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EVALUATION OF THE EFFECT OF DIFFERENT COMPOST FORMULATIONS AND CASING MATERIALS ON BUTTON MUSHROOM PRODUCTION

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ABSTRACT
This study was designed to determine the effect of compost formula and casing materials on growth and development of sporophore of *Agaricus bisporus* on paddy straw and dried tea leaf based composts. Locally available vermicompost (VSMS), garden soil (GS), tea waste (TW) and Spent mushroom compost of *Calocybe indica* (SMC) and their combination such as GS+ VSMS, TW+ VSMS, and SMC+ VSMS were used as casing materials for the cultivation of *A. bisporus*. In this study, seven casing soils formulation were used to determine their effect on fruiting. Growth parameters such as colonization days, time for pining, number of mushroom sporophore per bag, fruit body weight and yield rate were measured. The best result in fruit body number was recorded in paddy straw based compost encased with vermicompost. Due to low water potential garden soil required more time for pinning. Lowest fruit body was observed in tea leaf based compost encased with tea waste. Mixture of vermi + SMC as casing matter gave better result compared to SMC alone. The number of fruit body was significantly increased in addition of vermicompost with the other casing materials. The effect of compost formulations and casing materials on the biochemical constituents such as protein, carbohydrate content of fruit body was also evaluated. The protein and carbohydrate content of the mushroom samples grown in different casing materials did not show significant differences but it is significance in compost formulation levels. Among the compost formulations maximum protein content was measured in compost formulation I and minimum was recorded in compost II.

KEY WORDS: *Agaricus bisporus*, casing, compost, spent mushroom compost, vermicompost, tea waste.

INTRODUCTION

White button mushroom cultivation began in France two hundred years ago and gradually has developed into a thriving industry in the world (Piet *et al*., 1998). There are four main steps involved in mushroom cultivation: composting, conditioning, spawning and casing. Composting prepares a selective medium that favour growth of the mushrooms relative to other microorganisms (Kariaga *et al.,* 2012). The composting procedure involves two to three weeks of uncontrolled self- heating, followed by pasteurization. During self-heating the thermophilic fungus *Scytalidium thermophilium* becomes abundant and the density of this fungus in finished compost is a positive correlate of the yield of mushrooms (Gerben *et al.,* 1995, Kariaga *et al*., 2012). *S. thermophilium*has been confirmed to enhance and promote the growth of *Agaricus bisporus* (Gerben, 1993). It has been suggested that this fungal species provides a trigger for enhanced growth of button mushrooms (Gerben *et al*., 1995). The basic function of the thermophilic fungi is to utilize and exhaust the readily available carbohydrates and the free ammonia in the compost.Thermophilic *Scytalidium* varieties possess the largest biomass and create selectivity in the compost (Gerben *et al.,* 1995; Kariaga *et al.,* 2012). Significance of thermophilic fungi in mushroom compost preparation and measurement of microbial biomass is still an issue (Salar *et al*., 2007). The preparation of proper mushroom compost is still therefore a prerequisite for high yields of *Agaricus bisporus*. In Europe horse manure is commonly used because it is readily available. Compost for cultivation of the white button mushrooms, *A. bisporus*, is prepared from a mixture of organic materials subjected to a composting process making it selective for growth of *A. bisporus* (Colak, 2004). *A. bisporus* have a requirement for a separate ‘‘casing layer’’ that has specific physical, chemical and microbiological properties which stimulate and promote the initiation of primordial (Taherzadeh *et al*., 2013). Mushroom mycelial growth and mushroom development is not only related to genetic factors but also depends on environmental, chemical, and microbiological conditions (Pardo, 2004). Casing soil protects the compost against desiccation and supports the mushroom against pests and diseases and provides support for developing sporophores and gas exchange for development and growth mushrooms (Colauto *et al.,* 2011). Casing layer provide an environment change in which the mushroom shifts from a vegetative stage to a reproductive one. This happens due to microorganisms in the casing soil (Gulser *et al.,* 2003). Bacteria in the casing soil influence productivity, product quality and uniformity. Waste paper as an alternative casing materials was recorded by Sassine *et al.* (2005). Many materials, alone or in combination, have been used as casing both commercially and experimentally, although only very few have been shown to be of practical application (Gulser *et al.,* 2003; Peyvast *et al*, 2011). Spent mushroom Compost as a casing soil material can be used to reduce the cost of production and it can reduce pollution (Pardo *et al.,* 2008 and 2011).

Keeping this in mind, the present study has been designed to evaluate the best compost materials and best casing materials for growth of *A. bisporus* in North Bengal.

