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Assessment and selection of best indigenous isolates of milky Mushroom

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Abstract
Mushroom is become an important food complement in these days as a rich source of vitamins, minerals etc. Milky mushroom is of Indian origin and requires a smooth substrate like paddy straw (lignocellulolytic substrates), under the climatic condition with a relative humidity (80-90%) and medium temperature (30-35°C) for better growth. There are fourty commonly known different species of milky mushroom out of which only four are of edible nature viz., *C. indica*, *C. gambosa*, *C. ionides*, and *C. carnea*. As each and every mushroom possesses a good nutritive value, whereas milky mushroom is a rich source of Vitamin-B2, E, A and C along with minerals such as, Phosphorous Potassium Calcium Zinc Iron and Selenium. Therefore, the present work was directed to evaluate four strains of milky mushroom viz., CI-1, CI-2, CI-3 and CI-4 procured from tropical mushroom research station, OUAT Bhubaneswar, mushroom laboratory of IGKV, Raipur, local market of Kolkata and local market of Bolpur respectively, according to the climatic condition of Bolpur. In this concern, we have observed the parameters such as, growth of mycelium, days required for mother pawn and seed spawn preparation, days required for sporophore formation, number of fruiting bodies, yield and biological efficiency. It was found that the CI-3 and CI-1 strains performed better than that of CI-2 and CI-4 under this evaluation based on these parameters of *C. indica.*

Keywords: Biological efficiency, *Calocybe Indica*, lignocellulolytic, strain, tropical mushroom.

1. Introduction
Mushrooms are treated as macro fungi belongs to phylum Ascomycota or Basidiomycota which are visible fruiting bodies with sexual reproductive structures. Its cultivation is eco-friendly, due to microbial process agricultural wastes are transformed into valuable food which are rich in proteins, vitamins and minerals and can be beneficial to human health. Mushroom farming requires upright space, short time and barely water. So, it has the potential of being a major crop in the upcoming years (Prakasam, 2012) [13].

In many countries mushroom is treated as major farming, studies revealed that mushroom farming can acquire third position after crop and livestock farming in terms of profitable farming, (Prakasam, 2012) [13]. In many developing countries, the advance technology towards mushroom farming has an auspicious scope to fulfil the nutritional necessities without disturbing the land. China is growing more than twenty edible mushrooms at commercial level and became foremost producer of mushrooms, which includes *Calocybe indica* (Wu *et al*., 2013) [19]. After china USA is the second producer in the world (Kumar *et al*., 2013) [11].

Cultivation of edible mushrooms was started in India initially with temperate mushroom like button mushroom (*Agaricus bisporus*). Nevertheless, our country can build rapid growth in the Mushroom trade by growing the tropical and sub-tropical mushrooms. It is reported that from the period 2010-2017 India produced about 0.13 million tons of mushroom and this field of cultivation has achieved an average growth rate of 4.3 per cent. There was much variation in the variety wise contribution of the mushrooms and the contributions were reported as, 73 per cent from white button mushroom, 16 per cent from oyster mushroom, 7 per cent from paddy straw mushroom and about 3 per cent from milky mushroom.

Milky mushroom is a temperate mushroom, which needs relative humidity 80 to 90 per cent and temperature of 30-35°C for its better growth. This mushroom is ideal for growing in hot weather and it has a healthy fruiting body, striking colour, delicious taste, sustainable yield unique character and excellent life span as compared to other mushrooms (Amin *et al*., 2010) [1]. In West Bengal, *Calocybe indica* has been cultivated on a variety of agricultural wastes such as, garden and forest wastes. However, no work has been done on the other available strains of *C. indica.*

Therefore, the present work was an evaluation to refine the best strain of the same from the locally available strains of *C. indica* viz., CI-1, CI-2, CI-3, CI-4.

These strains were procured from tropical mushroom research station, OUAT Bhubaneswar (CI-1), mushroom laboratory of IGKV, Raipur (CI-2), local market of Kolkata (CI-3) and local market of Bolpur (CI-4), to evaluate their growth behavior, morphometric characters, yield and biological efficiency under the climatic condition of Bolpur, West Bengal.

