroom at commercial level. Different substrates can be used for cultivation of milky mushroom but at commercial level rice straw is widely used and types of bed preparation to increase the yield and biological efficiency of milky mushroom. It is generally cultivated in summer season, for the growth of mushrooms straw substrates play an important role different types of agriculture waste products like wheat and paddy straw were used for cultivation of milky mushroom. Substrate does not need to be Compost as it is used in case of button mushroom, use of compost as substrate can reduce basic structural components by secreting different enzymes. Trivedi *et al.*, (1991) used nine substrates for the cultivation of milky mushroom and found that wheat straw as better substrate and similar result was reported by Doshi *et al.*, (1989). Purkayastha and Chandra (1985) reported that chopped paddy straw is suitable substrate for cultivation of milky mushroom. Krishnamoorthy (1981) suggested that chopped paddy straw substrates can be sterilized by placing in autoclave and in pasteurization chamber at 15lb pressure to improve the yield of oyster mushroom.

Growth of Milky mushroom favours 30-35ºC temperature and 70-80% relative humidity for cultivation (Singh *et al.*, 2009; Amin *et al.*, 2010; Upadhyay, 2010 and Gitte *et al.*, 2014). Nowadays popularity of milky mushroom was seen in north India because of its higher biological efficiency attractive colour, better storing quality, simple growing procedure and ability to grow on different type of Agro-wastes as it was more popular in south India during the last decade. Panda and Biswas (2021) studied the suitable method of sterilization for casing material and appropriate thickness of casing layer. They used FYM, soil and sand as casing materials and sterilized chemically by using formalin. They evaluated four casing thickness viz., 0.5, 1.0, 1.5 and 2.0 inch for proper growth of *C. indica*. They found that soil and sand (as casing material) with a thickness of one produces the maximum biological efficiency. The climatic condition of West Bengal favours for production of milky mushroom at commercial level. Different substrates can be used for cultivation of milky mushroom but at commercial level rice straw is widely used. Types of bed preparation to increase the yield and biological efficiency of milky mushroom However, no research has been conducted on the Types of bed preparation for cultivating milky mushroom in West Bengal. This study was designed to evaluate the suitable method of bed preparation of straw substrate for better growth and yield of milky mushroom.

MATERIALS AND METHODS

A. Culture collection

The young fruiting bodies of milky mushroom were collected from Kolkata mushroom market during rainy season of 2019-20. The collected fruiting bodies was brought to the laboratory and isolated.

B. Preparation of media

For isolating mushrooms mycelium and maintaining the pure culture of milky mushroom Potato Dextrose Agar (PDA) medium was used.

Isolation and purification of the culture

In a clean laboratory, preparation of tissue culture carried out by taking young fruiting bodies and clean with the water and 0.1% $HgCl_2$ after drying a small portion of vegetative tissue is taken by cutting from the joint of stripe and pileus then those cut portions are transferred into medium containing potato dextrose agar for further growth then those plates were kept in BOD for incubation. Within a week mycelium were spread over media so slant is prepared from pure culture petri plates by using inoculating needle in laminar air flow chamber under aseptic condition.

Substrates used for spawn preparation

Substrates used for preparation of spawn were treated as seed in this regards many researchers were used different types of cereal grains for spawn preparation (Amle *et al.*, 2007 and Senthilnambi *et al.*, 2011). Due to available at low price wheat grain, paddy grain, bajra grain and sorghum grains were used in this work. Collected grains were washed under tap water to remove inert materials then allow to boil in hot water for half an hour. After the process of boiling excess water were drain out and spread over clean polythene sheet for drying after drying $CaCO_3$ and $CaSO_4$ were added at 2% and 0.5% concentration per kg of grains. Then these dry grains were poured into spawn bottles and spawn packets with plugging the mouth of the bottle and packets by non-absorbing cotton and wrapping with paper by using rubber band. Then sterilize in autoclave at 121°C for 120 minutes. Before inoculating of mushroom mycelium these sterilized spawn materials were allow to cool after cooling inoculation is done and kept in BOD for incubation then fully colonized spawn bottles and packets were used for mushroom production.

Substrates used for cultivation of *Calocybe indica*

Substrate preparation and Spawning

Mainly paddy straw is used in this study to select proper method out of three methods used *viz*; Rolled paddy straw, Bundle and Chopped paddy straw. These substrates were treated with various sterilization techniques *viz*; hot water treatment, steam sterilization, formalin and Bavistin and by using combination of limestone bleaching powder and IndofilM-45. After sterilization substrates were packed in polythene bags of 2.5-3.0 kg quantity. Layer method is used for spawning five layer is used during spawning @5% spawn rate.

Casing. Soil, sand and farm yard manure are used as casing materials that can gives support to fruiting bodies, and allows to escape gases from substrates those prepared casing materials were spread over substrate bags in a range of 0.5-2.0 inch casing thickness.

Watering and Harvesting. Watering is done by sprinkling on the casing layer on daily basis and mushrooms were harvested by twisting the fruiting bodies and data pertaining to this work is recorded.

Table 1: List of treatment combinations.