MATERIALS & METHODS

Inoculum and spawn preparation

The pure mycelial culture of *Agaricus bisporus* was obtained from Directorate of Mushroom Research, Solan, HP, India and maintained at 25°C in PDA and MEA media. Pure culture was stored at 15° C. The preparation of mycelial inoculum in Petri dishes and its conservation in test tube slants was performed according to Singh (Singh *et al*., 2009). Spawn was prepared by boiling wheat grains for 15 minutes followed by draining and cooling and calcium carbonate (5g/kg) and gypsum (25g/kg) were added, mixed well and then transferred into 65 to 75 cm clear double polypropylene bags, with a mean thickness of 0.6 mm and its upper portion was plugged with non absorbent cotton plug and were covered with paper. The grain filled bags were sterilized in autoclave at 15 lb pressure (121° C) for 40 min and allowed to cool at room temperature. The mycelial inoculum discs (5 mm) of 10 days old were aseptically inoculated in the spawn bags and incubated at 25± 2°C in shaded chamber for mycelial growth.

Experimental design

Locally available paddy straw and pruned dried tea leaf was used for compost preparation for growing *A. bisporus*. Experiments were conducted in a completely randomized design with 10 replicate of each compost formulation level and seven casing treatment level with six replicates in each treatment. Polyethylene bags of 60 × 30 cm size were used and cylindrical mushroom beds were prepared following layer methods of spawning @ 5%. Beds were made in which each bed contain 2kg of compost. A moisture content of about 40% was allotted in the compost prior to spawning. The inoculated bag was perforated with teasing needles. The bags were incubated in dark room to complete the spawn run and maintained temperature at 23°C with relative humidity of 85-90 %. After 18 days, when the beds were fully colonized by the vegetative mycelium, the upper surface of each bag was opened and the surface was applied with casing soil to a thickness of 2-3 cm over the spawn run compost. Before placement of casing layer the upper compost was ruffled. The beds were uniformly and regularly sprayed with water to keep the surface of compost moist. The primordial initiation was observed and young mushrooms fruit body was harvested. After each harvest, more watering was done in the next 2 days. The different casing materials like tea garden soil, tea waste, vermicompost, SMC were applied to the mushroom beds and its effect on sporophore development recorded.

Compost preparation

For composting raw materials such as paddy straw, wheat bran, manure was mixed and kept as stack and periodically turned for uniform fermentation. Rice straw and dried tea leaf both were moistened to the 50 % of their capacity on a concrete floor. Preliminary stack was prepared by adding wheat bran @ 8kg/250kg and urea @ 3 kg/250 kg of both basic materials such as paddy straw and tea leaf. The whole material was kept in stack form for five days untouched. The stack size was 4ft ×5ft. 1st turning was done after 5 days. During 1st turning, the inner temperature of the compost was raised upto 85°C. Aeration in composting materials was done by breaking and mixing the stack materials uniformly and kept them for 3 days. Free turning was done every three days interval up to 11 days. At 11th and 17th day gypsum @5kg/250 kg of paddy straw was mixed. The free turning was continued up to 23 days when the excess ammonia was completely released from the compost and the compost was used for spawning directly. In case of tea leaf based compost it took more time comparatively and was pasteurized by steaming in a closed chamber at 55-69° C for 1 hour.

Measuring of inner compost temperature

Inner compost temperatures were measured as an indicator of thermophilic microbial activity within the compost stack. Temperatures were measured at three heights of the compost stack 30 cm, 60 cm and 90 cm of the stack. The three heights were determined just in the middle of the compost pile. The temperature was measured every day at 24 hours intervals.

Casing materials

Vermicompost (VSMS), tea waste (TW), tea garden soil (GS), spent substrate of *C. indica* (SMC) were used as primary casing materials. Garden soil and tea waste was pasteurized with 5% formaldehyde. The vermicompost was prepared using the SMS of *Pleurotus Sp.* For conversion of SMS into vermicompost it took 45 days. The SMS of *C. indica* was collected from the mushroom unit, Department of Botany, University of North Bengal, after the fourth flush and was kept for 10 days for further composting and then used for experiment.

Spawning and cultivation

Both types of composts were spawned @ 5%. Spawning room was arranged with near about 25°C temperature and 80% relative humidity without ventilation. After mycelial growth, 3 cm layer of casing material was used to cover the colonized compost. The data concerning pin head formation time of *A. bisporus* was recorded as days after casing. Watering was done after casing.

Harvesting and maintenance

The development of primordial initiation after casing was varied on compost type. Regular watering was done to keep the casing soil moist to encourage mushroom growth and help to keep the humidity high. Mushroom was harvested before opening of the pileus. From one bed young mushrooms were harvested every day. No remnants of harvested sporophore were allowed to remain in the compost bed. After each harvest, the casing soil was slightly ruffled. Total weight of all the fruiting bodies harvested from bed was measured as total yield of mushroom.