In Birbhum subdivision of West Bengal, the temperature ranges from 25.5 to 41.5°C with a relative humidity of about 70-85 per cent. The climatic situation of Birbhum is suitable for cultivation of most of the agricultural and horticultural crops which produces some agro-wastes out of which a less amount is used and the remaining wastes causes implications to the environmental conditions. So, to generate some revenue as well as maintenance of these wastes, the present work suggests some easiest ways which can be followed by the farmers.

Materials and methods

Studies on the evaluation of some Indigenous Isolates of *Calocybe indica* (P and C) for cultivation in Birbhum, West Bengal were conducted and following materials and methods were used.

Sources of culture and substrates

Pure cultures of 4 Indigenous Isolates of milky mushroom (*C. indica* P&C) CI-1 procured from Tropical Mushroom Research station, OUAT Bhubaneswar, CI-2 procured from mushroom laboratory of IGKV, Raipur, CI-3 acquired from Kolkata CI-4 collected from local market Bolpur, All the strains were maintained on sterilized PDA (potato dextrose agar) medium, medium slants and kept at room temperature during the entire period of investigation. Sub-sequent culturing was done after every 3 months. The *C. indica* was cultivated on the paddy straw substrate which is agro-waste of paddy crop and was collected from Agricultural farm, Visva Bharati.

Experimental location

Experiments pertaining to the cultivation of *C. indica* (P&C) were conducted in a mushroom cultivation house located in the Experiential teaching unit, Mushroom Laboratory, Department of Plant pathology, Institute of Agriculture, Visva Bharati, Sriniketan.

Procedures of spawn preparation

A spawn is a pure fungal culture grown on soften grains in a sterilized condition. During the present study, mother spawn of four strains of *C. indica* (CI-1, CI-2, CI-3, CI-4) was prepared from pure cultures by following the method of (Munjal, 1973) [12]. Commercially seed spawn was prepared by pouring softened wheat grains in polyethylene bags @ 200gm grain per bag then bags were sterilized at 121° C for 15 minutes. Allow it for cooling, kept in a laminar airflow chamber then 10-15g of mother spawn were added in to the bags. Grain bags covering with fully white mycelial growth used for experiment work.

Preparation of substrate

Agricultural waste like paddy straw was collected from the field then substrate was finally cut into small pieces of 4-5 cm and then exposed to hot water for sterilization. The substrates were soaked in a plastic drum for 24 hours containing cold water. Then, the substrates were allowed to boil in boiling water (80-90 ºC) for 40-60 minutes. Remove Excess water and then the substrates were spread over cemented floor to get moisture about 60 percent.

Preparation of bags

It is a process of familiarizing spawn into the substrate to get uniform growth to get fruiting bodies. 2kg of wet substrate was packed in polyethylene bags with 4 to 5 layers of spawning with a dose of 5% on dry weight basis of the substrates. After spawning, the lower and corners portion of the bags were made10 to 15 small holes to drain out of excess water. Then, the bags were bind with rubber bands.

Spawn run, casing and maintenance of bags

Spawn run stands for uniform growth of mycelium on the substrate, the spawn bags were placed in a controlled environment where temperature and relative humidity is maintained for complete colonization then casing process is require to cover the upper most layer of the spawn bags just after the completion of the spawn run over the substrate. Casing is the process use to cover the top layer of spawn bags by using soil, sand, FYM or combination of soil, sand and FYM which have good water holding capacity applied with a layer of 3 to 4 centimeter. The casing materials were sterilized in autoclave at 121.6ºC for one hour before use then regularly water is added on the casing layer to get the reproductive phase of mycelium in the form of sporophore. After the process of casing followed by sprinkling of water regularly, several needle shaped pin heads are formed on the top layer of casing after few days those needle shaped pin heads are turned into large fruiting bodies which are ready for harvesting.

Measurement of yield attributes

The healthy fruiting bodies are plucked by twisting the sporophore in both direction without disturbing the nearer unmatured fruiting bodies. The cleaned fresh sporophores are packed in a polythene of 500gm measured by using electrical weighing machine. Biological efficiency is calculated by the method proposed by Chang *et al*. (1981) [3] for the yield of sporophores from each bag of all the replication used were expressed in grams and the effectiveness of mushroom and substrate combination were calculated and expressed as B.E.