Treatment	Combination
A1B1	Rolled Paddy straw + hot water
A1B2	Rolled Paddy straw + steam sterilization
A1B3	Rolled Paddy straw + chemical
A1B4	Rolled Paddy straw + combination of limestone, bleaching powder, IndofilM-45
A2B1	Chopped paddy straw + hot water
A2B2	Chopped paddy straw + steam sterilization
A2B3	Chopped paddy straw + chemical
A2B4	Chopped paddy straw + combination of limestone, bleaching powder, IndofilM-45
A3B1	Bundle Paddy Straw + hot water
A3B2	Bundle Paddy Straw + steam sterilization
A3B3	Bundle Paddy Straw + chemical
A3B4	Bundle Paddy Straw + combination of limestone, bleaching powder, IndofilM-45

Analytical procedure. The experiment was performed in factorial set-up in Completely Randomized Block Design (C. R. D.) with 3 replications (equal number of replications). The basic aim was to determine the best combination of method of substrate preparation and method of substrate sterilization. This can be achieved by getting the best combination from the interaction of the both.

Tukey HSD Test. Pairwise comparisons of significant treatments can be made using the most common method of comparison *i.e.*, Tukey HSD test. The following steps are involved in this test:

Step 1: Obtain the ANOVA and determine the significant sources of variations.

Step 2: If between variance (treatments) is significant at specified level of significance i.e., α, then pairwise comparison generally known as post hoc test is performed.

Step 3: The following information are required to compare the means:

Means of each treatment, Number of groups per treatment, MSE (mean sum of square due to error), Treatment degrees of freedom

$$HSD = \frac{M_i - M_j}{\sqrt{\frac{MSE}{N}}} \qquad (1)$$

The notation used in equation (1) bears the following abbreviations:

M*i*, M*j* are the mean of i^{th} and j^{th} treatments respectively, MSE = mean sum of square due to error, N = number of groups per treatment.

Step 4: Arrange the treatments in decreasing order so that M*i* should be greater than M*j*.

Step 5: Obtain the Tukey's HSD test statistic using equation (1).

Step 6: Obtain the *p*-value of Tukey's HSD test for the specified value of level of significance.

Step 7: If the *p* value is less than 0.05, then it is treated as significant otherwise not.

RESULTS AND DISCUSSIONS

The overall ANOVA is significant at 1 per cent level of significance which allows us to further compare the treatments or pair of treatments to get the better one, to test the pairwise combination of the treatments we have used Tukey HSD. As per our objective, we have used a total 12 number of treatment combinations for which there would be $\binom{12}{2} = 66$ pair of treatments combinations to be compared. These pairs are compared at 5% level of significance by means of Tukey HSD and we have reported the treatment combinations which are significantly different from each other in the present study.Various methods of substrate preparation such as rolled paddy straw, paddy straw without chopping(bundle) and chopped paddy straw were used to evaluate growth and yield performance of *C.indica*. In this connection, we have tied to achieve the objective by means of the following characteristics: (*i*) time requited for spawn run, (*ii*) days required for pin head initiation, (*iii*) number of days required to harvest, (*iv*) number of fruiting bodies, (*v*) yield and (*vi*) biological efficiency.

A. Time required for spawn run

We generally prefer the minimum days or time to spawn run, the treatment which takes minimum number of days would be the best treatment combination and preferred over the others. In the present work, we have found that treatment A (different methods of substrate preparation), B (different methods of substate sterilization) and A×B (interaction between method of substrate preparation and sterilization) are significant as reported in Table 2. Here, if interaction A×B is significant, then we compare only the interactions as per our objective.

The treatments A, B and interaction (A×B) is significant at 1% and 5% level of significance respectively, it enables us to compare the interactions pairwise. It has been observed that treatment combination A2B4 i.e., chopped paddy straw treated with a combination of chemicals, limestone, bleaching powder and Indofil M-45 took minimum average number of days i.e., 22.30 days to spawn run. The best treatment combination is highlighted in Table 3 and we have reported only the significant treatment combinations in this table. The plot of pairwise comparison obtained by means of Tukey HSD to find the treatment which takes the minimum days for spawn run is obtained (Fig. 1). It is worth mentioning that the treatment pair which is outside the zero line in Fig. 1 is significantly different from each other and the treatment combination which crosses the zero line is not significantly different.

Table 2: Analysis of variance for days required for spawn run.

S.V.	df	SS	MSS	F value	*p*-value
A	2	24.22	12.11	14.53	0.0001
B	3	90.22	30.07	36.09	0.0001
A*B	6	16.44	2.74	3.29	0.0166
Error	24	20.00	0.83		
Total	35	150.88			

Table 3: Pairwise comparison by Tukey HSD.