Biochemical analysis of fruit body grown on different substrate combination

Protein content of fruit body in different stages in fresh as well as dry form was determined following the protocol proposed by Lowry *et al.*, (1951).

Carbohydrate content of different parts of fruit body in both forms, fresh and dry was measured. Ethanol (95%) was used for extract preparation in both total sugar and reducing sugar. Total sugar was estimated at 630nm using Anthrone's reagent. Reducing sugar estimation was done

using 2 ml of alkaline copper tartrate in 2ml of ethanolic extract of sample. Determination of reducing sugar content using arseno molybdate was carried out at 620 nm following Nelson-Somogyi Method as described by Plummer (1978).

RESULTS & DISCUSSION

Inner compost temperature profiles of composts during composting

The inner compost temperature of the mean values of 9 measuring points was determined for the three different composting formulations compost I i.e. paddy straw based compost, compost II *i.e.* Tea leaf based compost and compost III *i.e.* Paddy straw and tea leaf based compost. Average temperatures of the compost of every day for the three different compost formulas are given in Fig 1. The highest temperature of all compost formulas were recorded at 7-10th day stage. It might probably be due to the activity of fungal diversity. Increase temperature at this stage is an indicator for rapid and exothermic microbial activity within compost. Similar result was found by Colak, (2004). This time period may be an optima activity stage of thermophilic fungi. This stage is a crucial stage for decomposition for carbohydrates necessary to produce a selective substrate environment for mushroom growing (Yalinkilic *et al*., 1994).

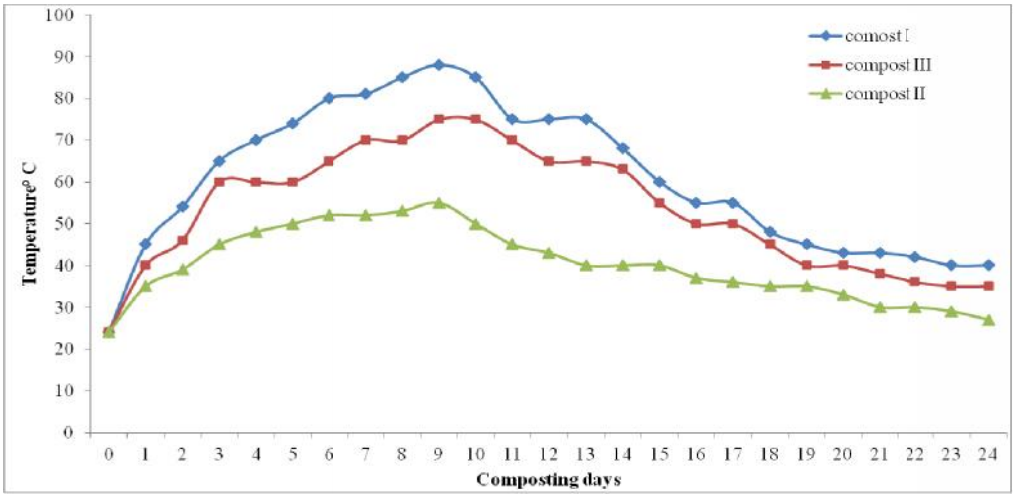


FIGURE1. Inner compost temperature profile during the composting period of three different compost formulas

Effect of compost and casing materials on yield

It has been recorded that the compost formulas have effect on growth parameters like number of mushroom per bag and yield rate. However no significant effect was recorded on single fruit body weight for these different casing materials. The effect of compost formulation on fruit body development is presented in Fig. 2. Results regarding the effect of compost formulas on single fruit body weight are presented in Fig. 3. The growth and productivity of *A. bisporus* was influenced by biological and physio-chemical characteristics of compost (Kariaga *et al*., 2012). The fresh weight of fruit body and yield was influenced by compost type. Similar result was recorded by Meire *et al.*, (2008) when they *cultivated A. bisporus* in two different compost formulas. Dias *et al*. (2003) analyzed the production of *Pleurotus sajor-caju* in different agricultural residues (bean straw corn straw and coffee hulls) and also observed mycelium growth, production and biological efficiency differences depending on the compost used. Maio *et al*. (2008) stated that the influence of substrate composition on the growth velocity of the mushroom *Pleurotus ostreatus* and observed a significant correlation between substrate formulation and the nutritional composition of the mushroom. Waste tea leaves based composts and wheat straw based compost was used in cultivation of *A. bisporus* and found the similar results in terms of pin head formation time and yield (Baysal *et al.*, 2007, Toker *et al*., 2007). The yield of mushroom was influenced by casing materials described by Zied, *et al.*, (2010). Different casing materials support different populations of bacteria, are related to the numbers of initials of fruiting (Noble *et al.*, 2003). Porosity and chemical composition also influence the primordial initiation and yield of *A. bisporus* (Taherzadeh *et al*., 2013). High salt content, of casing layer can affect the mycelial growth, formation of primordial, and mature fruit bodies (Taherzadeh *et al*., 2013). Kalha *et al.* (2011) and Kumar *et al.* (2012) reported that the various supplement and biofertilizer in casing layer have the effect on the yield of *C. indica.* The thickness of casing layer also affected the yield and bioefficiency as recorded by Subramanian *et al.* (2015).