Analytical procedure (Rangaswamy 2020) [14]

Suppose we have t treatments and if we are concerned with comparison of all possible pairs of these treatment means, then we can use Duncan's Multiple Range (DMRT) test. It can be used irrespective of the significance of F value. We have followed the steps to compare the treatments as,

Step 1: Arranged the treatment means according to their ranks it means they are ordered in descending order.

Step 2: Obtained the standard error of mean by the formula given as,

$$\text{Se}(\bar{Y}) = \sqrt{\frac{s_e^2}{r}} = \sqrt{\frac{EMS}{r}}$$, where s_e^2 and EMS

are equivalent and it stands for Error Mean Square or MSE in ANOVA table.

Step 3: From statistical tables of DMRT, we have obtained the significant studentized ranges (r_p) for p = 2, 3, …, t, treatments and error degrees of freedom.

Step 4: Obtained the shortest significant ranges

(R_p) as, $R_p = r_p \times \text{Se}(\bar{Y})$.

Step 5: From the largest mean subtracted the R_p for largest p. declared all the means less than this value significantly different from this largest mean. For the remaining treatments whose values are larger than the difference (Largest mean-largest R_p), compared the differences with appropriate R_p values. E.g., If two treatments are remaining compare R_2; if three are remaining compare with R_3.

Step 6: From the second largest mean subtracted the second largest R_p and compared the treatments.

Step 5: Continued this process until all the treatment pairs are covered.

Step 7: The results can be presented by using either the line or the alphabet notation to indicate the significant and non-significant pair of treatments. Here we have used both the notation in a single graph.

Result and Discussion

According to the characters observed the analysis was performed and the results of these parameters are given in this section.

Length of mycelium on PDA medium

Table 1: Analysis of variance table for the length of mycelia

Sources of Variation	Df	Sum Squares	Mean Squares	F-value	*p*-value
Treatments	3	7.689	2.563	39.43	0.001**
Error	16	1.04	0.065		
Total	19	8.729	2.628		

The overall ANOVA is significant at 1 per cent level of significance (Table-1). It allows us to further compare the treatments, or pair of treatments. To test the pairwise combination of the treatments we have used DMRT and found that the treatment pairs viz., C3, C1 fall under one group (i.e., a), it means these treatments are at par and remaining treatments viz., C1, C2 and C4 were significantly different from each other, it indicates that the average length of mycelia was significantly different for these treatments. From Table-2, the average maximum length of the mycelium was observed for the treatment C3 but it is at par with the treatment C1. Thus, we would take the maximum average length of mycelium for the treatment C1 to be the highest, because the above treatment mean i.e., for C3 (6.56) was non-significant.

