Bangladesh J. Bot. **49**(2): 387-393, 2020 (March)

EFFECTS OF COMPOSITION, AGE AND STERILIZATION TECHNIQUES OF MOTHER CULTURE ON THE GROWTH AND YIELD OF *VOLVARIELLA VOLVACEA* (BULL.) SINGER

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Keywords: Biological efficiency, Mother culture medium, Sterilization technique, *Volvariella volvacea,* Yield attributes

Abstract

The research work was carried out to investigate the efficacy of different mother culture media *viz.*, rice straw (T-1), rice straw and rice bran (T-2), rice husk (T-3), rice grain (T-4), maize grain (T-5), and rice straw and wheat bran (T-6) and the impact of age of mother culture and substrate sterilization techniques *viz.*, sun dried for 8 hrs covering with transparent polythene (A-1), black polythene (A-2), blue polythene (A-3) sheet, autoclave for two hrs at 121°C (A-4), and hot water for one hr (A-5) for the commercial cultivation of *Volvariella volvacea* (Bull.) Singer. The maximum mycelium run rate and minimum days required for completing the mother culture were recorded in T-4. The lowest days required for primordial initiation (DRFPI) was 6 in T-1 and T-2, whereas highest DRFPI was recorded in T-3. The maximum (13.33) days required for first harvest (DRFFH) and lowest (109) number of effective fruiting bodies (NEFB) were recorded in T-3. The minimum (10.67 days) DRFFH was found in T-2 and maximum (239.30) NEFB was recorded in T-1. The lowest length and diameter (LFB and DFB) were recorded in T-5 (3.03 cm) and T-1 (1.66 cm), while highest LFB and DFB were observed in T-3 (3.20 and 2.39 cm). Maximum biological yield and efficiency were observed in rice straw and wheat bran materials. The highest NEFB, DFB, biological yield and efficiency were recorded in 30 days old of mother culture. The results revealed that combined rice straw and wheat bran were the excellent mother culture medium and 30 days old was the best age for the commercial production of paddy straw mushroom. Considering the experimental results on the sterilization techniques it may be suggested that hot water sterilization of rice straw substrate was the best sterilization technique for the commercial production and yield improvement of *V. volvacea*.

Introduction

Volvariella volvacea (Bull.) Singer known as straw mushroom is one of the most important edible mushrooms specially in China, Thailand, Vietnam, Taiwan and Indian sub-continent (Singha *et al.* 2013). It is very much preferable for its attractive fruiting bodies as well as unique taste. It belongs to the class Basidiomycota, order Agaricales, and family Pluteaceae. It grows on almost all types of cellulosic agricultural waste material like rice straw, wheat straw and banana leaves etc. (Amin *et al.* 2008). In Bangladesh rice straw is widely used as the substrate for the commercial cultivation of *V. volvacea*, because this substrate is available, cheap and has no proper utilization. The production of paddy straw mushroom cannot be increased to industrial level because of its inconsistent control of yield (Amin *et al*. 2007, Thevasingh *et al*. 2007). The biological efficiency of straw mushroom is very poor, may be due to lack of suitable substrate selected for the cultivation of this mushroom or lack of information for the production. In order to increase the production of straw mushroom, there is need to improve on cultivation and procedure. Growing mushroom on simple substrate alone sometimes cannot provide enough nitrogen required for its optimal growth. Supplements may be added to obtain higher yields. Wheat bran, rice bran

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and maize powder are used for supplementation with the substrate at different levels. These supplements also raise the nutritional status of the mushroom (Alam *et al.* 2011, Yoon *et al.* 2012). The quality mother culture medium for the production of spawn is directly influenced by their quality and yield of mushroom cultivation.