Development of sporophore of *A. bisporus* on paddy straw based compost

Time periods of pin head formation and developmental parameters of fruit body of *A. bisporus* in paddy straw based compost (compost I) encased with different casing materials is presented in Table 1. Among the casing materials vermicompost was the most suitable casing material that needed 10-14 days for pining. Tea Garden soil was least suitable for pin head formation time as it required 21-24 days. The maximum sporophore formation was recorded as 36-50 number/bag with vermicompost as casing material. Least result regarding sporophore formation was recorded in tea waste. Maximum total yield of fruit body (1506gm) was obtained from the bag encased with vermicompost followed by SMC + vermicompost (1460gm), SMC alone and SMC+ tea waste (1370gm), tea garden soil (1220gm) and finally tea waste (1160gm). The

sporophores grown in paddy straw compost encased with different casing materials is presented in Fig. 4 (A-G).

Development of sporophore of *A. bisporus* on dried tea leaf based compost

Time periods of pin head formation and number of pinhead appeared in 1st flush and other developmental parameters of *A. bisporus* in dried leaf based compost (compost II) encased with different casing materials such as vermicompost, tea waste, tea garden soil and SMC are given in Table 2 and the sporophore grown in this compost is presented in Fig.4 (H-N). Vermicompost yielded the most suitable casing material with 19-23 days for pining. From Table 2 it is clear that the maximum sporophore formation was recorded as 6-12/bag with vermicompost followed by SMC (6-10/bag) and tea waste (4-7/bag). The minimum result regarding to sporophore number (3-5/ bag) formation was recorded in garden soil. Maximum total yield (1028gm) was obtained from the bag encased with vermicompost followed by SMC + vermicompost (690gm), SMC (590gm), tea garden soil vermicompost (530gm), tea garden (500gm), and tea waste + vermicompost (440gm). The minimum yield was obtained in the tea waste treated bag as 400gm.

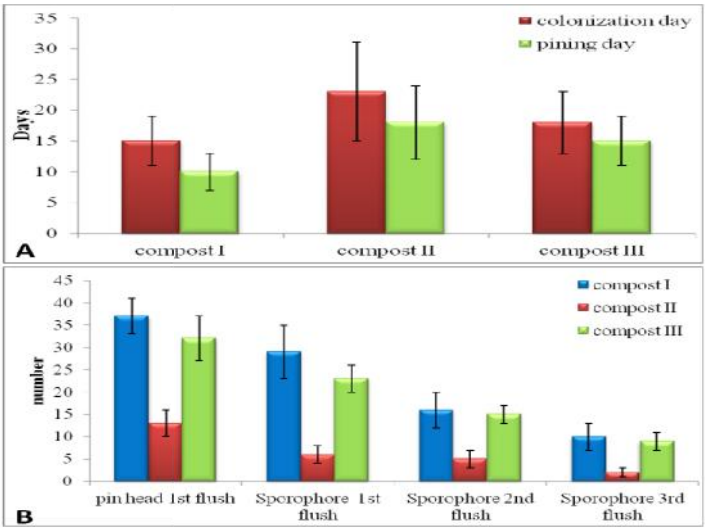


FIGURE2. Growth and mycelial development (A) and yield (B) of *A. bisporus* in three successive flushes in three different compost formulas encased with vermicompost.

