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Evaluation of Different Sterilization Techniques for Yield and Biological Efficiency of Milky White Mushroom (*C. indica* P&C)

Elangovan M.
Department of Plant Pathology, PSB, Visva-Bharati, West Bengal-731 236, India.
Corresponding author email id: elangomarimuthu96@gmail.com

Abstract **– The experiment was attempted to evaluate the most suitable surface sterilization technique for production of milky mushroom (Calocybe indica), under the agro-climatic condition of lateritic zone of West Bengal, different sterilization techniques i.e. hot water treatment, modified hot water treatment, chemical treatment (75 ppm bavistin+500 ppm formalin) and CaO @ 2% were assessed to know their effects on spawn run period, duration of pinhead formation, days for first harvest. Among the different substrate surface sterilization techniques chemical sterilization technique showing highest yield (677 g) and biological efficiency (67.7%) followed by hot water treatment 594g yield and 59.4% biological efficiency. The lowest yield (368.83g) and biological efficiency (36.88%) were obtained from substrate sterilized with 2% calcium oxide. The substrate surface sterilized with modified hot water treated beds shows early spawn run period (15.33 days) but chemically treated substrate beds viewing early pinhead formation (14.62 days), sporophore maturation period (9.33 days) and highest number of sporophore production (10.33). The longest spawn running period (20.00 and 20.67 days), primordia initiation (19.40 and 20.38 days) were occur in both the beds treated with 2% CaO and untreated controlled beds. Sporophore maturation period, number of fruiting bodies, yield and biological efficiency was low and almost par in both beds treated with 2% CaO as well untreated control beds. Thus, among the various substrate surface sterilization technique, chemically surface sterilized beds (75 ppm bavistin+500 ppm formalin) showing superiority than all other treatments with the highest yield (677 g), biological efficiency (67.7%) as well as sporophore maturation period, number of fruiting body.**

Keywords **– Milky Mushroom, Sterilization, Bavistin, CaO, Yield, Biological Efficiency.**

I. INTRODUCTION

Among the all known mushrooms such as *Agaricus spp., Pleurotus spp., Volvariella spp., Calocybe indica* is a potential new edible mushroom in the world trade. It is excellent source of proteins, vitamins, minerals, fibre, and carbohydrate and also free of cholesterol and fat. Milky mushroom (*C.indica*) originated from India and reported first time by Purkayastha and Chandra in 1970s **(Purkayastha & Chandra, 1974)**. It is milky white in colour, fleshy, robust in nature and also can grow in tropical and subtropical region. Production of milky mushroom was first attempted by Purkayastha and Chandra in 1974 in West Bengal. Many researchers have been attempted to cultivation of milky mushroom in various aspects. But information available in West Bengal about milky mushroom production is scanty. Commercial cultivation of this mushroom was attempted by several researchers and first ever mushroom variety *Calocybe indica* var. APK-2 was released by Krishnamoorthy in 1998 **(Krishnamoorthy & Balan, 2015)**. Milky mushroom production in the country was started in 1970s with effective utilization and recycling of agricultural waste and agro-industrial waste for enhance income and sustainability. Every year India produces about 600 million tonnes of agricultural wastes and a major part of it is left out to decompose naturally or burnt *in situ*. This can effectively be utilized to produce high quality food such as mushrooms followed by utilization of spent mushroom substrate by converting it into organic manure for crop plants **(Singh & Kamal, 2012)**. Substrates are the most important

substance to harbouring of most of the micro-organism such as fungi, bacteria and viruses etc. To avoid other growing unwanted micro flora during the mushroom cultivation substrate should be surface sterilized with various methods. The experiment was attempted to evaluate most suitable substrate surface sterilization technique for successful mushroom cultivation in agro-climatic zone of lateritic belt of West Bengal.

II. Materials and Methods

Culture:

The pure culture of *Calocybe indica* was isolated from fresh sporophore obtained from mushroom laboratory, Department of Plant Protection, Palli Siksha Bhavana, Visva-Bharati University by using tissue culture method **(Jonathan *et al.*, 2009)**.

Spawn:

Half boiled wheat grains were used as spawn substrate with following standard procedure described by **Munjal (1973)**. Grains of wheat mixed with 2% calcium carbonate and gypsum which helps to maintaining the pH 7.0 and keep the grains separating each other. Inoculation of culture was done in laminar air flow chamber and allows it for mycelial formation and beds spawn were prepared from mother spawn.

Substrate:

Freshly chapped paddy straw (10-15 cm) were used as substrate for production of milky mushroom by following procedure given by **(Marimuthu *et al.*, 2002)** with some modifications.

