

Research Article

EVALUATION AND STUDIES OF DIFFERENT STRAINS OF WHITE BUTTON MUSHROOM *Agaricus bisporus* (Lange.) Sing.

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ABSTRACT

Mushrooms are being used since the time immemorial. The several fungi are edible mushrooms and are saprophytic basidiomycetes, which have been successfully cultivated at commercial level worldwide using lignocellulose wastes as substrates for their cultivation. Nowadays, mushrooms are popular valuable foods because they are low in calories, carbohydrates, fat, and sodium, they are also cholesterol-free. Besides, mushrooms provide important nutrients, including selenium, potassium, riboflavin, niacin, vitamin D, proteins, and fiber. All together with a long history as food source, mushrooms are important for their healing capacities and properties in traditional medicine. There are different kinds of edible mushrooms cultivated all over the world. Among them *Agaricus* is the most popular and accepted edible mushroom. *Agaricus bisporus* commonly known as white button mushroom belongs to phylum Basidiomycota. Mushrooms are the health food of the world keeping in its importance, the studies of growth behavior, yield potential, effect of different culture media, pH and temperature on mycelia growth of different strains (Delta, NBS-5, ICAF, A-15, and MC-465) of *A. bisporus* were tested. Five culture media were tested for the radial growth and measured the mycelia growth, on MEA all-strains Delta, NBS-5, ICAF, A-15, and MC-465 were obtained fast and full growth (9 cm). The effect of different pH were tested for the radial growth and measured the mycelial growth. The growth of mycelial in 8pH shows good performance in all the strains of *A. bisporus* followed by 7 pH. The effect of different temperature was also evaluated. Mycelial growth of all the strains shows better results on 25°C. Among various strains of *Agaricus bisporus*, strain Delta produced maximum mushroom yield and number of fruiting bodies i.e., 20.28 kg per q of compost and 104.00 respectively as compared to NBS-5, ICAF, A-15, and MC-465 strains, whereas minimum fruit body was observed in MC-465 (85.50) with 14.11 kg of production per 100 kg of compost. In the growth behavior, NBS-5 gave the highest body weight followed by the other strains. Maximum average body length and stripe was found in MC-465 strain of *Agaricus bisporus*.

KEYWORDS: *A. bisporus* strains, culture media, pH and temperature, morphological characteristics, mycelial growth, yield.

Citation

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INTRODUCTION

Mushrooms are part of fungal biota characterized by wonder. They comprise delicious, nutritionally rich, medicinally important, non-conventional source of human food. *Agaricus bisporus* (Lange) Sing is popularly known as the button mushroom is an edible fungus belongs to phylum Basidiomycota. This mushroom is extensively cultivated throughout the world and contributes about 40% of the total world production of mushroom. Button mushroom is one of the largely growing mushrooms and has the good demand in the market and world trade too. Mushrooms are the health food of the world. The indoor cultivation of *Agaricus bisporus* was however started in caves in France around 1810 [1]. It was first cultivated in India more than 3 decades ago, at Chambaghat, Solan. In India, mushroom cultivation has great potential due to favorable weather conditions, abundant cheaper agro wastes as well as cheaper availability of labour. Now a day's mushroom cultivation has been adopted by progressive farmers as a cottage industry, which not only provides them an additional source of income but they could also recycle the farm waste which was earlier sold by them at much low cost. *Agaricus bisporus* is one of the most acceptable edible fungi contributing 31.8 % of total world mushroom production [2]. The three major mushroom producing countries are China, USA and Netherland which account for more than 60 per cent of the world production. Punjab has become one of leading states in white button mushroom production with 58000 tons/ annum [3]. In mushroom cultivation, mushroom mycelium absorbs food from the natural or semi-synthetic composts on which they grow. The mycelium branches and produces enzymes that digest complex carbohydrate, lipids and protein, which are then easily absorbed by the hyphae. The mycelium penetrate the compost during spawn run stage, mushroom growth take place, and energy stored until fruiting bodies are formed. The fruiting stage is the formation of the primordial, formed from an aggregation of mycelium after casing soil. The food experts also appreciate the food value of mushroom because of its less calorific value (27–30 kcal/100 g) with a low amount of fat (1.3–8% of dry weight mushrooms) and digestible carbohydrate and very high content of protein (20–40% on dry weight basis), have a balanced composition of vitamins and minerals like phosphorous, potassium, sodium, calcium, magnesium, zinc, iron, copper, Manganese etc.. They also contain 5–15% dry matter and are rich in fiber [4]. Their amino acid composition is better when compared to that of vegetables like potatoes and carrots. It produces enzymes, which degrade lignocellulosic materials for their own growth and fruiting. Mushrooms are well-known for its medicinal properties, anti-diabetic, cardiovascular and immune modulating effect in addition to prevent the risk of cancer and controlling blood sugar level [5] with significant antioxidant activity recorded in both wild and cultivated species [6]. The alkaloid, phenolic and organic acid contents in mushroom contribute to the antioxidant and free radical scavenging properties of mushrooms according to their ability to capture metals, inhibit lipo-oxidase and scavenge free radicals [7]. The edible ones those consumed by tribal and local communities, in particular, which could impact the health and nutrition, as because it contains significant protein, flavonoid, β -carotene, lycopene, etc. usually not available in a daily diet. However, only a few selected species have been worked out to estimate antioxidant components such as phenols, flavonoid, carotene, etc including its antioxidant properties [8,9].