Combination	Difference	Lower	Upper	*p*-value	Combination	Difference	Lower	Upper	*p*-value
A2:B3-A1:B1	3.3333	0.6459	6.0208	0.0069	A2:B4-A1:B2	3.3333	0.6459	6.0208	0.0069
A3:B3-A1:B1	4.0000	1.3125	6.6875	0.0008	A3:B4-A1:B2	6.3333	3.6459	9.0208	0.0000
A1:B4-A1:B1	3.0000	0.3125	5.6875	0.0197	A2:B4-A2:B2	3.0000	0.3125	5.6875	0.0197
A2:B4-A1:B1	4.3333	1.6459	7.0208	0.0003	A3:B4-A2:B2	6.0000	3.3125	8.6875	0.0000
A3:B4-A1:B1	7.3333	4.6459	10.0208	0.0000	A3:B4-A3:B2	5.6667	2.9792	8.3541	0.0000
A3:B4-A2:B1	5.6667	2.9792	8.3541	0.0000	A3:B4-A1:B3	5.3333	2.6459	8.0208	0.0000
A3:B3-A3:B1	3.0000	0.3125	5.6875	0.0197	A3:B4-A2:B3	4.0000	1.3125	6.6875	0.0008
A2:B4-A3:B1	3.3333	0.6459	6.0208	0.0069	A3:B4-A3:B3	3.3333	0.6459	6.0208	0.0069
A3:B4-A3:B1	6.3333	3.6459	9.0208	0.0000	A3:B4-A1:B4	4.3333	1.6459	7.0208	0.0003
A3:B3-A1:B2	3.0000	0.3125	5.6875	0.0197	**A3:B4-A2:B4**	**3.0000**	**0.3125**	**5.6875**	**0.0197**

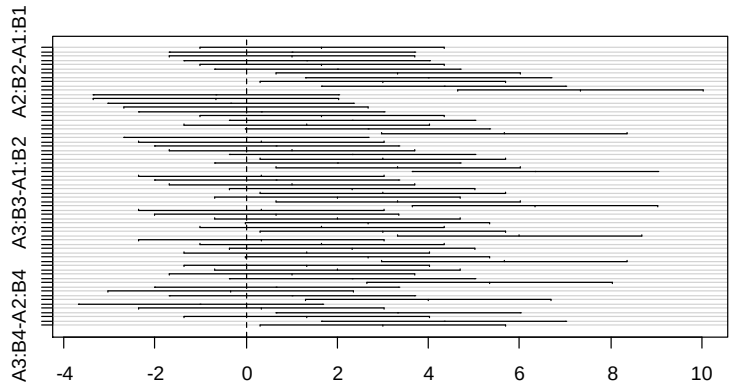

Fig. 1. Plot of pairwise comparison of the interaction between treatment A and treatment B.

B. Time required for pin head initiation

Here the minimum days or time to pin head initiation is preferred, the treatment which takes minimum number of days would be the best treatment combination and preferred over the others. In the present work, we have found that treatment A (different methods of substrate preparation), B (different methods of substate sterilization) are significant and A×B (interaction between method of substrate preparation and sterilization) is non-significant as reported in Table 4. Here, if interaction A×B is non-significant, then we compare A and B separately. In case of different methods of substrate preparation,A1(rolled paddy straw) was found to be the best method that took the minimum days for pin head initiation (Fig. 2).For different methods of substate sterilization, B2(steam sterilization) was found to be the best method that took the minimum number of days for pin head initiation (Fig. 3). The plot of pairwise comparison obtained by means of Tukey HSD to find the treatment which takes the minimum days required for pinhead initiation of treatments A is given in Fig. 2. The plot of pairwise comparison obtained by means of Tukey HSD to find the treatment which takes the minimum days required for pinhead initiation interaction of treatments B (Fig. 3).

Table 4: Analysis of variance for days required for pin head initiation.

S.V.	df	SS	MSS	F value	*p*-value
A	2	272.08	136.04	370.121	0.0001
B	3	45.36	15.12	41.133	0.0001
A*B	6	5.49	0.91	2.489	0.0514
Error	24	8.82	0.37		
Total	35	331.75			

Table 5: Pairwise comparison of treatment A by Tukey HSD.

Combination	Difference	Lower	Upper	*p*-value
A2-A1	-3.075	-3.6931	-2.4569	0
A3-A1	-6.72583	-7.34393	-6.10774	0
A3-A2	-3.65083	-4.26893	-3.03274	0

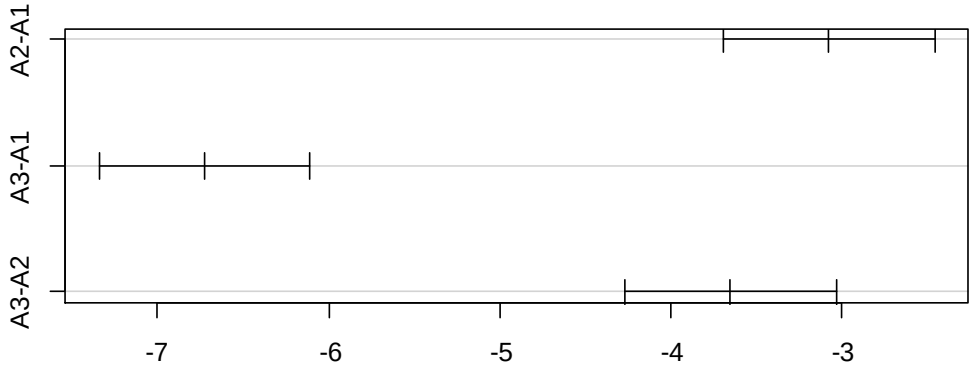

Fig. 2. Plot of pairwise comparison of treatment A.

Table 6: Pairwise comparison of treatment B by Tukey HSD.

