

Profitable Eco-friendly Bio conversion of “White Button Mushroom” (*Agaricus bisporus*)

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Abstract: The large scale production of mushrooms has opened up new vistas of export earnings to improve the economic status of our country. With the view point of profitable eco-friendly bioconversion of *Agaricus bisporus* (Lange) Imbach, the study conducted upon the morphological features of fruit bodies of different strains, the yield of different strains, growth rate of different strains in compost and casing soil of *A. bisporus* in which, strain P1 and NCS 5 were found superior over the other strains. The yield of strains varied from 11.0 kg to 13.75 kg per quintal compost. The strains NCS 5 (13.75 kg) and P1 (13.10 kg) were statistically at par over the check. [Nature and Science. 2009;7(8):26-35]. (ISSN 1545-0740).

Key words: Profitable, Eco-friendly, Bioconversion, White Button Mushroom

Introduction

Mushrooms are non-conventional source of human food. These are used as food since the beginning of human civilization. These are delicious, nutritionally rich and have their own importance as medicines. These are excellent low caloric meat substitute. The widespread use of mushrooms in ancient times is also confirmed by the hypothesis of Wason (1971) that the “Soma” of Rigveda was a preparing of mushroom, *Amanita muscaria*. For the profitable eco-friendly bioconversion of lignocellulosic wastes of agro-industry, the production of mushroom is regarded as the second most commercial microbial technology next to the yeast. The large scale production of mushrooms has opened up new vistas of export earnings to improve the economic status of our country.

Mushroom has been defined as a “Macro-fungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand” (Chang and Miles, 1993). These are non-chlorophyllic plants, which occur seasonally all over the world with quite different characters like shape, size, colour, appearance and edibility, depending on their habitat.

Taxonomically (Alexopoulos et al, 1996) these are species of phylum Basidiomycota and Ascomycota. Nearly 300 species belonging to genera of the edible mushrooms are reported in India, out of

the 2000 species occurring in the world. Out of these total edible mushrooms, 80 species have been grown experimentally, 20 are being cultivated commercially and 8 are being produced on large scale all over the world. Some species of cultivated edible mushrooms whose production has reached on industrial scale are *Agaricus bisporus*, *Agaricus bitorquis*, *Flammulina velutipes*, *Hypsizygus marmoreus*, *Lentinula edodes*, *Pleurotus ostreatus*, *Tremella fuciformis*, *Volvariella volvacea* (Chang and Miles, 2004).

In India, *A. bisporus* (white button mushroom), *Pleurotus spp* (oyster mushroom) and *Volvariella volvacea* (tropical mushroom) are cultivated commercially. Annual increment of world *Agaricus* production during 1975-1997 was 5.3% (Delcaire, 1978 and Chang, 1999).

The world production of *Agaricus* was about 2 million metric tons (MT) in 1997 with a comparison of world production of all cultivated edible mushrooms, the percentage of world total production of *Agaricus* decreased from 73.1 in 1975 to 31.8% in 1997, even though the actual production increased from 900.0 thousand MT in 1981 to 1955.9 thousand MT in 1997, a 2.2 fold increase, and the annual increase percentage during the period 1975 to 1997 was 5.3%. This is mainly attributable to the increasing production of other cultivated edible mushrooms, such as the drastically increased production in Asia in recent years of *Lentinula*, *Pleurotus*, *Flammulina* and *Hypsizygus*, four speciality mushrooms.

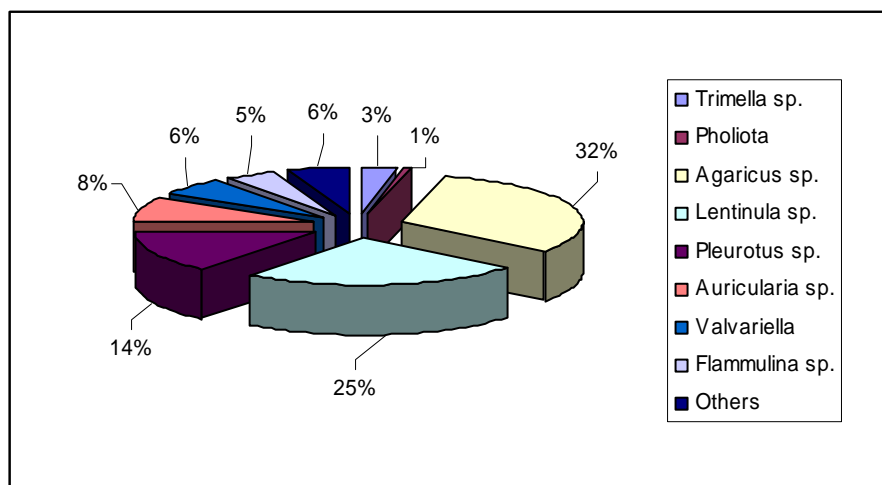


Figure1. Share of Different Mushrooms in Total World Production (6.15 Million Tonnes in 1997)