MATERIALS AND METHODS

The research work was carried out during two cropping years September 2020 and January 2021 and the parent culture of five strains of *Agaricus bisporus* (Lange) Sing. viz., Delta, NBS-5, ICAF,

A-15, and MC-465 were obtained from Mushroom Research and Training Centre (MRTC), G. B. Pant University of Agriculture & Technology, Pantnagar.

Preparation of culture media. The pure culture of cultivated mushrooms can be obtained on the following media. These media generally used as substrate for isolation, sub-culture, maintenance and preservation of mushroom culture.

Table 1. Preparation of culture media.					
Contents of media	Medium				
	MEA	PDA	CEA	YPDA	WEA
Malt extracts	25g	-	-	-	-
Peeled potato	-	250g	-	250g	-
Dextrose	-	20g	-	20g	-
Yeast extracts	-	-	-	1.0g	-
Wheat grain	-	-	-	-	100g
Ready synthetic compost	-	-	100g	-	-
Agar agar	20g	20g	20g	20g	20g
Distilled water	1L	1L	1L	1L	1L

Whereas, PDA- Potato dextrose agar, MEA- Malt extract agar, WEA- Wheat extracts agar, CEA- Compost extract agar, YPDA-Yeast extract potato dextrose agar, g-Gram, L-Litre.

Sliced potato was boiled in distilled water for 20-25 minutes till these become soft. Extract was filtered with a muslin cloth and add 20g dextrose and 20g agar powder to the filtrate over a hot plate by stirring. The final volume of the medium was adjusted to one liter by adding required amount of distilled water. The medium was taken in flask were plugged with non-absorbent cotton and sterilized in autoclave at 121°C (15lb/sq. inch pressure) for 30 minutes. Wheat grains were boiled in 1 liter distilled water for one hour. Filter the extract after 24 hour. Agar was added to the supernatant by stirring with a glass rod over a hot plate. The final volume of medium was adjusted to 1 liter by adding required amount of distilled water. The medium was then autoclaved as above. Malt extract medium was prepared by malt extracts 25g and Agar 20g was dissolved in 1liter warm distilled water. Compost extract agar medium was prepared by same method of wheat grain extract agar medium. Yeast Extract Potato Dextrose Agar (YPDA) was also prepared by PDA medium.

Maintaining temperature

In case of temperature effect was different strains (Delta, NBS-5, ICAF, A-15, and MC-465) of *Agaricus bisporus*. The inoculums were transferred in Petri-plates which poured by PDA in laminar flow. Then the inoculated Petri-plates took in incubator where different temperature were 15°C, 20°C, 25°C and 30°C and then observing the radial growth of fungi or mushroom.

Maintaining pH in medium

The pH was maintaining in potato dextrose agar media with the help of base and acids. Firstly the medium was made without agar and adjusted the pH (6, 7, 8 & 9) with the help of pH meter. After adjusting the pH mixed the agar-agar and then sterilized the medium in autoclave at 121°C (15lb/ sq. inch pressure) for 25-30 minutes. After autoclaved melt medium poured in sterilized petriplates in laminar flow and took for the solidification of media. After solidifying of medium in laminar flow then inoculate culture in Petri-plates and kept in incubator where temperature was 25°C.