Combination	Difference	Lower	Upper	*p*-value
B2-B1	-0.83222	-1.62063	-0.04382	0.0359
B3-B1	-2.36333	-3.15174	-1.57493	0.0000
B4-B1	-2.76444	-3.55285	-1.97604	0.0000
B3-B2	-1.53111	-2.31952	-0.74271	0.0001
B4-B2	-1.93222	-2.72063	-1.14382	0.0000
B4-B3	-0.40111	-1.18952	0.387293	0.5094

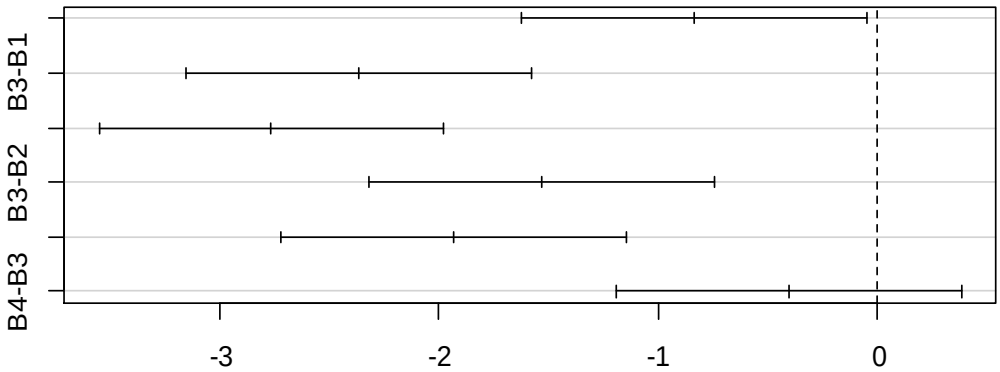

Fig. 3. Plot of pairwise comparison of treatment B.

C. Number of days required to harvest

Here we prefer the minimum days required to harvest after pin head initiation, the treatment which takes minimum number of days would be the best treatment combination and preferred over the others. In the present work, we have found that treatment A (different methods of substrate preparation), B (different methods of substate sterilization) and A×B (interaction between method of substrate preparation and sterilization) are significant as reported in (Table 7). Here, if interaction A×B is significant, then we compare only the interactions as per our objective. The chopped paddy straw substrate treated with steam sterilization took minimum time (16.60) days period for harvesting after pin head initiation. The treatment combination is highlighted in Table 8.

Table 7: Analysis of variance for days required to harvest.

S.V.	df	SS	MSS	F value	*p*-value
A	2	145.8	72.9	156.3	0.0001
B	3	49.02	16.34	35.03	0.0001
A*B	6	33.48	5.58	11.96	0.0001
Error	24	11.19	0.47		
Total	35	239.49			

Table 8: Pairwise comparison of interactions by using Tukey HSD.

Combination	Difference	Lower	Upper	*p*-value	Combination	Difference	Lower	Upper	*p*-value
A2:B1-A1:B1	-6.1700	-8.1806	-4.1594	0.0000	A1:B3-A3:B1	2.0767	0.0661	4.0873	0.0386
A3:B1-A1:B1	-8.0800	-10.0906	-6.0694	0.0000	A2:B2-A1:B2	-4.3033	-6.3139	-2.2927	0.0000
A1:B2-A1:B1	-3.0000	-5.0106	-0.9894	0.0008	A3:B2-A1:B2	-5.2500	-7.2606	-3.2394	0.0000
A2:B2-A1:B1	**-7.3033**	**-9.3139**	**-5.2927**	**0.0000**	A1:B3-A1:B2	-3.0033	-5.0139	-0.9927	0.0008
A3:B2-A1:B1	-8.2500	-10.2606	-6.2394	0.0000	A2:B3-A1:B2	-4.7000	-6.7106	-2.6894	0.0000
A1:B3-A1:B1	-6.0033	-8.0139	-3.9927	0.0000	A3:B3-A1:B2	-5.7800	-7.7906	-3.7694	0.0000
A2:B3-A1:B1	-7.7000	-9.7106	-5.6894	0.0000	A1:B4-A1:B2	-3.1067	-5.1173	-1.0961	0.0005
A3:B3-A1:B1	-8.7800	-10.7906	-6.7694	0.0000	A2:B4-A1:B2	-4.7000	-6.7106	-2.6894	0.0000
A1:B4-A1:B1	-6.1067	-8.1173	-4.0961	0.0000	A3:B4-A1:B2	-6.1100	-8.1206	-4.0994	0.0000
A2:B4-A1:B1	-7.7000	-9.7106	-5.6894	0.0000	A1:B3-A3:B2	2.2467	0.2361	4.2573	0.0195
A3:B4-A1:B1	-9.1100	-11.1206	-7.0994	0.0000	A1:B4-A3:B2	2.1433	0.1327	4.1539	0.0296
A1:B2-A2:B1	3.1700	1.1594	5.1806	0.0004	A3:B3-A1:B3	-2.7767	-4.7873	-0.7661	0.0021
A3:B2-A2:B1	-2.0800	-4.0906	-0.0694	0.0381	A3:B4-A1:B3	-3.1067	-5.1173	-1.0961	0.0005
A3:B3-A2:B1	-2.6100	-4.6206	-0.5994	0.0042	A1:B4-A3:B3	2.6733	0.6627	4.6839	0.0032
A3:B4-A2:B1	-2.9400	-4.9506	-0.9294	0.0010	A3:B4-A1:B4	-3.0033	-5.0139	-0.9927	0.0008
A1:B2-A3:B1	5.0800	3.0694	7.0906	0.0000					