In 1986, the United States was the largest producer of *Agaricus* (266.7 thousand MT), followed by China (254.6 thousand MT) and France (170.0 thousand MT). However, in 1999-2000 China produced 637.3 thousand MT and jumped to become the largest producer of this important mushroom, followed by United States, Netherlands and France.

In India, Sharma (1997) has reported nearly 85% of the total production (40,000 MT) of edible mushrooms was contributed by *A. bisporus*.

The cultivation of white button mushroom is mostly confined to small, seasonal growing units which are mostly unpasteurised compost and producing 10-14 kg mushroom/qt. compost. However, 18-22 kg mushroom/qt. compost had been harvested under controlled conditions using the pasteurised compost by a limited number of commercial growing units but in the developed countries the production is 25-30 kg mushroom/qt. compost (Chaddha and Sharma, 1995).

Mushroom cultivation involves a number of operations. After the completion of spawn run the crop enters the reproductive phase leading to the production of fruit bodies. Even after the colonization of compost, fructification will not take place unless the colonized compost is covered with casing, a process of applying the cover on the surface of

compost, is done by a suitable casing mixture. By applying casing layer, which is nutritionally poor, stress conditions for the production of fruit bodies are created. Controlled water supply (spraying of water) for the growth and development of fruit bodies and also to maintain humidity and temperature in the crop room by evaporative cooling is done 2-3 times every day. It also provides a medium of low osmotic value and above all physical support to fruit bodies.

Agaricus is restricted to the saprophytic with a chocolate brown spore print and usually an annulus (ring) around the stalk. The epithet *bisporus* refers to the two spored basidia lining the gills. It is also known as white button mushroom due to its colour and shape. When the mushroom is bruised it becomes brown due to oxidation.

The yield and quality of the mushroom produced is determined by three factors –

- 1) The genetic makeup of the mushroom strain
- 2) The environmental conditions in which the mushroom is grown.
- 3) The physiological and nutritional requirement of different strains.

There are many parameters which can influence the quality of mushroom. In our country, introduction of exotic strains and then evaluation for selection of

better strain is still a continuing process. Some isolated attempts to obtain improved strains through multispores, monospores and tissue culture has started to get better yield. Introduction of better quality strains from other countries cannot solve the problem of better quality strains in our country due to altogether different conditions of growing mushrooms than those 2 of Europe and America. Therefore; we need to develop better performing strain in terms of yield, quality and wider adaptability under diverse growing conditions.

Mushrooms are capable of producing the highest quantity of protein per unit area and time from the worthless agro-wastes. Thus, these are recognised as the alternate source of good quality protein.

Mushrooms contain 20-35% protein on dry weight basis which is higher as well as of higher quality than those of the vegetables and fruits. Many vitamins such as Thiamine (B1), Riboflavin (B2), Nicotinic acid, Pantothenic acid, Vitamin C and Vitamin K are also present in the mushrooms (Manning, 1985). These are good sources of minerals. *Agaricus bisporus* is reported to contain a considerable amount of potassium, phosphorus, copper and iron (Manning, 1985). Mushrooms have long been considered to have medicinal value.

Bioactive polysaccharides having antitumor and anticancer effects are isolated from mushroom species are *Coriolus versicolor*, *Lentinula edodes*, *Schizophyllum commune*, *Ganoderma lucidum*, *Aagaricus baize*, *Grifola frondoca* (Chang and Miles, 2004).

Many bioactive substances with immune-modulating effects have been isolated from mushrooms include polysaccharides, glyco-proteins, triterpenoids and fungal immune-modulating proteins.

In view of the above background, present studies were undertaken with the following objectives:

- (1) To study the morphological features of fruit bodies of different strains.
- (2) To study the growth rate of different strains in compost and casing.
- (3) To study the yield performance of the strains.