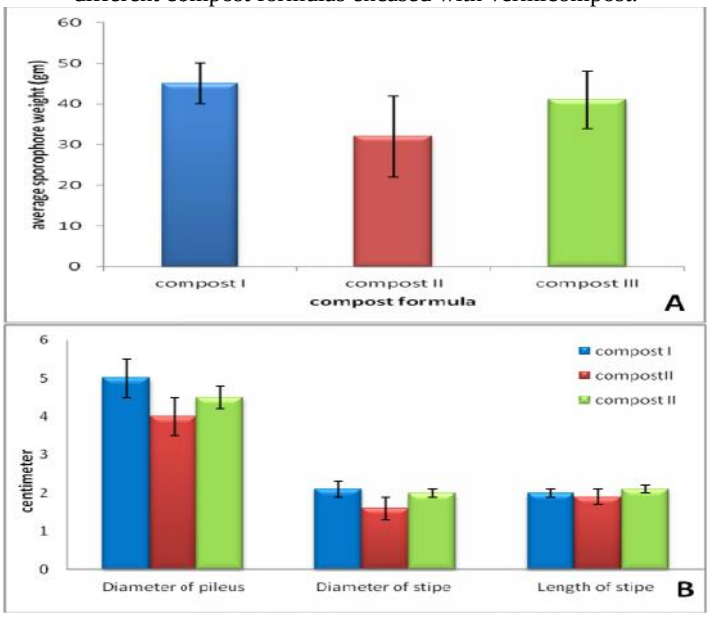
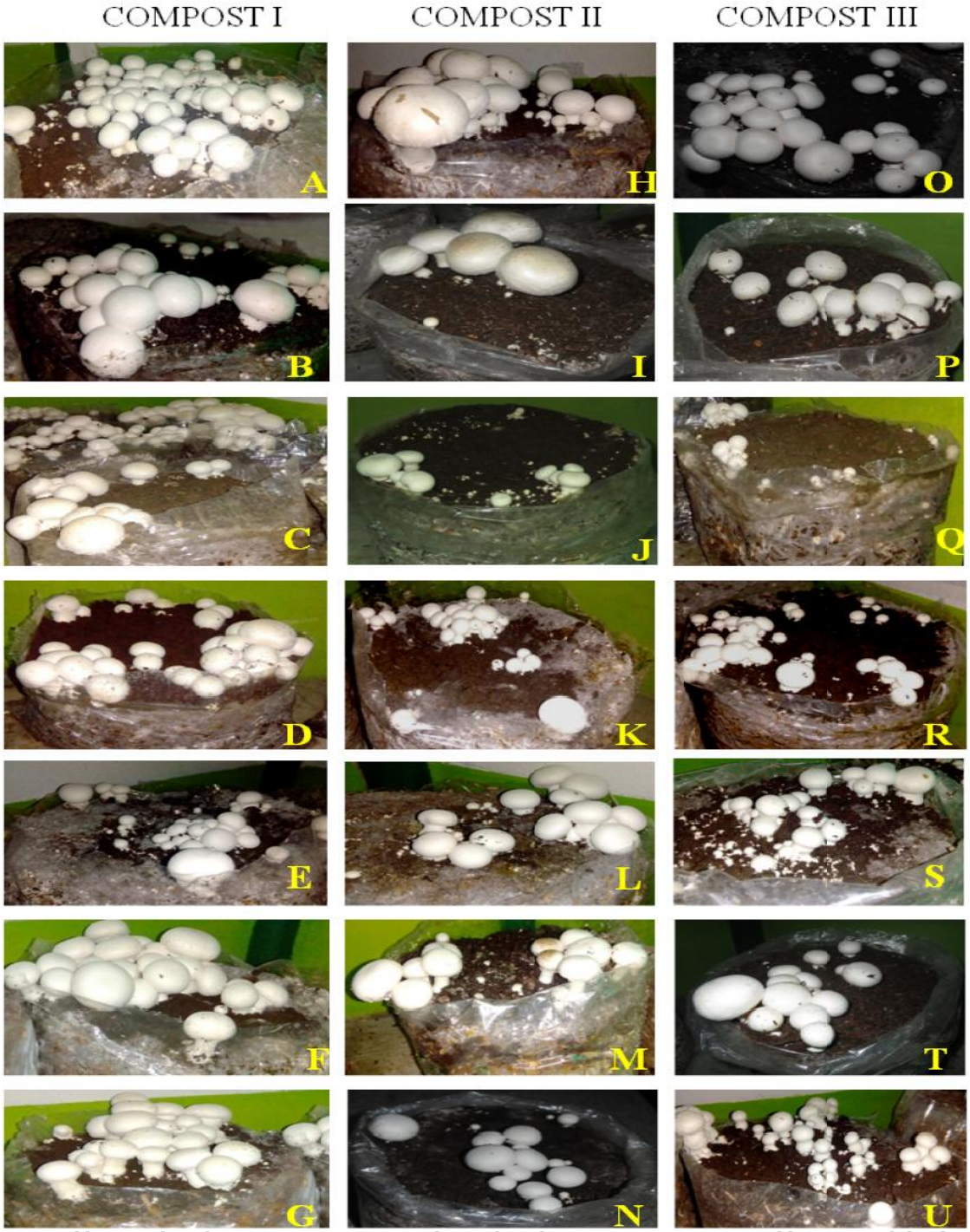