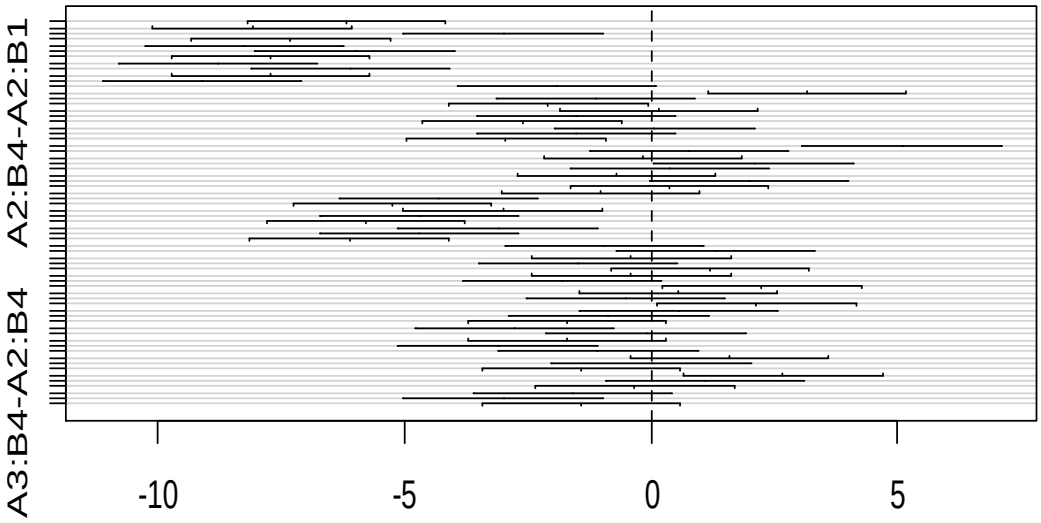

Fig. 4. Plot of pairwise comparison of the interactions between treatment A and treatment B.

The plot of pairwise comparison obtained by means of Tukey HSD to find the treatment which takes the minimum days to harvest is given in Fig. 4.

D. Number of fruiting bodies

Here we prefer the maximum average number of fruiting bodies, the treatment which gives maximum average number of fruiting bodies would be the best treatment combination and preferred over the others. In the present work, we have found that treatment A (different methods of substrate preparation), B (different methods of substate sterilization) and A×B (interaction between method of substrate preparation and sterilization) are significant as reported in (Table 9). Here, if interaction A×B is significant, then we compare only the interactions as per our objective. Maximum number of fruiting bodies found on chopped paddy straw substrate treated with hot water (12.30 Nos). The best treatment combination is highlighted in Table 10.

Table 9: Analysis of variance for Number of fruiting bodies.

S.V.	df	SS	MSS	F value	*p*-value
A	2	36.15	18.075	155.37	0.0000
B	3	20.43	6.81	58.54	0.0000
A×B	6	37.37	6.229	53.54	0.0000
Error	24	2.79	0.116		
Total	35	96.74			

Table 10: Pairwise comparison by Tukey HSD.

Combination	Difference	Lower	Upper	*p*-value	Combination	Difference	Lower	Upper	*p*-value
A2:B1-A1:B1	**-5.3300**	**-6.3341**	**-4.3259**	**0.0000**	A2:B2-A1:B2	-1.9967	-3.0008	-0.9925	0.0000
A3:B1-A1:B1	-3.9967	-5.0008	-2.9925	0.0000	A3:B2-A1:B2	-3.6667	-4.6708	-2.6625	0.0000
A1:B2-A1:B1	-1.6633	-2.6675	-0.6592	0.0002	A1:B3-A1:B2	-2.3300	-3.3341	-1.3259	0.0000
A2:B2-A1:B1	-3.6600	-4.6641	-2.6559	0.0000	A2:B3-A1:B2	-4.0000	-5.0041	-2.9959	0.0000
A3:B2-A1:B1	-5.3300	-6.3341	-4.3259	0.0000	A3:B3-A1:B2	-4.0033	-5.0075	-2.9992	0.0000
A1:B3-A1:B1	-3.9933	-4.9975	-2.9892	0.0000	A1:B4-A1:B2	-3.0000	-4.0041	-1.9959	0.0000
A2:B3-A1:B1	-5.6633	-6.6675	-4.6592	0.0000	A2:B4-A1:B2	-2.6633	-3.6675	-1.6592	0.0000
A3:B3-A1:B1	-5.6667	-6.6708	-4.6625	0.0000	A3:B4-A1:B2	-2.0000	-3.0041	-0.9959	0.0000
A1:B4-A1:B1	-4.6633	-5.6675	-3.6592	0.0000	A3:B2-A2:B2	-1.6700	-2.6741	-0.6659	0.0002
A2:B4-A1:B1	-4.3267	-5.3308	-3.3225	0.0000	A2:B3-A2:B2	-2.0033	-3.0075	-0.9992	0.0000
A3:B4-A1:B1	-3.6633	-4.6675	-2.6592	0.0000	A3:B3-A2:B2	-2.0067	-3.0108	-1.0025	0.0000
A3:B1-A2:B1	1.3333	0.3292	2.3375	0.0033	A1:B3-A3:B2	1.3367	0.3325	2.3408	0.0032
A1:B2-A2:B1	3.6667	2.6625	4.6708	0.0000	A3:B4-A3:B2	1.6667	0.6625	2.6708	0.0002
A2:B2-A2:B1	1.6700	0.6659	2.6741	0.0002	A2:B3-A1:B3	-1.6700	-2.6741	-0.6659	0.0002
A1:B3-A2:B1	1.3367	0.3325	2.3408	0.0032	A3:B3-A1:B3	-1.6733	-2.6775	-0.6692	0.0002
A3:B4-A2:B1	1.6667	0.6625	2.6708	0.0002	A2:B4-A2:B3	1.3367	0.3325	2.3408	0.0032
A1:B2-A3:B1	2.3333	1.3292	3.3375	0.0000	A3:B4-A2:B3	2.0000	0.9959	3.0041	0.0000
A3:B2-A3:B1	-1.3333	-2.3375	-0.3292	0.0033	A2:B4-A3:B3	1.3400	0.3359	2.3441	0.0031
A2:B3-A3:B1	-1.6667	-2.6708	-0.6625	0.0002	A3:B4-A3:B3	2.0033	0.9992	3.0075	0.0000
A3:B3-A3:B1	-1.6700	-2.6741	-0.6659	0.0002					