Edible fungi belong to widely different taxonomic subdivisions among the Ascomycotina and Basidiomycotina. *Agaricus bisporus* is a secondary homothallic basidiomycetes belonging to the order agaricales and family agaricaceae (Webster, 1980 and Alexopoulos et al., 1996).

This mushroom is one of the most acceptable edible fungi contributing 31.8% of total world mushroom production (Chang and Miles, 2004). Although cultivation of this mushroom is being practised since 17th century. A brief account of the pertinent literature available on various aspects of present studies has been reviewed.

Morphological:

Atkinson (1961) described different species of *Agaricus* on the basis of morphological characteristics. However, a detailed outline to study the fungal fruiting bodies was documented by Smith and Smith (1973). Mehta and Dhar (1991) studied vegetative and sporophore characters of 15 different multispore cultures namely, MS 1 to 15 of *Agaricus bisporus* (Lange) Sing and they found an average of pileus diameter 3.43 cm, pileus thickness 0.98 cm, stipe length 3.3 cm, stipe diameter 1.49 cm and gill cavity 3.55 cm. Thakur and Dhar (1993) studied morphology of *Agaricus* strains and they reported the average pileus diameter 3.78 cm, pileus thickness 1.08 cm, stipe length 2.79 cm, stipe diameter 1.64 cm and gill cavity 4.05 cm.

Growth rate in compost:

Straatsma et al. (1991) studied growth of *A. bisporus* mycelium on sterile compost. The mycelium on sterile compost extended at a linear rate of 4mm/day. Mehta and Dhar (1991) recorded mycelial growth rate of multispore cultures after 7 days of incubation. Singh (1994) studied the growth rate of different strains of *A. bisporus* (P1, P2, MS 39, NCS 5, NCS 15, NCS 12 AND S 11) on compost up to 17 days from inoculation and found maximum and minimum growth rate per day as 4.62 and 2.88mm, respectively for the strains NCS 12 and P2. Singh (1998) working with *A. bisporus* strains X 13, X25, NCS 12, NCS 5, NCS 11, S 11, AbP 7, U 3, MS 39, P1 and one strain of *A. bitorquis*, NCB 13 reported

that strains MS 39 and U 3 showed maximum growth in compost after 17 days of inoculation and NCS 12 followed by NCB 13, X 25, S11, P 1 and AbP 7 were also grown. The average downward mycelial growth rate of multisporous cultures after 7 days of incubation. The average downward mycelia growth varied between 2.0- 4.3 cm.

Growth rate in casing soil:

Before initiation of fruiting, the growth of fungus forms mycelia pad or stroma in casing soil. The growth of *Agaricus bisporus* in casing soil was recorded to be 5 mm/day by Rainey et al. (1986).

Singh (1994) studied the growth rate of different strains of *A. bisporus* (P1, P 2, MS 39, NCS 5, NCS 15, NCS 12 and S11) in the casing soil upto 17 days from inoculation and reported that the maximum and minimum growth rate per day occurred in the strains P1 (4.03mm) and S 11 (1.76mm), respectively. Singh (1998) reported that in casing soil maximum growth rate per day was found in the strain 512-H whereas, NCS 5 showed minimum growth rate per day.

Yield of strains:

Jin (1990) studied the yield performance of fluffy and appressed type mycelium of the same strain of *A. bisporus*, he reported that fluffy type tended to give high yield whereas, the appressed type showed good quality. Mehta and Dhar (1991) evaluated 9 strains of *A. bisporus* for yield performance. The strains NCS 14, NCS 5, NCS 11, NCS 6 and NCS 15 were at par in terms of yield and yielded significantly higher than S 1, MS 39, P2 and NCS 12. Singh (1990, 1991) conducted the yield evaluation trials of different strains of *A. bisporus*. He reported that strains S 11 was introduced in sixties while strains RRL 89, S 22 and S 649 were introduced during 1975- 1983. The strain S 11 and S 310 were good yielders, while TM 7 and L 20 were identified as moderate yielders.

Methodology:

The experiment was conducted at "Mushroom Research and Training Centre", Centre of Advanced Studies in Plant Pathology, G. B. Pant University of Agriculture and Technology, Pant Nagar.

A. Selection of the strains:

Four strains of *A. bisporus* namely P1, NCS5, NCS12 and S11 (check) selected based on their yield performance under All India Coordinated Mushroom Improvement Project at G. B. Pant University of Agriculture and Technology, Pantnagar (UK).