Table 2: Groups of the treatments

Treatments	Length	Groups
C3	6.56	a
C1	6.30	a
C2	5.52	b
C4	5.00	c

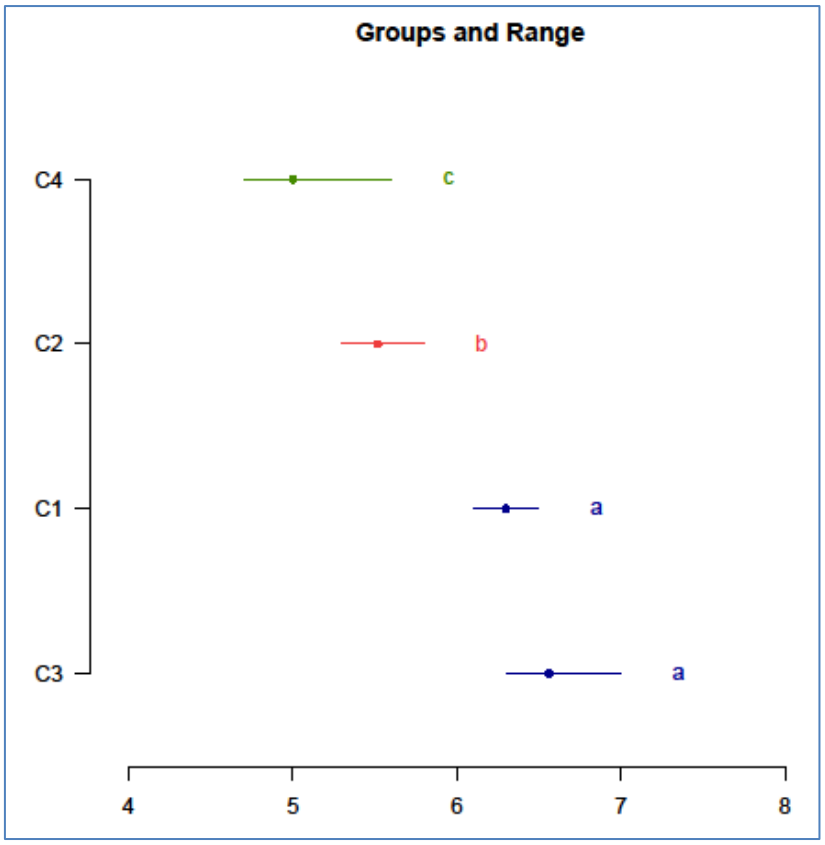

Fig 1: Plot of treatment means with the name of the respective group

(ii) Days required for preparation of mother spawn

Table 3: Analysis of variance table for Days required for preparation of mother spawn

Sources of Variation	Df	Sum Squares	Mean Squares	F-value	*p*-value
Treatments	3	88.2	29.4	7.171	0.00288
Error	16	65.6	4.1		
Total	19	153.8	33.5		

C3 & C4 fall under one group (i.e., a) it means C3 and C4 treatments are at par and C1, C2 fall under one group (i.e., b) it means C1 and C2 treatments are at par but C3 and C1 are significantly different from each other, it indicates that the average Days required for preparation of mother spawn was significantly different for these treatments. From Table-4, the minimum average Days required for preparation of mother spawn was observed for the treatment C2 (20.80) which was at par with C1 (21.0) but C1 was significantly different from C3. So, the minimum average days required for preparation of mother spawn was observed for the treatment C1 (21.0).

Table 4: Groups of the treatments

Treatments	Avg. DRFPMS	Groups
C4	25.8	a
C3	24.0	a
C1	21.0	b
C2	20.8	b

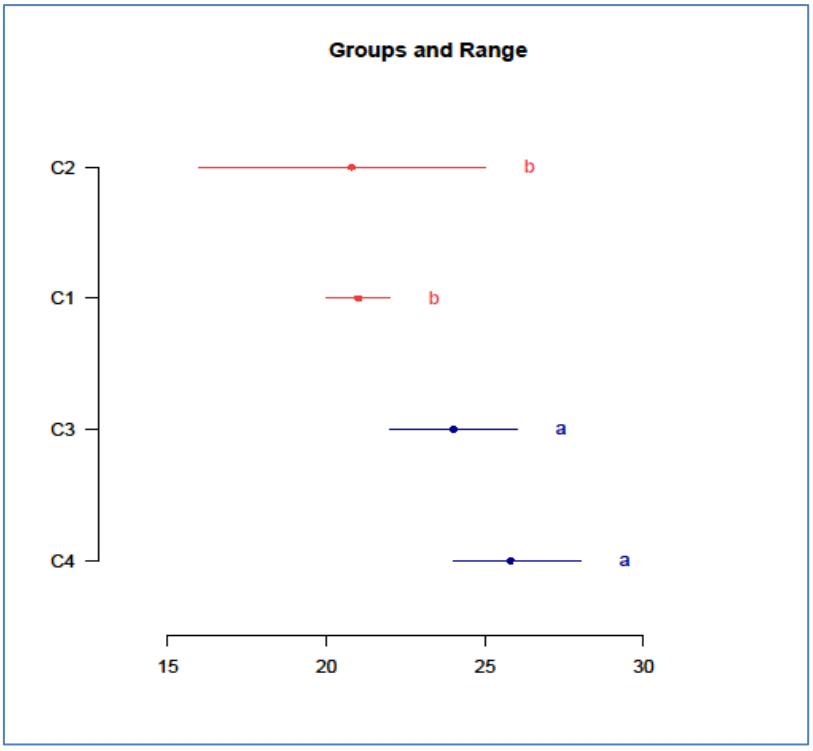

Fig 2: Plot of treatment means with the name of the respective group

(iii) Days required for preparation of seed spawn

Table 5: Analysis of variance table for Days required for preparation of seed spawn