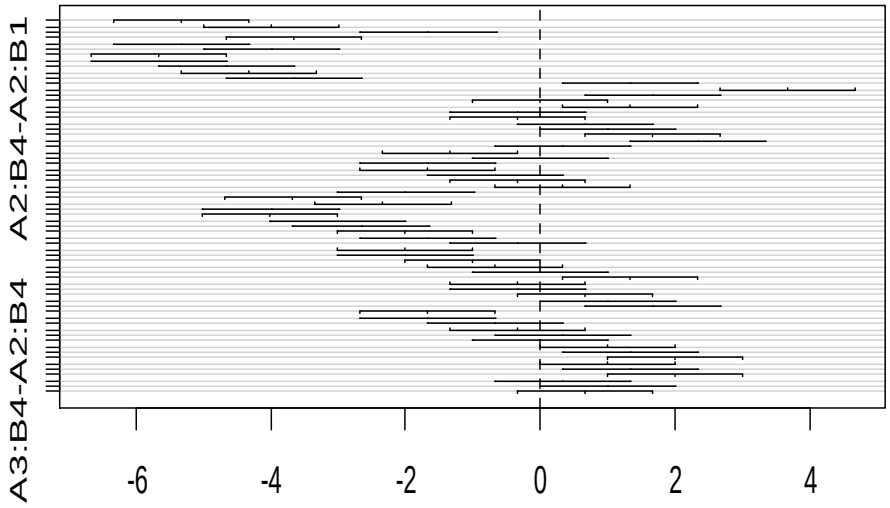

Fig. 5. Plot of pairwise comparison of the interactions between treatment A and treatment B.

The plot of pairwise comparison obtained by means of Tukey HSD to find the treatment which shows average number of fruiting bodies is given in Fig. 5.

E. Performance based on yield

Here we prefer the maximum average yield of fruiting bodies, the treatment which gives maximum average yield of fruiting bodies would be the best treatment combination and preferred over the others. In the present work, we have found that treatment A (different methods of substrate preparation), B (different methods of substate sterilization) and A×B (interaction between method of substrate preparation and sterilization) are significant as reported in Table 11.

Table 11: Analysis of variance for Yield.

S.V.	df	SS	MSS	F value	*p*-value
A	2	363858	181929	93.523	0.0001
B	3	50047	16682	8.576	0.0005
A×B	6	174558	29093	14.956	0.0001
Error	24	46687	1945		
Total	35	635150			

Here, if interaction A×B is significant, the substrate chopped Paddy straw treated with hot water results maximum yield i.e., A2B1 (1193.30 gm) the treatment combination is highlighted in Table 12, followed by chopped paddy straw treated with steam sterilization A2B2 i.e. (1060.00gm) and chopped paddy straw treated with chemicals, Formalin and Bavistin i.e. A2B3 (1028.30gm) similar findings were reported by Purkayastha and Chandra (1985). The plot of pairwise comparison obtained by means of Tukey HSD to find the treatment which shows average yield of fruiting bodies is given in Fig. 6.

Table 12: Pairwise comparison by Tukey HSD.