B. Morphology:

The morphology of various structures of the fruiting body produced by the different strains were studied selecting 100 fruiting bodies per strain randomly per day from flush of a crop grown on pasteurized compost. The pileus, stipe and gill cavity of each fruiting body was measured with the help of Vanier Callipers, to know the morphological difference between the strains.

C. Growth rate of strains in compost:

Glass tubes (180 mm long) plugged at one end with cotton wool. 4 g. of spawn were placed at the bottom of the test tube and then covered to a depth of 100mm compost. All the treatments were replicated three times. The first observations were made after 5 days of inoculation and the next four observations were made at interval of three days each. The pH and humidity of the compost used for the experimentation was 7.20 and 73.0% respectively.

Growth rate of strains in casing:

The experiment was laid down using glass tubes as earlier experiment on growth rate of strains in compost. Instead of compost, a casing mixture of FYM+ soil (3:1) treated with Formaldehyde (4%) was used. Each treatment was replicated three times and kept in a B.O.D incubator at $20 \pm 2^\circ$ C for growth. The first observation was made five days after inoculation followed by four observations at an interval of three days each.

Yield of strains:

The yield of strains was assessed by growing them on pasteurized synthetic compost. The synthetic compost of 2.2% nitrogen level was prepared as per following formulation:

Wheat straw	: 1000 kg
Chicken manure	: 600 kg
Urea	: 14.5 kg
Wheat bran	: 100 kg
Gypsum	: 50 kg

For compost making, short composting method was employed giving 7 days out and 6 days indoor composting period. Casing mixture (FYM+ soil) 3:1 sterilized with 4 % Formaldehyde was used. 10kg compost was filled in polythene bags of 70 X 45 cm in size and spawning was done using 0.75% grain spawn of compost weight. Each treatment was replicated 3 times and bags were kept in crop room at prevailing temperature of $20 \pm 2^\circ\text{C}$. The 3.5 cm thick casing was done on 17th day from the date of spawning. The yield of strains obtained from 30 days harvesting period were compared with each other.

Statistical analysis:

Statistical analysis of the data was done as per the requirement of the experiment. Critical differences (CD) were calculated at 5% level of significance for comparison of differences between the treatment means.

Results:

The structures of fruit body like diameter, thickness gill cavity of pileus length and diameter of stipe were measured and the data recorded are summarized in Table 1.

Table1. Structural Measurements of Different Strains of *A. bisporus*

S. No.	Strains	Pileus (cm)			Stipe (cm)	
		Diameter	Thickness	Gill cavity	Length	Diameter
1.	P1	1.90	1.10	0.80	2.30	1.50
2.	NCS5	3.00	0.90	1.60	1.60	1.45
3.	NCS12	3.60	1.00	0.70	0.70	2.55
4.	S11 (Check)	2.30	0.80	2.00	2.00	1.60
	C D at 5%	0.314	0.236	0.518	0.570	0.399

A perusal of the data in Table 1 shows that highest pileus diameter (3.60 cm) recorded from strain NCS 12. There was significant difference among the treatments. The lowest pileus diameter was recorded in P1 (1.90cm). The thickness and of pileus did not vary significantly. However, the strain P1 (1.10 cm) and S11 (0.80 cm) were scaled maximum and minimum, respectively for thickness.

However, the strain S 11 (check, 1.75 cm) and NCS 12 (0.70cm) proved to be maximum and minimum size for gill cavity of the pileus. The maximum and minimum length of stipe was measured in the strains P1 (2.30cm) and NCS 12 (0.70 cm), respectively whereas, the length of stipe measured in strain S11 (check, 2.00 cm) and NCS (1.45 cm), respectively. The strain S 11 (check, 1.60cm) and P1 (1.50cm) were at par in diameter of stipe.

Growth rate of strains in compost:

The growth rate of strains in compost plays an important role in mushroom production. The fast growing strains reduce the period required for casing and ultimately it results early fruiting in contrary to slow growing strains. Therefore, this experiment was aimed to assess the growth rate of strains. The compost filled in test tubes were inoculated with grain spawn of different strains and incubated for 17 days at $20 \pm 2^\circ\text{C}$. The growth rate recorded at 5th, 8th, 11th, 14th and 17th days of incubation is summarized in Table 2.