Different sterilization methods are often used for cultivation of oyster mushroom production and its yield improvement (Khan *et al*. 2011). Using such appropriate methods, spawning will assure better resistance against any disturbance of competitive microorganisms. Khan *et al.* (2011) also reported that sterilization of substrates is much more appropriate method for effective and smooth cultivation of mushrooms to remove the existence of a number of microorganisms. But in Bangladesh condition, no research work has been conducted on the effect of different sterilization methods on *V. volvacea* cultivation. Therefore, this research work was undertaken to select an ideal material for mother culture medium and to evaluate the suitable age of mother culture and substrate sterilization technique for the commercial cultivation of *V. volvacea.*

Materials and Methods

The fruiting bodies of *Volvariella volvacea* (Fig. 1) was obtained from the National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka, Bangladesh. This experiment was carried out at the laboratory of Mycology and Plant Pathology, Department of Botany, Jahangirnagar University, Savar, Dhaka. Rice straw was used as substrate in this study.

Fig. 1. The fruiting bodies of *Volvariella volvacea.*

Six different mother culture media were selected for this experiment. The mother cultures were prepared by the following manner, T-1 = Rice straw were chopped into 3 - 4 cm and added equal amount of water, T-2 = Mix chopped rice straw of 3 - 4 cm with rice bran at 3 : 1 ratio and added equal amount of water,T-3 = Rice husk soaked in water for three hrs and then drain out the excess water, T-4 = Rice grain boiled for 1.5 - 2 hrs and then drain out the excess water, T-5= Maize grain boiled for 1.5 - 2 hrs and then drain out the excess water,T-6 = Mix chopped rice straw of 3 - 4 cm with wheat bran at 3 : 1 and then added equal amount of water. Polypropylene bags of (7" × 10") were filled with 230 g of medium for T-1, T-2 and T-6, 170 g for T-3 and 300 g for T-4 and T-5. The necks of the bags were heat resistant plastic. A hole of about 2/3 deep of the volume of the bags was made at the centre with a sharp end stick for space to put inoculums. The neck was plugged with cotton and covered with brown paper and tied with robber. The packets were sterilized in an autoclave for one hr at 120°C under 1 kg/ pressure. After

sterilization the packets were cooled for 24 hrs and transferred to a clean bench. PDA culture medium containing mycelium of *V. volvacea* was placed aseptically in the hole of mother culture packet and again plugged as mentioned above. Then the inoculated packets was placed in the culture room at 26 - 37°C. To determine effective age of mother culture rice straw and wheat bran mother culture medium (T-6) was prepared as mentioned above and kept 15, 20, 25, 30 and 35 days after inoculation.

For determination of effective substrate sterilization technique, rice straw was chopped into 3-4 inches in length and treated with five different treatments (A) in the following manner: A-1, sun dried for 8 hrs covering with transparent polypropylene (pp); A-2, sun dried for 8 hrs covering with black pp; A-3, sun dried for 8 hrs covering with blue pp; A-4, mixing with equal amount of water poured the chopped rice straw into a net bag and autoclave for two hrs at 121°C and A-5, poured the chopped rice straw into a net bag and treated with hot water for one hr and allowed to drain out the excess water by hanging the bag for 20 hrs.

Transparent polythene sheet was placed in the incubation room and then both end open box was placed on the sheet. The size of the box was 100 cm (length) × 30 cm (width) × 30 cm (height). The first layer was filled with 4 cm sterilized rice straw then inoculated with mother culture and covered with 4 cm sterilized rice straw and again inoculated with mother culture covered with 3 cm sterilized rice straw and after inoculating with mother culture the 3rd layer was covered with 1cm sterilized rice straw. The mother culture was taken out of the packet followed by placing on a tray breaks into small pieces and placed in each layer at 2 - 3 cm intervals at peripheral region. The whole bed was covered with a transparent polythene sheet. After 8 days, the sheet was removed for sufficient aeration, light, temperature and humidity, so that fructifications could be ensured. Temperature and relative humidity during the experiment were 27 - 38°C and 85 - 95%, respectively and mushrooms were harvested at egg stage.

The experiment was laid out in completely randomized design with three replications. Data on mycelium growth rate, days to complete mycelium running, days required to primordial initiation, days to required first harvest, number of effective fruiting bodies, length and diameter of fruiting bodies, biological yield and efficiency were noted. Data were analyzed according to standard methods using the MSTAT-C program. Means were compared using Duncan's multiple range test.