FIGURE3. Effect of compost formulas on fruit body morphological features; Single sporophore weight (A); Diameter (cm) of pileus and size of stipe of sporophore of *A. bisporus grown* in three different compost formulas encased with vermicompost (B)

Development of sporophore of *A. bisporus* on combined paddy straw and dried tea leaf based compost

On paddy straw and tea leaf based compost (compost III), vermicompost was recorded as most suitable casing material that required 15-17 days for pining. Time periods for pin head formation and number of pinhead appeared in 1st flush and other developmental parameters of *A. bisporus* in compost III encased with different casing materials is given in Table 2. The sporophore grown on compost III is presented in Fig.4 A-U).The maximum

sporophore formation range was recorded as 26-32 number/bag with vermicompost followed by tea waste (21-25/bag) and SMC (19-23/bag). The least result regarding sporophore number formation was recorded as 5-18/bag in tea waste casing material. Maximum total yield (1228gm) was obtained from the bag encased with vermicompost followed by SMC + vermicompost (1210gm), SMC (1060 gm) and vermi +tea waste (980gm), tea garden soil + vermicompost (970gm), tea garden soil (910gm). The minimum total yield was recovered in tea waste casing as 870gm.



*compost I-Paddy straw based compost, compost II-tea leaves based compost, compost III-paddy straw and dried tea leaf based compost

FIGURE4. Fruit body of *A. bisporus* in three different types of compost formulas encased with different casing materials, compost I (A-G); compost II (H-N); and compost III (O-U). casing materials ; vermicompost (A,H,O); garden soil (C,J,Q); tea waste (D,K,R); garden soil + vermicompost (E,L,S); tea waste + vermicompost (F,M,T); spent compost + vermicompost (G,N,U).

TABLE 1. Pin head formation and development details of *A. bisporus* on paddy straw based compost (compost I) using locally available casing materials

Casing Materials	days for primordial initiation	No. of pin head	No. of sporophores/ bag in 1st flush	Production/bag (gm)			Total yield/bag (gm)
				1st flush	2nd flush	3rd flush	
Vermicompost	10-14	37-53	36-50	586±27.81	500±23.2	420±28.8	1506
Tea Garden soil	21-24	27-31	18-27	500±36.57	440±28.7	380±54.0	1220
Tea waste	19-23	26-34	15-18	515±36.09	380±41.4	270±37.1	1160
SMC	16-20	27-39	24-36	521±34.66	460±43.8	390±35.6	1370
SOIL+ VSMS	14-17	28-39	25-31	520±35.84	460±31.8	320±24.5	1300
Tea + VSMS	16-19	38-46	32-37	530±34.86	500±33.1	340±27.1	1370
SMC+VSMS	14-18	32-43	30-41	550±34.26	500±33.8	410±27.0	1460

TABLE 2. Pin head formation and development details of *A. bisporus* on tea leaves based compost (compost II) using locally available casing materials

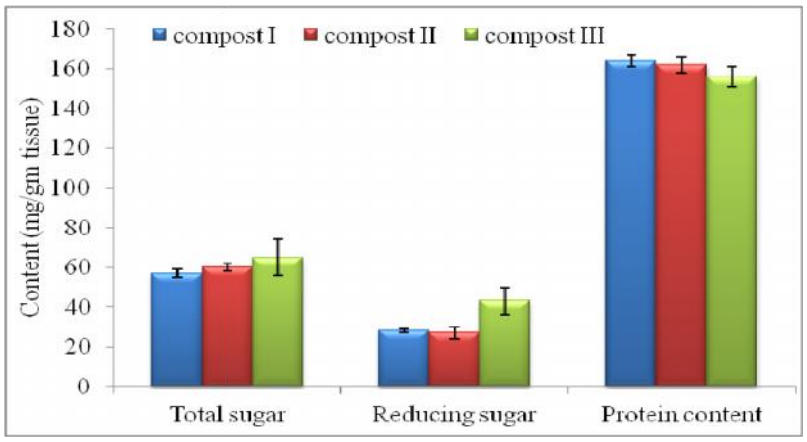
Casing Materials	days for primordial initiation	No. of pin head	No. of sporophores/ bag in 1st flush	Production (gm)/bag			Total yield /bag g (gm)
				1flush	2flush	3flush	
Vermicompost	18-23	13-21	6-13	300±21.03	220±20.05	100±9.66	1028
Tea Garden soil	21-25	6-8	3-5	190±20.76	200±17.95	110±10.54	500
Tea waste	17-21	6-10	4-7	210±20.71	100±13.17	90±5.96	400
SMC	19-23	10-16	6-10	270±42.67	200±31.56	120±14.29	590
SOIL+ VSMS	18-21	8-10	4-8	210±25.21	210±24.98	110±7.68	530
Tea + VSMS	17-19	7-11	6-9	220±26.50	120±14.15	100±10.85	440
SMC+VSMS	17-22	11-18	6-12	290±28.13	260±28.25	140±15.71	690

TABLE 3. Pin head formation and development details of *A. bisporus* on paddy straw and tea leaves based compost (compost III) using locally available casing materials.