Preparation of spawn: The spawn was prepared at Mushroom Research and Training Centre (MRTC) Pantnagar, using grains of wheat and by following standard method [10]. Healthy wheat grains were washed and boiled (grain: water, 1:25, w/v) to soften the seeds without damaging of the seed coat and the grains were allowed to dry on sieve overnight. Next day the mixing of boiled grains was done with calcium carbonate and calcium sulfate @ 3gm and 12gm per Kg wheat grains, respectively. Chemically mixed grains then placed in glass bottles and plugged with the help of nonabsorbent cotton. Prepared bottles of grains were sterilized by using autoclave (22 psi steam pressure, 121°C for 20 minutes). After sterilization bottles were shaken properly to avoid aggregation of grains. Next day, bits of 12-15 days old pure culture used for inoculation of grained bottles then bottles were shaken vigorously to spread the mycelium properly in bottle and after 10 days inoculated bottles were incubated at $25 \pm 1^\circ\text{C}$. It took 25 days for bottled grains to be entirely covered by mycelium. This is called master spawn. Master spawn was further used in preparation of commercial spawn.

Compost preparation: *Agaricus bisporus* (Lange) Sing. require a selective medium for its growth, therefore, synthetic compost was prepared by long method of composting according to formula given by [11].

Wheat straw - 1000 kg

Wheat bran - 150 kg

Urea - 18 kg

Gypsum - 35 kg

Wheat straw was spread over the cemented platform. Water was sprinkled over the straw by pipe and frequently turned by forks till sufficient moisture was absorbed. Wetting of straw was continued up to 48 hours. After two days, required quantities of different materials viz., urea and wheat bran except gypsum were thoroughly moistened with water and heap was made. The prepared mixture was filled in the rectangular blocks (moulds). While filling the blocks materials were slightly pressed on the sides and kept loose in the centre. The compost was decomposed by total seven turning and each turning was done at 3 days interval. Gypsum was mixed during 3rd turning and at each turning, water should be sprinkled to make up the loss of moisture content due to evaporation.

Spawning: Process of mixing spawn with compost is termed as spawning. The mixing of compost and spawn was done @ 1 kg grain spawn per quintal compost and spawned compost filled in polythene bags (60 x 60cm size) @ 10 Kg per bag.

Spawn run: The spawned bags were kept in the crop room for spawn run. Spawn run is the spread of mycelium of the mushroom throughout the compost. During spawn run, in crop room the temperature and relative humidity ranged between 20-24°C and 85-95%, respectively.

Preparation and treatment of casing mixture: By mixing of 2 years old FYM (Farm Yard Manure) and 2 years old spent mushroom compost in 1:1 ratio, casing mixture was prepared. Formaldehyde (4%) was used for the treatment of casing mixture. Then mixture was covered with polythene sheet for 48 hours.

Casing: After complete spawn run a layer of casing mixture was used to cover the surface of compost. The bags were opened and 5 cm thick casing mixture was applied to the surface of the spawn run compost. Uncased compost produces hardly any mushrooms [12].

Watering: Water was sprayed regularly 2 times per day after casing for good case run and growth of fruiting bodies.

Case run: Case run is the growth of mushroom mycelium through the casing mixture. The environmental conditions for case run were similar to spawn run.

Cropping and Harvesting

During cropping season (fruit body growth and development) temperature and relative humidity ranged between 16-20°C and 80-90%, respectively. Pinheads (initial of fruit body) matured within 3 - 4 days of its initiation. Mature fruiting bodies of the mushroom were harvested at button shaped stage. Mushrooms, after maturation, were harvested by holding the fruiting body between forefinger and thumb, and rotating in clockwise or anticlockwise direction and the soiled stem portion was cut with sharp edged knife.

RESULTS AND DISCUSSION

Effect of culture media

The results presented in Table 1 revealed that the studies of effect of different culture media on mycelia growth of strains (Delta, NBS-5, ICAF, A-15, and MC-465) of *A. bisporus*. Five culture media were tested for the radial growth and measured two days interval. The result showed the mycelial growth on MEA of all five strains Delta, NBS-5, ICAF, A-15, and MC-465 were obtained fast and full growth (9 cm) completed in 9,11,12,13 and 14 days respectively. The growth of mycelial in PDA medium was good performance in all strains of *A. bisporus* followed by the YPDA, CEA and WGEA culture media. The mycelial growth was slow on WGEA medium and full growth completed in 18 days in case of MC-465 strain. The similar works of [13] had been observed mycelial growth on different growth media and under different culture media conditions was investigated in 7 strains of edible fungi. Mycelial growth rates were investigated higher on WDA (wheat/dextrose/agar) medium than on PDA (potato/dextrose/agar) or MPA (malt/ Soya peptone/agar) media in all strains. The radial growth produced by various strains of *A. bisporus* was earlier studied by [14,15] for identification of homokaryons and heterokaryons in strains of *A. bitorquis* on the basis of growth rate.