Sources of Variation	Df	Sum Squares	Mean Squares	F-value	*p*-value
Treatments	3	33.2	11.07	4.427	0.019
Error	16	40	2.5		
Total	19	73.2	13.57		

C4 fall under one group (i.e., a) and C1, C2 and C3 fall under one group (i.e., b) it means C1,C2 and C3 treatments are at par but treatment C4 was significantly different from C1, it indicates that the average Days required for preparation of seed spawn was significantly different for this treatment. From Table-6, the minimum average Days required for preparation of seed spawn was observed for the treatment C3 (19.20) which was at par with C1and C2.

Table 6: Groups of the treatments

Treatments	Avg. DRFPSS	Groups
C4	22.8	a
C1	20.6	b
C2	20.6	b
C3	19.2	b

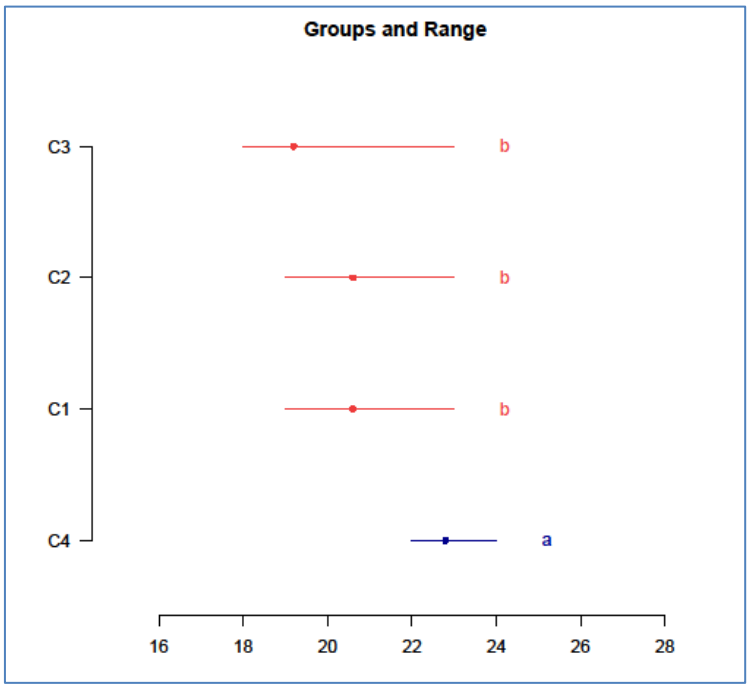

Fig 3: Plot of treatment means with the name of the respective group

(iv) Days required to spawn run

Table 7: Analysis of variance table for Days required to spawn run

Sources of Variation	Df	Sum Squares	Mean Squares	F-value	*p*-value
Treatments	3	113	37.67	20.64	0.0000
Error	16	29.2	1.83		
Total	19	142.2	39.5		

C4 fall under one group (i.e., a) and C1, C2 fall under one group (i.e., b) it means C1,C2 treatments are at par but treatment C4 was significantly different from C1 and C2 and C3 are significantly different, it indicates that the average days required for spawn run was significantly different for this treatments. From Table-8, the minimum average days required to spawn run was observed for the treatment C3 (22.40).