Combination	Difference	Lower	Upper	*p*-value	Combination	Difference	Lower	Upper	*p-value*
A2:B1-A1:B1	**403.33**	**273.49**	**533.18**	**0.0000**	A2:B3-A3:B1	216.00	86.15	345.85	0.0002
A1:B2-A1:B1	147.00	17.15	276.85	0.0173	A3:B2-A1:B2	-137.00	-266.85	-7.15	0.0324
A2:B2-A1:B1	270.00	140.15	399.85	0.0000	A3:B3-A1:B2	-196.33	-326.18	-66.49	0.0007
A2:B3-A1:B1	238.33	108.49	368.18	0.0000	A3:B2-A2:B2	-260.00	-389.85	-130.15	0.0000
A3:B1-A2:B1	-381.00	-510.85	-251.15	0.0000	A1:B3-A2:B2	-209.33	-339.18	-79.49	0.0003
A1:B2-A2:B1	-256.33	-386.18	-126.49	0.0000	A3:B3-A2:B2	-319.33	-449.18	-189.49	0.0000
A2:B2-A2:B1	-133.33	-263.18	-3.49	0.0405	A1:B4-A2:B2	-196.67	-326.51	-66.82	0.0006
A3:B2-A2:B1	-393.33	-523.18	-263.49	0.0000	A2:B4-A2:B2	-209.33	-339.18	-79.49	0.0003
A1:B3-A2:B1	-342.67	-472.51	-212.82	0.0000	A3:B4-A2:B2	-234.00	-363.85	-104.15	0.0001
A2:B3-A2:B1	-165.00	-294.85	-35.15	0.0054	A2:B3-A3:B2	228.33	98.49	358.18	0.0001
A3:B3-A2:B1	-452.67	-582.51	-322.82	0.0000	A2:B3-A1:B3	177.67	47.82	307.51	0.0023
A1:B4-A2:B1	-330.00	-459.85	-200.15	0.0000	A3:B3-A2:B3	-287.67	-417.51	-157.82	0.0000
A2:B4-A2:B1	-342.67	-472.51	-212.82	0.0000	A1:B4-A2:B3	-165.00	-294.85	-35.15	0.0054
A3:B4-A2:B1	-367.33	-497.18	-237.49	0.0000	A2:B4-A2:B3	-177.67	-307.51	-47.82	0.0023
A2:B2-A3:B1	247.67	117.82	377.51	0.0000	A3:B4-A2:B3	-202.33	-332.18	-72.49	0.0004

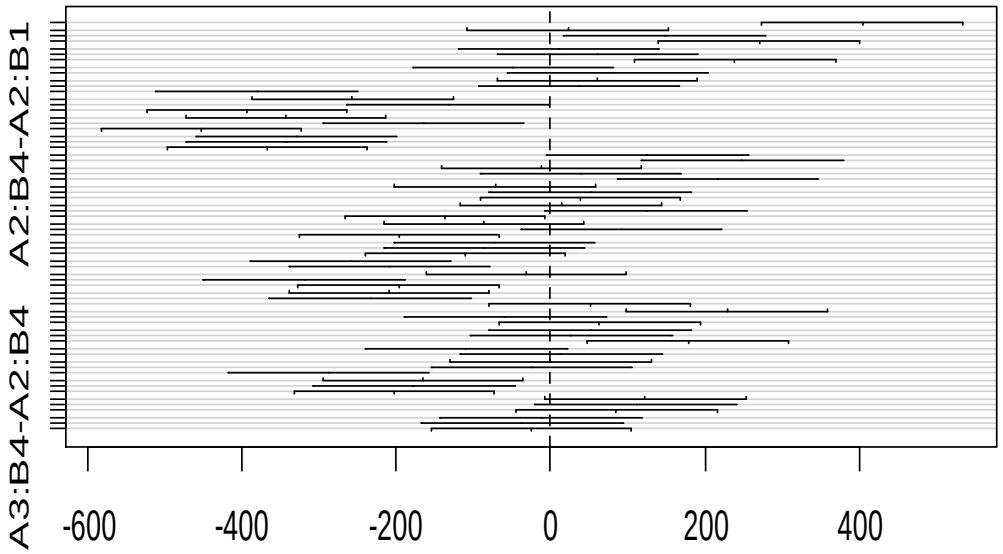

Fig. 6. Plot of pairwise comparison of the interaction of treatment A and treatment B.

F. Biological efficiency

Here we prefer the maximum average biological efficiency of fruiting bodies, the treatment which gives maximum average biological efficiency of fruiting bodies would be the best treatment combination and preferred over the others. In the present work, we have found that treatment A (different methods of substrate preparation), B (different methods of substate sterilization) and A×B (interaction between method of substrate preparation and sterilization) are significant as reported in (Table 13). Here, if interaction A×B is significant, maximum biological efficiency observed on the substrate chopped paddy straw treated with hot water results maximum yield i.e., A2B1 (119.3%) the best treatment combination is highlighted in Table 14, followed by chopped paddy straw treated steam sterilization A2B2 i.e. (106.0%) and chopped paddy straw treated with chemicals chemical Formalin and Bavistin i.e. A2B3 (102.8%) similar results were reported by Krishnamoorthy (1981).

Table 13: Analysis of variance for biological efficiency.

S.V.	df	SS	MSS	F value	*p*-value
A	2	3639	1819.3	93.523	0.0001
B	3	500	166.8	8.576	0.0005
A×B	6	1746	290.9	14.956	0.0001
Error	24	467	19.5		
Total	35	6352			

Table 14: Pairwise comparison of interactions by using Tukey HSD.