Table 2. Effect of different culture media on mycelial growth of strains of <i>Agaricus bisporus</i>						
Sl No.	Contents of media	Medium				
		Delta	NBS-5	ICAF	A-15	MC-465
1	MEA	9	11	12	13	14
2	PDA	10	12	13	13	16
3	YPDA	12	12	13	14	16
4	WGEA	12	13	16	16	18
5	CEA	12	13	15	16	17
	S.Em.±	1.110555				
	CD 5%	3.276136				
	CV	5.674155				

Effect of temperature

The results presented in Table 2 revealed that the studies of effect of different temperature on mycelia growth of strains (Delta, NBS-5, ICAF, A-15, and MC-465) of *A. bisporus*. Four different temperatures were tested for the radial growth and measured two days interval. The result showed the mycelial growth of all five strains Delta, NBS-5, ICAF, A-15, and MC-465 on 25 °C was obtained fast and full growth (9cm) completed in 9, 11, 13, 14 and 14 days respectively, followed by the growth of mycelial at 20°C and 30°C the mycelial growth was slow for strain MC-465 and took 19 days for full growth. On 15°C mycelial growth shows less

effective and full growth of all the strains were completed in 13,15,16,18 and 17 days respectively. The temperature differs for mycelium growth on different strains of *A. bisporus*. Similar result given by [16], where it had been observed mycelium growth on different temperature, that investigated in vitro mycelium growth of *Agaricus bisporus* strains ABI05/03, ABI-06/04, ABI-04/02, ABI-06/05 and ABI01/01 was evaluated performed by means of measurements of four diameters of the colonies, every 48 hours, during 12 days of incubation in darkness under 20°C and 25°C, it was verified that: mycelium growth of *A. bisporus* is influenced by the temperature of incubation; temperature of 25° C was more favourable to the mycelium growth of all *A. bisporus* strains; under temperature of 20° C, the best growth was obtained with strains ABI-06/ 05 and ABI-01/01 and, under temperature of 25°C, strain ABI-01/01 showed significantly higher growth than all other strains.

Table 3. Effect of different Temperature on mycelial growth of strains of <i>Agaricus bisporus</i>						
Sl. No.	Temperature	Strains				
		Delta	NBS-5	ICAF	A-15	MC-465
1	15 °C	13	15	16	18	17
2	20 °C	10	11	14	15	18
3	25 °C	9	11	13	14	15
4	30 °C	12	14	16	16	19
	S.Em.± CD 5% CV	0.862812 2.545293 5.213142				

Effect of pH

The results presented in Table 3 revealed that the studies of effect of different pH on mycelia growth of strains (Delta, NBS-5, ICAF, A-15, and MC-465) of *A. bisporus*. pH were tested for the radial growth and measured two days interval. The result showed the mycelial growth on 8 pH of all five strains Delta, NBS-5, ICAF, A-15, and MC-465 were obtained fast and full growth.

Table 4. Effect of different pH on mycelial growth of strains of <i>Agaricus bisporus</i>						
Sl. No.	pH	Strains				
		Delta	NBS-5	ICAF	A-15	MC-465
1	6	13	14	15	18	19
2	7	12	13	15	16	17
3	8	11	12	14	16	16
4	9	12	13	14	17	18
	S.Em.± CD 5 % CV	0.881917 2.601654 5.137417				