Table 8: Groups of the treatments

Treatments	Avg. DRFSR	groups
C4	29	a
C1	25.2	b
C2	24.6	b
C3	22.4	c

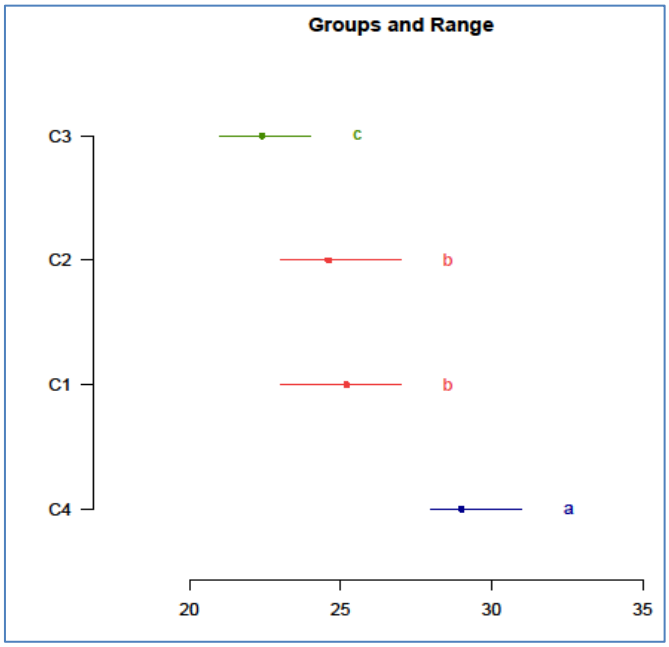

Fig 4: Plot of treatment means with the name of the respective group

(v) Days required for fruiting body formation

Table 9: Analysis of variance table for Days required for fruiting body formation

Sources of Variation	Df	Sum Squares	Mean Squares	F-value	*p*-value
Treatments	3	322.6	107.53	37.73	0.0000
Error	16	45.6	2.85		
Total	19	368.2	110.38		

The treatment C4 fall under one group (i.e., a) and C1, C2 and C3 fall under one group (i.e., b) it means C1, C2 and C3 treatments are at par but treatment C4 was significantly different from C1. it indicates that the average days required for fruiting body formation was significantly different for this treatment. From Table-10, the minimum average days required to spawn run was observed for the treatment C1 (43.00).

Table 10: Groups of the treatments

Treatments	Length	Groups
C4	51.6	a
C1	43	b
C2	42.6	b
C3	41.6	b

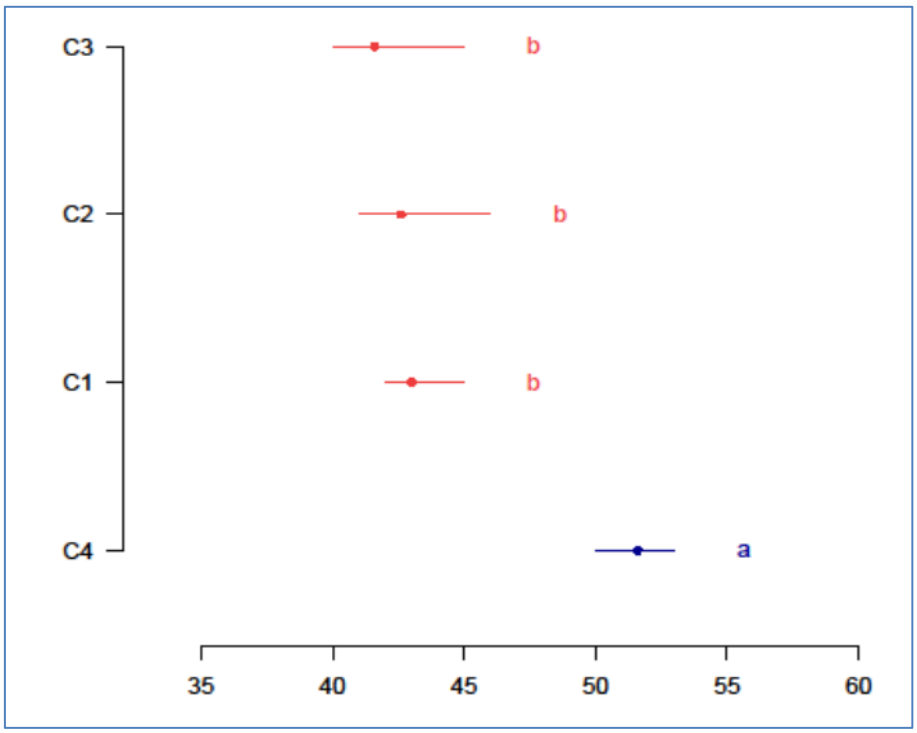

Fig 5: Plot of treatment means with the name of the respective group

(vi) Number of fruiting bodies

Table 11: Analysis of variance table for Number of fruiting bodies

Sources of Variation	Df	Sum Squares	Mean Squares	F-value	*p*-value
Treatments	3	73	24.333	18.36	0.0000
Error	16	21.2	1.325		
Total	19	94.2	25.658		

C4 fall under one group (i.e., c) and C1 and C2 fall under one group (i.e., b and C3 treatments are fall under one group (i.e., a) it means C3 and C2 are significantly different from each other and C2 and C1 are at par but C1 and C4 are significantly different from each other. it indicates that the average number fruiting body was significantly different for treatment C3. From Table-12, the maximum average number fruiting body was observed for the treatment C3 (12.40).