Combination	Difference	Lower	Upper	*p*-value	Combination	Difference	Lower	Upper	*p*-value
A2:B1-A1:B1	**40.3333**	**27.3488**	**53.3179**	**0.0000**	A2:B3-A3:B1	21.6000	8.6155	34.5845	0.0002
A1:B2-A1:B1	14.7000	1.7155	27.6845	0.0173	A3:B2-A1:B2	-13.7000	-26.6845	-0.7155	0.0324
A2:B2-A1:B1	27.0000	14.0155	39.9845	0.0000	A3:B3-A1:B2	-19.6333	-32.6179	-6.6488	0.0007
A2:B3-A1:B1	23.8333	10.8488	36.8179	0.0000	A3:B2-A2:B2	-26.0000	-38.9845	-13.0155	0.0000
A3:B1-A2:B1	-38.1000	-51.0845	-25.1155	0.0000	A1:B3-A2:B2	-20.9333	-33.9179	-7.9488	0.0003
A1:B2-A2:B1	-25.6333	-38.6179	-12.6488	0.0000	A3:B3-A2:B2	-31.9333	-44.9179	-18.9488	0.0000
A2:B2-A2:B1	-13.3333	-26.3179	-0.3488	0.0405	A1:B4-A2:B2	-19.6667	-32.6512	-6.6821	0.0006
A3:B2-A2:B1	-39.3333	-52.3179	-26.3488	0.0000	A2:B4-A2:B2	-20.9333	-33.9179	-7.9488	0.0003
A1:B3-A2:B1	-34.2667	-47.2512	-21.2821	0.0000	A3:B4-A2:B2	-23.4000	-36.3845	-10.4155	0.0001
A2:B3-A2:B1	-16.5000	-29.4845	-3.5155	0.0054	A2:B3-A3:B2	22.8333	9.8488	35.8179	0.0001
A3:B3-A2:B1	-45.2667	-58.2512	-32.2821	0.0000	A2:B3-A1:B3	17.7667	4.7821	30.7512	0.0023
A1:B4-A2:B1	-33.0000	-45.9845	-20.0155	0.0000	A3:B3-A2:B3	-28.7667	-41.7512	-15.7821	0.0000
A2:B4-A2:B1	-34.2667	-47.2512	-21.2821	0.0000	A1:B4-A2:B3	-16.5000	-29.4845	-3.5155	0.0054
A3:B4-A2:B1	-36.7333	-49.7179	-23.7488	0.0000	A2:B4-A2:B3	-17.7667	-30.7512	-4.7821	0.0023
A2:B2-A3:B1	24.7667	11.7821	37.7512	0.0000	A3:B4-A2:B3	-20.2333	-33.2179	-7.2488	0.0004

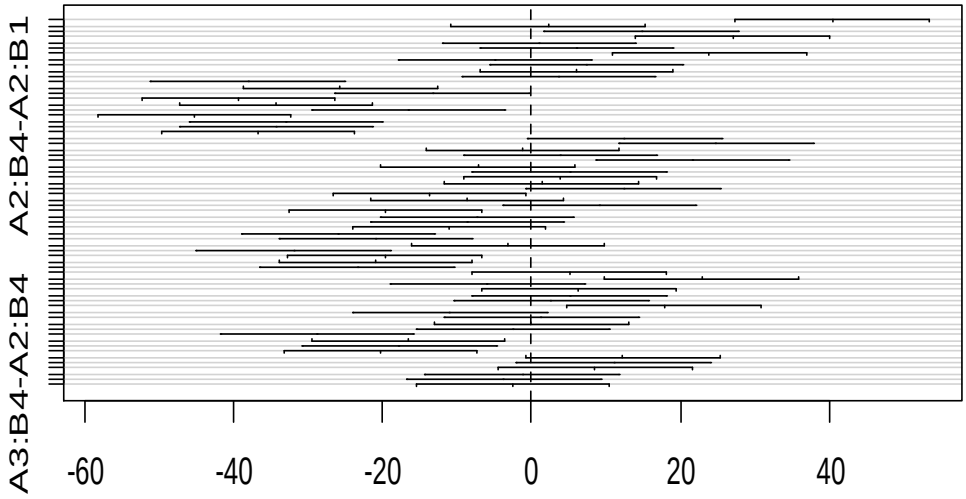

Fig. 7. Plot of pairwise comparison of the interactions between treatment A and treatment B.

The plot of pairwise comparison obtained by means of Tukey HSD to find the best treatment which shows the maximum biological efficiency of fruiting bodies is given in Fig. 7.

CONCLUSION

The present investigation reflects that chopped paddy straw sterilized with the combination of chemicals, limestone, bleaching powder and Indofil M-45 *i.e.*, treatment A2B4 (22.30 days) took the minimum number of days for spawn run. Paddy straw treated with steam sterilization took the minimum number of days for pin head formation. Chopped paddy straw sterilized with steam took the minimum number of days to harvest i.e., A2B2 (16.60 days). The maximum average number of fruiting bodies (12.30 Nos.), maximum yield (1193.30g) and highest biological efficiency (119.30 %) were resulted in case of chopped paddy straw treated with hot water i.e., A2B1. So, the efficient cultivation of milky mushroom can be done by using chopped paddy straw by sterilizing it using hot water.

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