Casing Materials	days for primordial initiation	No. of pin head	No. of sporophore/ bag in 1st flush	Production (gm)/bag			Total yield/bag (gm)
				1st flush	2nd flush	3rd flush	
Vermicompost	15-17	32-45	26-32	500±33.16	448±27.16	380±23.04	1228
Tea garden Soil	18-24	14-24	11-15	420±34.48	290±24.23	200±20.26	910
Tea waste	18-21	26-32	21-25	400±39.36	320±36.40	150±19.27	870
SMC	16-23	22-36	19-23	490±43.09	340±28.01	230±24.31	1060
SOIL+ VSMS	16-19	17-27	14-19	440±28.25	310±27.85	220±20.09	970
Tea + VSMS	16-20	27-37	26-34	410±39.34	360±32.76	210±19.49	980
SMC+VSMS	15-19	28-41	26-39	500±32.38	360±32.69	350±27.65	1210

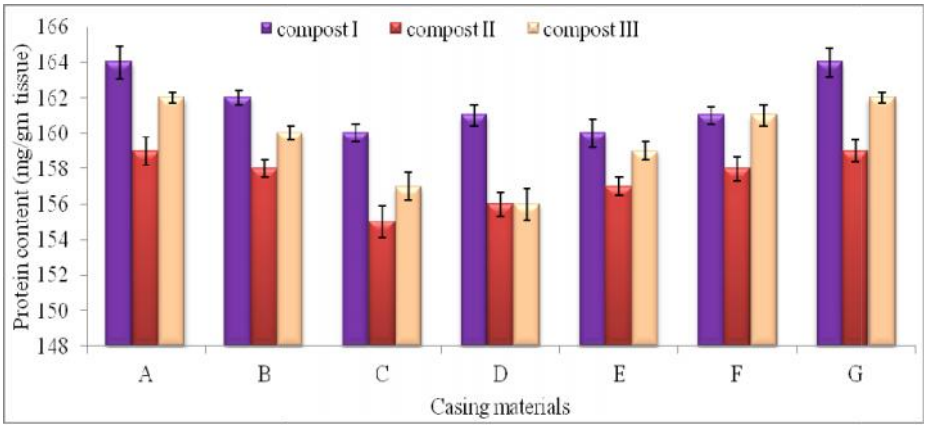
Effect of compost on biochemical constituents of fruit body of *A. bisporus*

Mushrooms grown in different compost formulas showed significant variations in protein content, however no such significant differences were recorded for different casing materials. Among the compost formulation the highest protein content of the young fruit body was observed as 164.67 mg/gt in paddy straw based compost and minimum protein content was determined as156.33 mg/gt in tea leaf based compost grown fruit body respectively. Protein and sugar content of young sporophore grown in three different compost formulas encased with vermicompost is presented in Fig.5. This study regarding the influence of compost on chemical constituents of fruit body was supported by the study of Dehariya *et al.,* (2013) and Merie *et al.* (2014). Among the casing materials vermicompost was recorded as best casing materials giving the maximum protein content as 164mg/gt in paddy straw based compost. The minimum protein content was measured as 153.67 mg/gt in fruit body grown in tea leaf based compost. The results regarding protein content (mg/gm tissue) of fruit body grown in three different compost-encased with different casing treatment is represented in Fig.6. The lowest total protein content was determined in the fruit body grown on compost II formula encased with tea waste. Fruit body grown in Compost II encased with vermicompost showed the total sugar content of 65 mg/gm tissue as maximum content and minimum was measured as 58.33mg/gm tissue in GS casing treatment. The results regarding carbohydrate content of fruit body grown in different compost encased with different casing materials is presented in Fig.7&8. Maximum total sugar content was observed in TW + vermi casing treatment and minimum in SMC treatment in compost III. The biochemical constituent of mushrooms depends on the growing substrate and the organic supplements also have effect on the non-enzymatic antioxidants and mineral expression fruit body of mushroom (Sharma *et al.,* 2013). The chemical constituents of fruit body grown in different substrates vary as reported by Shin *et al*., (2007) and Pani (2010). However, yield and nutritional values of *A. bisporus* cultivated on dried tea leaf based composts should be investigated in more detail.