Table 12: Groups of the treatments

Treatments	Length	Groups
C3	12.4	a
C2	9.8	b
C1	9.6	b
C4	7	c

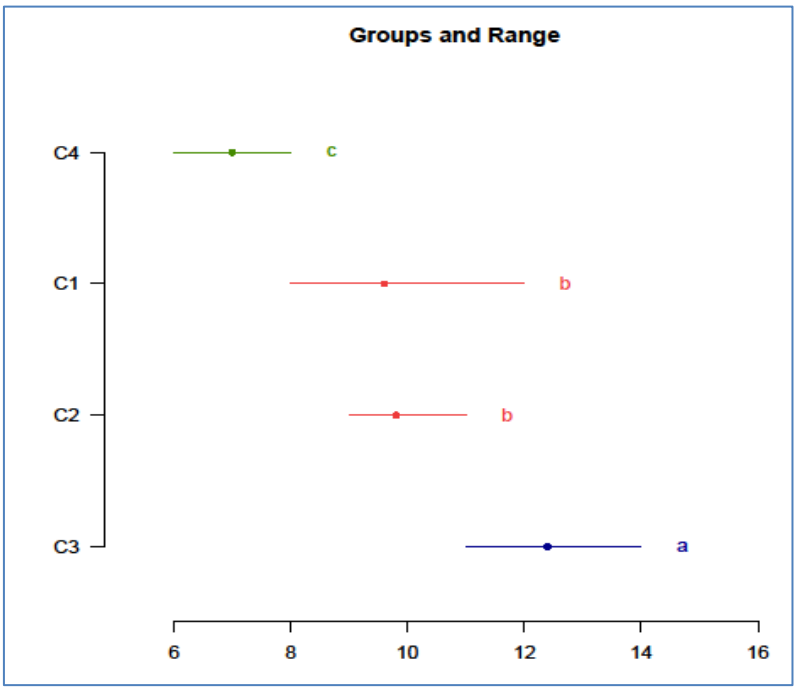

Fig 6: Plot of treatment means with the name of the respective group

(vii) Yield (gm)

Table 13: Analysis of variance table for yield

Sources of Variation	Df	Sum Squares	Mean Squares	F-value	*p*-value
Treatments	3	749985	249995	24.76	0.0000
Error	16	161575	10098		
Total	19	911560	260093		

C4 fall under one group (i.e., c) and C1 and C2 fall under one group (i.e., b and C3 treatments are fall under one group (i.e., a) it means C3 and C2 are significantly different from each other and C2 and C1 are significantly different and C1 and C4 are also significantly different from each other. it indicates that the average yield was significantly different for all the treatments. From Table-14, the maximum average yield was observed for the treatment C3 (1082.40).