The growth rate of strains varied significantly from each other at different intervals (Table 1). On 5th day of their growth, the strain NCS 5 (11.42mm), NCS 12

(11.40 mm) and S 11 (9.20mm, check) gave significantly higher growth rate in comparison to P1 strain. However, the strains NCS 12 (31.85), NCS 5 (29.25 mm) and S 11 (24.25mm, check) gave significantly higher growth rates than those of other strain which included P 1 (13.22mm) on 8th day of incubation. Thus NCS 12 has started superseding. The strain NCS 12 (49.65) superseded all the strains on 11th day of its growth. While on 14th day the growth rate of NCS 12 (60.45) and NCS 5 (59.45mm) were statistically at par and high than other strains. The similar growth trends of these two strains were observed on 17th day of incubation but the strains NCS 5 had superseded the strain NCS 12.

It may be concluded from the above results that the strains NCS 5 and NCS 12 were statistically higher in growth rates at different intervals and on the basis of per day as well.

Growth rate of strains in casing:

The quick growth of strains in casing soil results early formation of stroma from which the fruit bodies come out. Therefore, the present study was undertaken to determinate the growth of strains in casing as described in "Material and Methods". The growth of strains in casing measured at different intervals for a period of 17th days is given in Table 3.

Table2. Growth Rate of Different Strains of *A. bisporus* in Compost

Observation	Average growth rate (mm) in 17 days from inoculation			
	P1	NCS 5	NCS 12	S 11 (Check)
I 5 th day	4.81	11.42	11.40	9.20
II 8 th day	13.22	29.25	31.85	24.25
III 11 th day	37.45	35.05	49.65	38.45
IV 14 th day	52.85	59.45	60.45	53.25
17 th day	67.20	80.45	78.65	70.85
Growth rate/day	3.94	4.73	4.62	4.16

CD at 5% for: Strains (A) : 3.15

Observations (B) : 3.52

Observations X Strains (A) X (B) : 7.05

Table 3. Growth Rate of Different Strains of *A. bisporus* in Casing Soil

Observation	Average growth rate (mm) in 17 days from inoculation			
	P1	NCS 5	NCS 12	S 11 (Check)
I 5 th day	6.01	10.05	6.83	3.00
II 8 th day	16.21	18.00	12.60	7.05
III 11 th day	35.65	31.45	25.45	15.80
IV 14 th day	46.22	43.65	34.22	21.05
V 17 th day	68.65	62.62	49.05	30.00
Growth rate/day	4.03	3.68	2.88	1.76

CD at 5% for: Strains (A) : 3.45

Observations (B) : 3.86

Observations X Strains (A) X (B) : 7.72

The data presented in Table 2, shows that the strains varied significantly from each other in terms of growth rate at different intervals. On 5th day of incubation the strain NCS 5 (10.05mm) gave significantly higher growth rate than S 11 (3.00 mm, check) while other strains NCS 12 (6.83mm) and P1 (6.01) were at par with each other. On 8th day of incubation, again NCS 5 (18.00mm) gave significantly higher growth rate as compared to strain S 11 (7.05mm) and NCS 12 (12.60mm) though it was at par with strain P1. But all the strains were significantly at higher at higher growth rate on 8th day as compared to 5th day. On 11th day of growth in casing the strain P1 (35.65) and NCS 5 (31.45mm) were statistically at par and significantly higher than

those of other strains. The strains NCS 5 and P1 (46.22mm) gave significantly higher growth rate than S11 (21.05mm, check) and NCS 12 (34.22 mm) on 14th day of incubation. On 17th day the growth of strain P1 (68.65mm) gave significantly higher growth rate than S 11 (30.00 mm, check) and NCS 12 (49.05) but was at par with the strain NCS 5 (62.62).

Yield of strains:

The yield performance of different strains was recorded using pasteurized compost. The experimentation was done on prevailing room temperature during January, 2006 onwards. The

humidity in crop room was maintained by sprinkling of water on walls, floor and beds. The yields obtained

from different strains are summarized in the table given below.

Table 4. Yields performance of different strains of *A. bisporus* in 30 days cropping period

S. No.	Strains	Average yield in kg/qt. Compost		
		Number	Weight	Weight / Fruit body (gm)
1.	P1	2280	13.10	5.74
2.	NCS 5	2482	13.75	5.53
3.	NCS 12	2230	12.50	5.60
4.	S 11 (check)	1920	11.00	5.72
	CD at 5 %	263.72	1.140	-

It is evident from the data in the above table that yield of strains varied from 11.00 kg to 13.75kg. The strains P1 (13.10 kg), NCS 5 (13.75 kg) and NCS 12 (12.50 kg) were statistically at par in terms of yield. The yield obtained from these strains was significantly higher than strain S 11 (check, 11.0 kg). The number of fruit bodies obtained from the strains NCS5, P1 and NCS 12 were significantly higher as compared to strain S 11(1920, check). It is interesting to record that the maximum weight per fruit body harvested was from the strain S 11 (check) followed by P1, a poor and a moderate yielders, respectively.