Results and Discussion

Effects of six different media of mother culture on the growth and yield of *V. volvacea* have been presented in Table 1. The highest mycelium run rate (MRR) was observed in T-4, which was statistically higher than all other treatments. The lowest MRR was observed in T-1, which resembled with Amin *et al*. (2010) findings on *Calocybe indica.* T-1 was statistically similar to T-2, T-3, T-5 and T-6. Significantly the lowest days required for completing mother (DRFCM) was recorded in T-4, which was statistically similar to T-5. Tripathy *et al.* (2009) obtained minimum and maximum DRFCM on bajra (14 days) and Bengal gram (21 days) on *Volvariella diplasia*. The days required for primordial initiation varied from 6 to 7.33 days in different mother culture media. These findings are similar to previous reports of Tripathy *et al.* (2011). The lowest and highest number of days required for first harvest were observed in T-2 (10.67) and T-3(13.33), respectively. T-2 is statistically similar to T-1, T-4, T-5 and T-6. The number of effective fruiting bodies ranged from 109 to 239.30 in T-3 and T -1, respectively. T-5 is statistically similar to T-3 and T-4. The minimum and maximum length of fruiting bodies were observed in T-5 and T-3. Highest diameter of fruiting bodies was found in T-3, which was significantly higher than all other treatments. Maximum biological yield was found in T-6 (1103 g), which was statistically similar

to T-1 and T-2, respectively. Tripathy *et al.* (2011) reported that highest biological yield of *V. volvacea* (1360 g) and *V. diplasia* (1243 g) was obtained from wheat grain with rice bran. A significant variation of biological efficiency was recorded in different selected mother culture media. Lowest (16.57%) and highest (27.58%) biological efficiency was observed in T-6 and T-5 (Fig. 2), respectively while Tripathy *et al.* (2009) recorded highest biological efficiency in wheat grain (16 %) and lowest in Bajra (6.4 %). The results indicated that combined rice straw and wheat bran was the excellent medium of mother culture for the commercial production of paddy straw mushroom.

Table 1. Effect of media of mother culture on the growth and yield of *Volvariella volvaceae*.

Media of mother culture	Mycelium run rate (cm/day)	Days required for completing mother	Days required for primordial initiation	Days required for first harvest	Number of effective fruiting bodies/ bed	Length of fruiting bodies (cm)	Diameter of fruiting bodies (cm)	Biological yield (g/bed)
T-1	0.72 b	20.00 a	6.00 c	11.00 b	239.30 a	3.05 a	1.66 b	939.30 ab
T-2	0.83 b	19.56 a	6.00 c	10.67 b	173.70 bc	3.11 a	1.69 b	928.70 ab
T-3	0.74 b	17.67 c	7.66 a	13.33 a	109.00 d	3.20 a	2.39 a	734.00 bc
T-4	1.27 a	11.00 d	6.66 bc	11.67 b	131.00 cd	3.08 a	1.74 b	728.70 bc
T-5	0.89 b	11.67 d	7.33 ab	11.67 b	126.30cd	3.03 a	1.77 b	663.00 c
T-6	0.76 b	18.56 b	7.33 ab	11.33 b	202.00 ab	3.12 a	1.78 b	1103.00 a
CV %	10.94	2.72	7.00	4.89	16.87	9.30	3.75	14.61

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. T-1 - rice straw, T-2 - rice straw and rice bran, T-3 - rice husk, T-4 - rice grain, T-5 - maize grain and T-6 - rice straw and wheat bran.

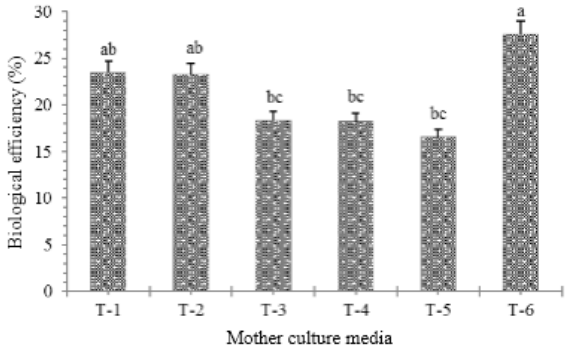

Fig. 2. Effect of various media of mother culture on the biological efficiency of *Volvariella volvacea.* T-1 - rice straw, T-2 - rice straw and rice bran, T-3 - rice husk, T-4 - rice grain, T-5 - maize grain and T-6 - rice straw and wheat bran.