Table 14: Groups of the treatments

Treatments	Length	Groups
C3	1082.4	a
C2	933.2	b
C1	775.4	c
C4	560	d

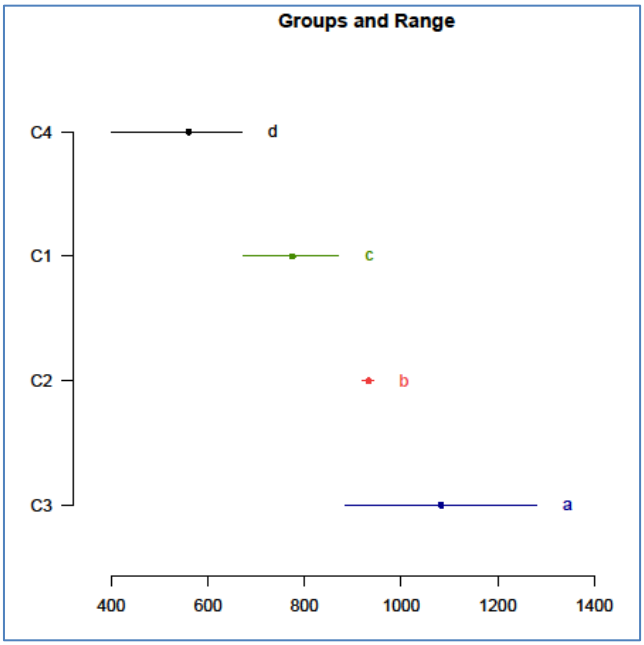

Fig 7: Plot of treatment means with the name of the respective group

(viii) Biological Efficiency (%)

Table 15: Analysis of variance table for Biological efficiency

Sources of Variation	Df	Sum Squares	Mean Squares	F-value	*p*-value
Treatments	3	7479	2493	24.68	0.0000
Error	16	1616	101		
Total	19	9095	2594		

The treatment C4 fall under one group (i.e., c) and C1 and C2 fall under one group (i.e., b and C3 treatments are fall under one group (i.e., a) it means C3 and C2 are significantly different from each other and C2 and C1 are significantly different and C1 and C4 are also significantly different from each other. it indicates that the average yield was significantly different for all the treatments. From Table-16, the maximum average yield was observed for the treatment C3 (108.24).

Table 16: Groups of the treatments

Treatments	Length	Groups
C3	108.24	a
C2	93.1	b
C1	77.54	c
C4	56	d

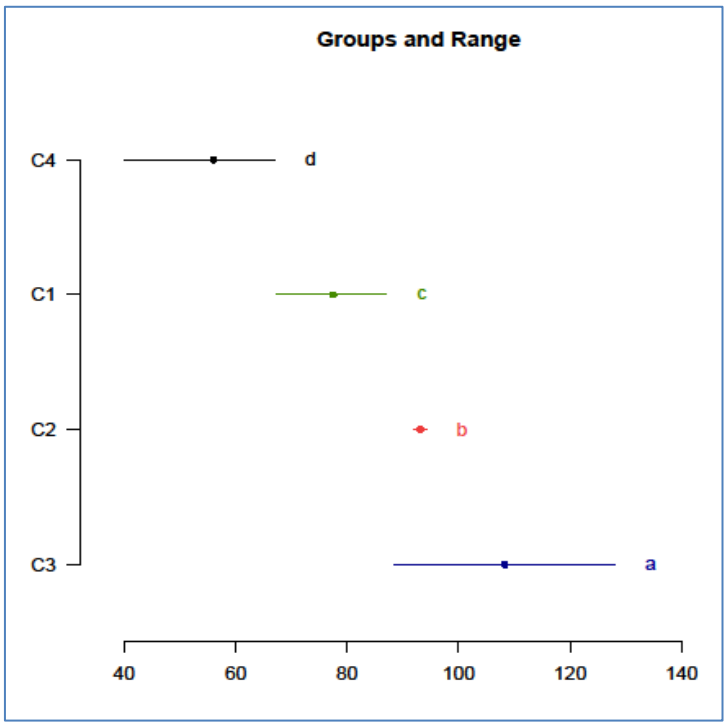

Fig 8: Plot of treatment means with the name of the respective group

Conclusion

Comparison of all the four isolates resulted that CI-1 (procured from Tropical Mushroom Research station, OUAT Bhubaneswar) has produced the maximum length when compared to remaining three isolates i.e., CI-2, CI-3 and CI-4.Isolate CI-1 (procured from Tropical Mushroom Research station, OUAT Bhubaneswar) required minimum days for preparation of mother spawn While isolate CI-2 (procured from Mushroom laboratory IGKV Raipur) has recorded the minimum days require for preparation of seed spawn, Isolate CI-3has recorded the minimum time for spawn run. isolate CI-1 has recorded the fruiting body formation in minimum no of days isolate CI-3 (collected from Kolkata) has recorded the maximum Number of fruiting bodies and also recorded the maximum yield and Biological efficiency of fruiting bodies among these isolates So we have concluded that isolate CI-3 was the best isolate.

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