To evaluate the yield and biological efficiency of milky mushroom (*C. indica*) chapped paddy straw substrate was surface sterilized with different techniques such as hot water treatment, modified hot water treatment, chemical treatment and soaked with 2% calcium oxide (CaO) and untreated control. Chapped paddy straw was boiled in hot water with 80°C for 30 to 45 minutes and shade dried up to attaining 60% moisture. In modified hot water treatment substrate soaked in cold water for 4 hours and again dipped in hot water with 80°C for 30 minutes. In case of chemical treatment of substrate surface sterilized with 75ppm bavistin and 500ppm formalin. For calcium oxide treatment substrate soaked in 2% calcium oxide (CaO) solution for overnight. Sterilized substrate filled in 16"×18" thickness polythene bag with 4% spawning with 15-20 holes. Each treatment has replicated with five times. All the bags were kept in cropping room for mycelial running and casing was done with sterilized casing soil after whole bags covered with white mycelial mattress. Following observations are recorded such as No. of days for mycelial formation, no of days for primordia initiation, days for sporophore maturation, stipe length, pileus diameter, yield and biological efficiency. All the data were noticed and analysed by using Completely Randomized Design (CRD) with standard methodology at 1% level of Critical Deference (CD). Total yield of mushroom was calculated by sum of all the four harvest. The following formula was used to calculate biological efficiency of mushroom, $\text{B.E}\ (\%) = \frac{\text{fresh Weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \times 100$

III. Results and Discussion

Effect of Mycelial Growth, Primordia Formation and Fruiting Body Maturity of Milky White Mushroom (C. Indica)

It was obvious from the table 1, modified hot water treatment showed an early spawn run period (15.33 days),

and followed by hot water treatment (16.33 days) and chemical treatment (17.33 days). Though the chemical treatment took maximum days for spawn run but, the chemically treated beds initiated early pinhead formation (14.62 days) and gave first harvest 9.33 days prior to modified hot water treatment (16.48 and 13.67 days) and hot water treatment (15.76 and 12.33 days). All the treatments except the 2% calcium oxide treatment were differed significantly from the control (Fig. 1).

Table 1. Effect different substrate surface sterilization techniques on growth of *C. indica*.

Treatments	Days for Spawn Run	Days for Primordia Initiation	Days for Fruiting Body Maturation
Hot water treatment	16.33	15.76	12.33
Modified hot water treatment	15.33	16.48	13.67
Chemical treatment	**17.33**	**14.62**	**9.33**
2% CaO	20.00	19.40	15.67
Control	20.67	20.38	15.33
SEm (±)	0.54	0.56	0.49
CD @1%	1.69	1.76	1.56
CV %	5.19	5.89	6.45

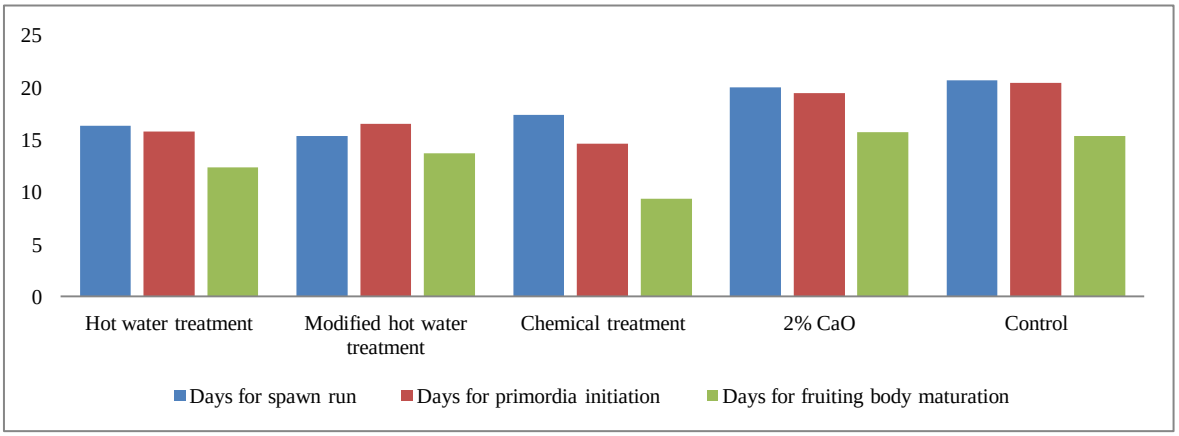

Fig. 1. Effects of various substrate surface sterilization techniques on growth of *C. indica.*

Effect of Substrate Sterilization Technique on Fruiting Body Production of Milky White Mushroom (C. Indica)

It was clearly understood from the table the effect of different substrate surface sterilization techniques were tested by the number of sporophore they produced and it was recorded that maximum number of sporophores (10.33) was obtained from the beds treated chemically followed by hot water treatment (8.67) and modified hot water treatment (8.33). The average number of sporophores obtained from the beds of 2% calcium oxide was found to be minimum 5.67 (Fig. 2).