Effect of age of mother culture on the growth and yield of paddy straw mushroom has been presented in Table 2. Five different ages of selected mother culture (rice straw and wheat bran) i.e. 15, 20, 25, 30 and 35 days were evaluated for the cultivation of *V. volvaceae*. The minimum days required for primordia initiation (DRFPI) and days required for first harvest (DRFFH) were recorded 8.33 and 11.67 days, respectively. The maximum DRFPI and DRFFH were observed in 15 days. The lowest length of fruiting bodies, diameter of fruiting bodies (DFB), number of effective fruiting bodies (NEFB), biological yield and biological efficiency were observed in 15 days old mother culture. The highest NEFB, DFB and biological yield were recorded in 30 days

old mother culture. The maximum biological efficiency (23.23%) was observed in 30 days old mother culture, whereas lowest (3.23%) in 15 days old of mother culture (Fig. 3). The results indicated that 30 days old was the best age for the commercial production of paddy straw mushroom. Thirbhuvanmala *et al*. (2012) observed that 20 days old mother culture gives optimum yield and growth followed by 15 and 25 days, respectively. Pani (2011) reported that biological efficiency was highest in 21 days old mother culture.

Table 2. Effect of age of mother culture on growth and yield of selected strain of *Volvariella volvacea*.

Treatments (days)	Days required for primordia initiation	Days required for first harvest	Number of effective fruiting bodies/ bed	Length of fruiting bodies (cm)	Diameter of fruiting bodies (cm)	Biological yield (g/bed)
15	14.67 a	20.67 a	28.00 d	1.95 b	1.33 c	129.30 d
20	11.67 b	16.67 b	81.67 c	2.92 a	1.73 ab	459.30 c
25	8.66 c	11.67 c	190.30 a	3.03 a	1.52 bc	812.70 b
30	8.33 c	11.67 c	193.30 a	2.95 a	1.84 a	929.30 a
35	8.33 c	11.67 c	118.30 b	2.84 a	1.56 b	517.70 c
CV %	8.10	7.41	10.69	10.22	6.79	9.66

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 3. Effect of various sterilization techniques to rice straw substrate on the growth and yield of *Volvariella volvacea*.

Sterilization technique	Days required for primordia initiation	Days required for first harvest	Length of fruiting bodies (cm)	Diameter of fruiting bodies (cm)	Number of effective fruiting bodies/bed	Biological yield g/ bed
A-1	7.33 a	11.67 a	2.45 d	1.84 b	32.33 d	241.7 c
A-2	7.33 a	11.33 a	3.27 b	2.22 a	77.33 c	542.7 b
A-3	7.33 a	11.33 a	2.65 d	1.79 b	41.00 d	244.7 c
A-4	8.33 a	11.67 a	2.98 c	1.87 b	119.00 b	532.3 b
A-5	8.33 a	11.67 a	3.55 a	1.94 ab	199.30 a	948.7 a
CV%	7.65	4.75	4.85	4.49	13.26	12.79

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Substrate was sterilized with sun dried for 8 hrs covering with transparent pp (A-1), covering with black pp (A-2), covering with blue pp (A-3), autoclave for two hrs at 121°C (A-4), and hot water for one hr (A-5) for the commercial production of *V. volvacea*. Primordia formation was observed in all experimental sets. Days required to primordia initiation were observed between 7.33 and 8.33 days. The A-1, A-2 and A-3 required 7.33 days, while A-4 and A-5 required 8.33 days for primordia initiation (Table 3). Days required for primordia initiation were statistically similar for all experimental sets. Patra and Pani (1995) reported that the time required for primordia initiation of *Calocybe indica* on paddy straw was 13 - 16 days. Similar findings were also reported by Moonmoon *et al*. (2008). Substrate containing glucose, fructose and trehalose produced the highest number of primordia, whereas abnormal fruiting bodies were produced in glycerol, xylose, sucrose and fructose. The results indicated that days required to first harvest was statistically similar in all the tested treatments. Length of fruiting bodies ranged from 2.45 to 3.55 cm (Table 3). The highest length of fruiting body was found in A-5 (3.55 cm) followed by T-2 (3.27 cm) and the lowest length of fruiting bodies were found in A-1, which was statistically