The environment, substrate and strain are equally important factors for the mushroom production. Since, the present studies were evaluated for their yield. The rest of the strains NCS12 were at par with that of check (S 11). Earlier workers Mehta and Dhar (1991); Singh (1990, 1991) found NCS 5 to be one of the best yielders.

Discussions & Conclusion

The number of fruit bodies obtained from the strains NCS 5, P1 and NCS 12 were significantly higher as compared to strain S 11 (1920, check). It is interesting to record that the strain S 11(check) followed by P1, a poor and a moderate yielders, respectively.

Serious efforts are being made world-wide to discover economic methods for upgrading the low cost bulk plant wastes such as cereal, straws, and leaves, wood bark etc. into higher value complex of

terrestrial plants is the most abundant biological materials on the earth. It consists of three major components cellulose, hemicelluloses and lignin. The major viable biological processes utilizing significant quantities of lignocelluloses wastes include ruminant's feeding and the cultivation of edible mushrooms. Only Button mushroom is being cultivated on wide scale in different parts of the world. The strains, which play an important role in mushroom production, were evaluated for variability in their morphological and agronomical aspects in present investigation are discussed herein light of the results obtained by other workers. Possible explanations have also been cited wherever feasible.

The compost, spawn of different strains and casing soil were purchased from Mushroom Research and Training Centre, Pantnagar to obtain the fruit bodies for morphological studies. The morphological features of fruit bodies of different strains viz., diameter, thickness and gill cavity of pileus, length and diameter of stipe measured in present investigation have at least, in part, in similarity with the works carried out by earlier workers (Mehta and Dhar, 1991; Thakur and Dahr, 1993). Their observations were based on 4 different strains of *A. bisporus* named P1, NCS 5, NCS 12 and S 11 (check) and recorded mean of different structures like pileus diameter, pileus thickness, stipe, length and diameter etc. which varied to limited level from the measurements of these structures by the authoress in present investigation. The variations might be due to harvesting of sporophores at different growth stage and size. Besides other factors, the growth rate of a strain in compost and casing plays an important role on yield of the cultivated mushrooms. Experiments were conducted to study the growth rate of different

strains in compost and casing. The yield performances of the strains were also assessed. In the present study the growth rate of strain were determined using synthetic compost and casing soil in test tubes spawned with the grain spawn. The growth rate of different strains, varied in compost and casing. In compost, NCS 5, NCS 12 and S 11 gave maximum growth rate on 5th day from inoculation. While, with approach of 8th day the strain S11 (check) slowed down trend was maintained till 17th day. Mehta and Dhar (1991) also recorded variations in the mycelia growth of multispore cultures on 7th day of incubation. However, no report is available in the literature so far on periodical growth rate of strain in compost. The casing used for *Agaricus* cultivation forms the most important part of commercial cultivation. In casing ,on 5th day of incubation the strains NCS 5, NCS 12, P1 and S 11 (check) gave equally the same growth rate i.e. 10.05mm, 6.83mm, 6.01mm, 3.00 mm respectively in comparison to other strain while on 11th day the maximum growth was observed in case of strain P1. On the 14th day the strain NCS 5 was found statistically at par with strain P1.

Finally, the strain P1 ranked first in terms of growth rate in casing per day. Rainey et al. (1986), reported growth of *A. bisporus* in casing soil 5 mm per day. The difference in growth rate of present investigation may be because of the difference in casing material used by the authoress and above earlier workers. There is no information in literature on growth rate of the strains being evaluated in present studies. The environment, substrate and strain are the equally important factors for the mushroom production. Since, the present studies were evaluated for their yield. The rest of the strains NCS 12 were at par with that of check (S11). Earlier workers Mehta and Dhar; Singh (1990, 1991); found NCS 5 to be one of the best yielders. It may be concluded from the foregoing discussions that the strain NCS 5 and P1 have superiority over the other strains studied in present investigations.

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