Effect of Substrate Sterilization on Yield and Biological Efficiency of Milky White Mushroom (C. Indica)

It was evident from the table 3 yield and biological efficiency was the main criterions to check the effect of various treatments on the production of *C. indica*. The maximum yield and biological efficiency were observed

from the chemically treated substrate, 677g and 67.70% respectively followed by hot water treatment 594g and 59.4%. The biological efficiency of modified hot water treatment and 2% calcium oxide were noticed less 40.75% and 36.88% which invites competitor moulds in beds. Minimum biological efficiency (34.9%) was obtained from untreated control. All the treatments differed significantly from each other (Fig. 3).

Table 2. Effect different substrate surface sterilization techniques on sporophore production of *C. indca*.

Sl. No	Treatments	No. of Fruiting Body
1.	Hot Water Treatment	8.67
2.	Modified Hot Water Treatment	8.33
3.	Chemical Treatment	**10.33**
4.	2% CaO	5.67
5.	Control	5.33
	SEm (±)	0.49
	CD @ 1%	1.56
	CV %	11.17

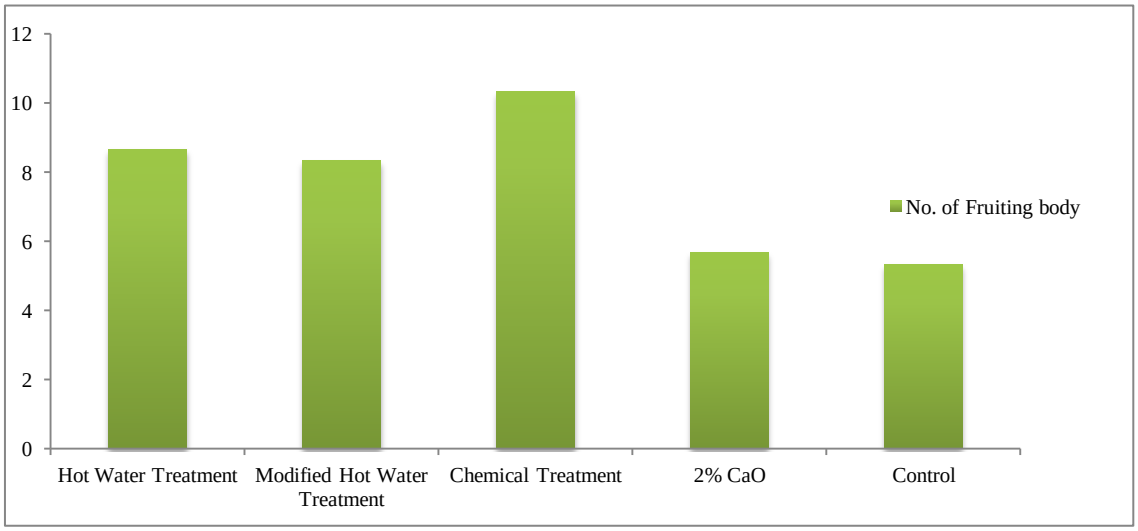

Fig. 2. Effect different substrate surface sterilization techniques on sporophore production.

The role of stipe length of sporophore was found to be less in terms of yield and biological efficiency and it was showed non-significant relationship among the treatments. Bigger size of sporophores (pileus diameter 4.7 cm) was noticed from the beds treated chemically. However, the beds treated with hot water produced smaller size of sporophore (pileus diameter 2.13 cm).

Substrate surface sterilization plays an important role in successful mushroom production. Surface sterilization of the substrate significantly influences the growth, yield, and biological efficiency and also reduces the contamination in the beds of *C. indica*. The results of the present studies clearly showed that chemical sterilization of the substrate initiate early pinhead, sporophore maturation, and highest number of sporophore, yield and biological efficiency followed by hot water treatment. Carbendazim primarily affected the cell and nuclear division of fungi which inhibit the growth of competitor mould fungi in beds. The present investigation corroborated with the findings of by **Trivedi *et al.*, (1991); Raina& Gupta, (2006) and Bhatt *et al.*, (2007)**.

Table 3. Effect of substrate sterilization on yield and biological efficiency of milky white mushroom (*C. indica*).

Treatments	Stipe (l) (cm)	Pileus (d) (cm)	Yield (g)	B.E %
Hot Water Treatment	7.37	2.13	594.00	59.40
Modified Hot Water Treatment	7.10	2.27	407.67	40.76*
Chemical Treatment	8.20	4.70	677.00	67.70
2% CaO	6.40	3.07	368.83	36.88*
Control	6.67	3.10	349.00	34.90*
SEm (±)	0.65	0.23	14.12	1.41
CD @ 1%	NS	0.71	44.51	4.45
CV %	15.84	12.77	5.10	5.10

*Beds contaminated with *Coprinus* sp.