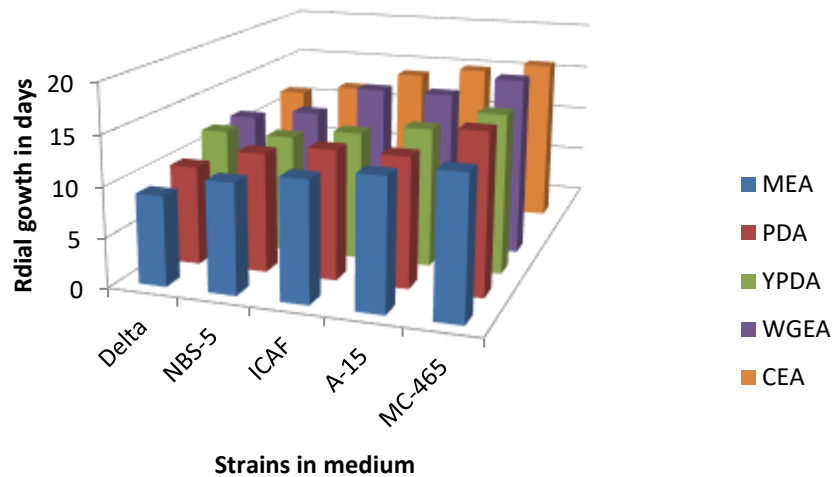


Fig. 1. Effect of culture media on different strains of *A. bisporus*

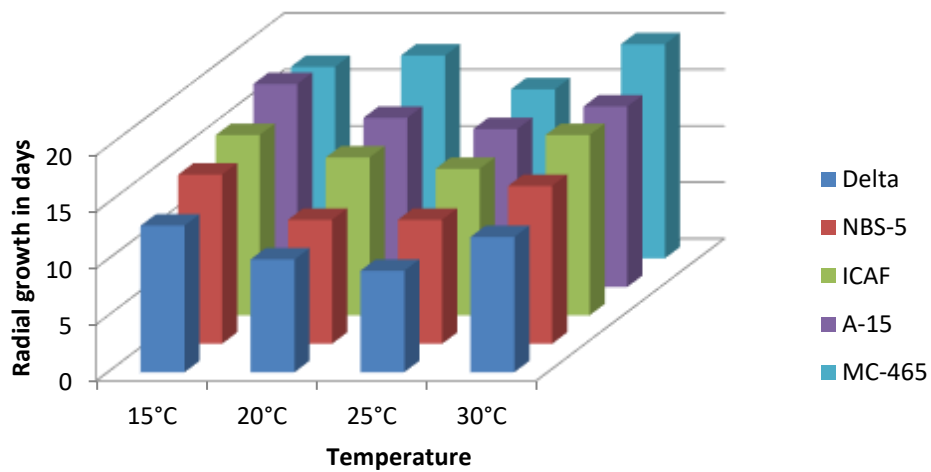
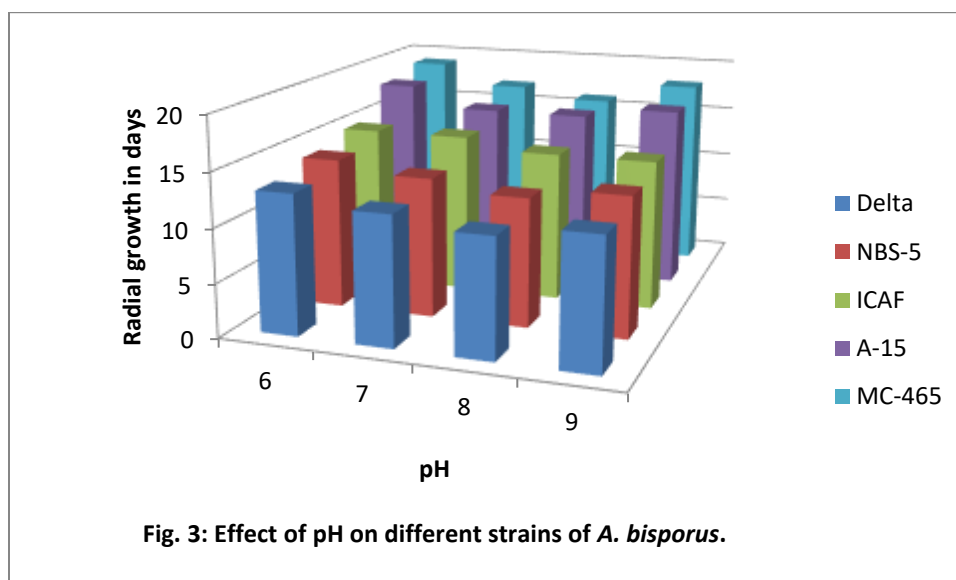


Fig. 2. Effect of Temperature on different strains of *A. bisporus*



The growth of mycelial in 8 pH was good performance in all strains of *A. bisporus* followed by the 7 pH. The mycelial growth was much slow on 6 pH and full growth completed in 19 days in case of strain MC-465. Similar works giving by [17] had been observed mycelium growth on different pH, which investigated the suitable pH (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5), for the growth of button mushroom. For studies on the suitable pH, the fungi were incubated at $24 \pm 1^\circ\text{C}$. The best pH level for fungal growth was 6.0. The maximum mycelial dry weight (100 mg) was obtained at pH 6.0, followed by pH 5.5 with 86.66 mg mycelial dry weight.