similar to A-3. Diameter of fruiting bodies ranged from 1.84 to 2.22 cm, respectively. Chang (1979) found that the days to first harvest was 8 to 17 and days required for total harvest 38 to 50. The number of the effective fruiting bodies varied significantly. The highest number of effective fruiting bodies was obtained in A-5 (199.3) and lowest number of effective fruiting bodies (32.33) was obtained in A-1. The A-5 was significantly higher than all the treatments. In case of biological yield significant variation was observed. Highest and lowest biological yield found in A-5 (948.7)

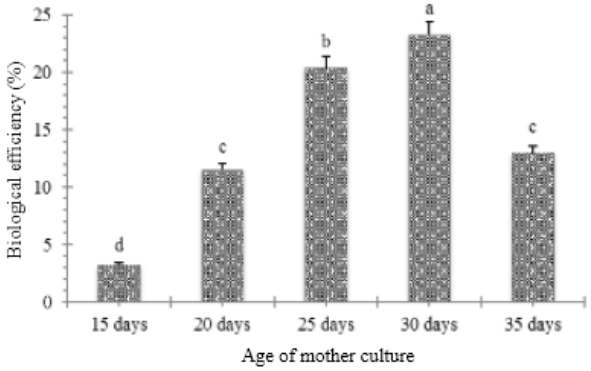

Fig. 3. Effect of age of mother culture on the biological efficiency of *Volvariella volvacea*.

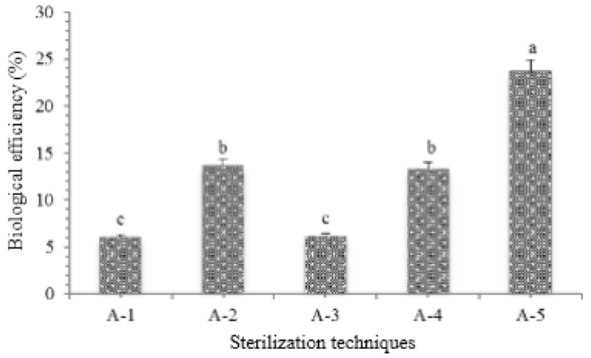

Fig. 4. Effect of various sterilization techniques to rice straw substrate on the biological efficiency of *Volvariella volvacea*.

and A-1 (241.7), respectively. The effective number of fruiting bodies *V. volvaceae* and biological yield was significantly higher in hot water treated substrate as compared to other sterilization techniques. The highest biological efficiency (23.72%) was recorded in A-5 that was significantly higher as compared to all other treatments (Fig 4). The findings of the present study are in conformity with those of previous studies using *V. volvacea* (Moonmoon *et al*. 2008). Khan *et al.* (2011) reported that laboratory autoclaving was most effective method with respect to yield of oyster mushroom. Hot water treated substrate degraded the cellulose, hemicellulose and lignin. The degraded carbohydrates served as energy sources for constructing the structural components of the fruiting body (Rajarathnam *et al*. 1986, Alam *et al*. 2010). Proper sterilization of substrates is much more appropriate method for effective and smooth cultivation of mushrooms. In view of the above, it could be concluded that preparation of mushroom bed with 25 to 30 days old mother culture give highest growth and yield and the sterilization of rice straw substrate with hot water for one hr was the best sterilization technique for the commercial production of *V. volvacea*.

Acknowledgements

This work was supported by a research grant from the Ministry of Science and Technology, Government of the People's Republic of Bangladesh (Agenda No.-39.009.006.01. 00.049.2013-2014/BS-1/435).

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(Manuscript received on 24 August, 2018; revised on 10 June 2019)