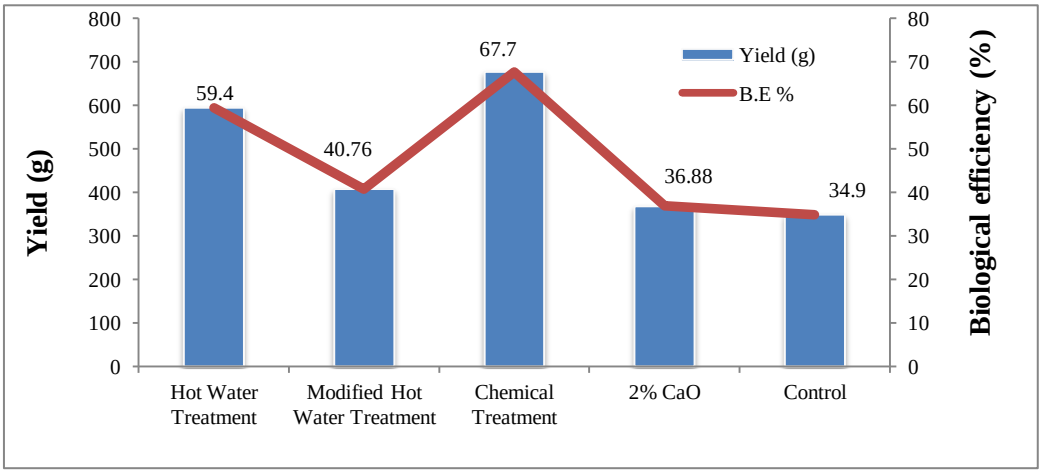

Fig. 3. Effects of various substrates surface sterilization on yield and biological efficiency of milky white mushroom (*C. indica*).

Few other researchers also recommended the substrate surface sterilization with 75 ppm bavistin and 500 ppm formalin for higher production of oyster mushroom **Krishnamoorthy *et al.*, (1991), Nallathambi & Marimuthu, (1994), Biswas, M.K., (2015).** Modifeied hot water treatment and calcium oxide @ 2% were not suitable under the agro-climatic condition of lateritic belt of West Bengal because both invite competitor moulds during cropping period. More water was found to be reained in the beds where 2% CaO was applied during sterilization. Hot water treatment of substrate sterilization was also gave substantial yield and suggested by several workers for milky mushroom cultivation. **(Purkayastha *et al.*, 1981), (Purkayastha, 1985), (Krishnamoorthy & Muthusamy, 1997), (Senthilnambi *et al.*, 2011), (Rawal & Doshi, 2014).**

IV. Conclusion

The present study concluded that modified hot water treatment showed an early spawn run period (15.33 days) followed by hot water treatment (16.33 days) and chemical treatment (17.33 days). Though the chemical treatment took maximum days for spawn run but, the chemically treated beds initiated early pinhead formation (14.62 days) and gave first harvest (9.33 days) prior to modified hot water treatment (16.48 and 13.67 days) and hot water treatment (15.76 and 12.33 days), maximum number of sporophore (10.33), yield (677g) and biological efficiency (67.7%) were obtained from chemically treated beds followed by hot water treated beds

594g yield and 59.4% biological efficiency. The minimum number of sporophore (5.67), lowest yield (368.83g) and biological efficiency (36.88%) were noticed from the beds treated with 2% calcium oxide. The chemically treated beds showed significant relationship among the all other treatments. The present findings of the investigation suggests chemical sterilization of substrate enhance the yield and biological efficiency of milky white mushroom (*Calocybe indica*) under the agro-climatic lateritic belt of West Bengal.

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AUTHOR'S PROFILE

Mr. Elangovan M., Obtained B.Sc., (Agri) from Annamalai University, Chidambaram, Cuddalore, Tamil Nadu. M.Sc., (Agri) from Visva-Bharati Central University, Shantiniketan, West Bengal. Did my Master research on Mushroom Cultivation especially different techniques to improve biological efficiency of Milky white mushroom. Cleared various exams like IARI-PhD Entrance 2019 and ICAR-ASRB NET 2019 and currently working as a Assistant Professor in Don Bosco College of Agriculture (Affiliated to Tamil Nadu Agricultural University), Sagayathottam, Tamil Nadu, India. Address for communication: 7/138, Kambaliampatty, Kottanatham Post, Vedasandur Taluk, Dindigul District, Tamil Nadu, Pin code – 624 620, Mobile No.: 9597469210. email id: elangomarimuthu96@gmail.com