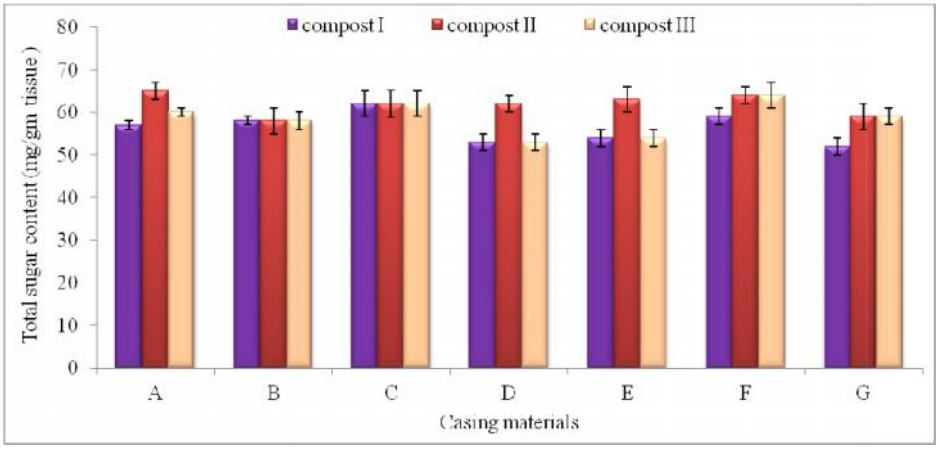
*compost I-Paddy straw based compost, compost II-tea leaves based compost, compost III-paddy straw and dried tea leaf based compost

FIGURE5. Effect of compost formulas on protein and carbohydrate content (mg/gt) in young fruit body grown in different compost encased with vermicompost



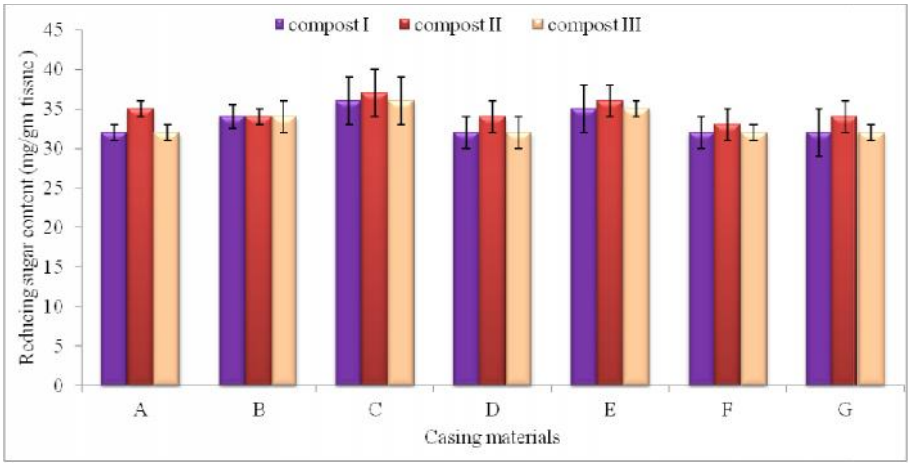
*compost I-Paddy straw based compost, compost II-tea leaves based compost, compost III-paddy straw and dried tea leaf based compost

FIGURE 6. Effect of casing materials on protein content in young fruit body grown on three different compost formulas and encased with different casing materials; such as Vermicompost (A), garden soil (B), Tea waste (C), Spent mushroom compost of C. indica (D), Garden soil and vermicompost (E), Tea waste and vermicompost (F), SMS and vermicompost (G).



*compost I-Paddy straw based compost, compost II-tea leaves based compost, compost III-paddy straw and dried tea leaf based compost

FIGURE7. Effect of casing materials on total sugar content in young fruit body grownon three different compost formulas and encased with different casing materials; Vermicompost (A), garden soil (B), Tea waste (C), Spent mushroom compost of C. indica (D), Garden soil and vermicompost (E), Tea waste and vermicompost (F), SMS and vermicompost (G).



*compost I-Paddy straw based compost, compost II-tea leaves based compost, compost III-paddy straw and dried tea leaf based compost

FIGURE8. Effect of casing materials on reducing sugar in young fruit body grown on three different compost formulas and encased with different casing materials; Vermicompost (A), garden soil (B), Tea waste (C), Spent mushroom compost of *C. indica* (D), Garden soil and vermicompost (E), Tea waste and vermicompost (F), SMS and vermicompost (G).

CONCLUSION

From the present study we can conclude that the paddy straw based compost encased with vermicompost is the best for cultivation of *A. bisporus*. The results in this study revealed that the casing material play a decisive role in increasing biological efficiency of white button mushroom. The nutritional value of fruit body also depends of the substrate on which it is grown.

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