Evaluation of high yielding strains of *Agaricus bisporus* (Lange) Sing

An experiment was carried out for five strains of *Agaricus bisporus* were evaluated for yield performance given in table 1. The highest yield were obtained from strain Delta with an average 20.28 kg/100 kg of compost followed by NBS-5 (20.05 kg/q) further other strains, ICAF (17.11 kg/q), A-15 (16.28 kg/q), and MC-465 (14.11 kg/q) gave minimum yield over all strains. Maximum number of fruiting body were produced in strain Delta with an average 104.00 per ten kilogram of compost followed by strain NBS-5 (99.00), ICAF (92.0) , A-15 (88.00) however minimum number of fruiting body was produced in MC-465 with an average of 86.0 per ten kilogram of compost. The average fruiting body weight was observed to be significantly higher in case of strain NBS-5 with an average fruit body weight of 20.50 gm followed by strain Delta (19.50 gm), ICAF (18.50 gm) and A-15 (18.50 gm), and minimum of MC-465 (16.50 gm).

Table 5. Yield performance of different strains of *Agaricus bisporus*(Lange) Sing.

Sl. No.	Strains	Number of fruiting body /10 kg of compost*	Yield* (Kg / q of compost)
1	Delta	104.00	20.28
2	NBS-5	99.00	20.05
3	ICAF	92.50	17.11
4	A-15	88.00	16.28
5	MC-465	85.50	14.11
	S.Em.±	0.48	0.07
	CD 5%	1.52	0.24

The present study showed that confirmative results with finding of [18]. had been observed yield potential of different six strains of *Agaricus bisporus* (S-649, S-46, U-3, Pant-52, and Pant-215) were evaluated for yield performance in terms of the number and weight of fruiting bodies at room temperature. The highest number of fruiting bodies (2161/100kg of compost) was recorded for U-3 followed by S-649 and Pant -215.

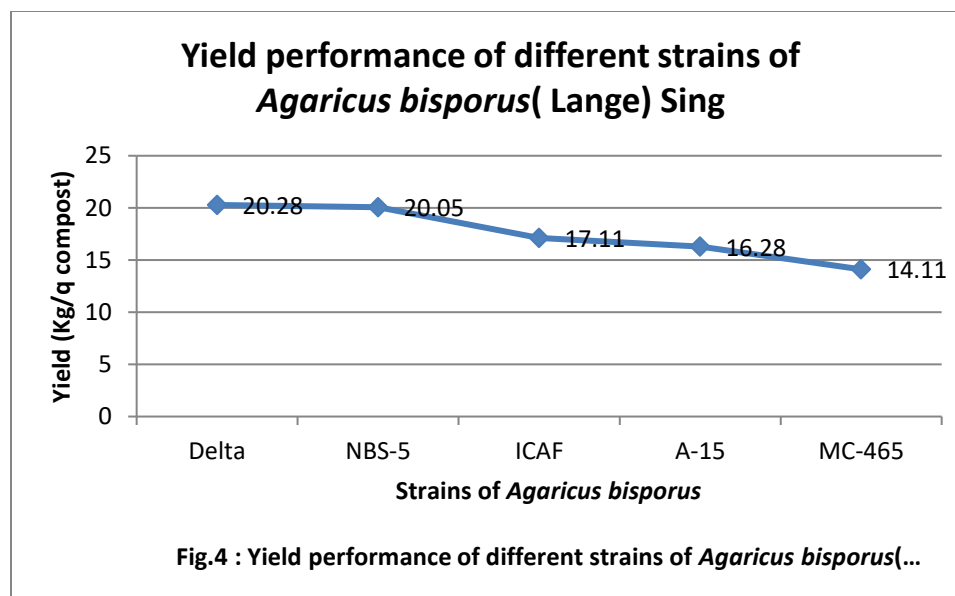


Table 6. Morphological characteristics of different strains of *Agaricus bisporus* (Lange) Sing

Strains	Avg. body weight. (gm)	Avg. body length (mm)	Avg. pileus diameter (mm)	Avg. thickness (mm)	Avg. pileus weight. (mm)	Avg. Stripe length (mm)
Delta	19.00±0.29	37.00±0.29	38.00±0.58	18.00±0.35	15.50±0.29	20.00±0.40
NBS-5	21.00±0.35	26.00±9.41	40.00±0.35	19.00±0.12	17.00±0.29	19.00±0.35
ICAF	18.00±0.17	39.00±0.29	37.00±0.23	16.50±0.29	14.50±0.87	21.00±0.58
A-15	17.00±0.46	38.00±0.58	35.00±0.46	15.00±0.40	14.00±0.40	22.00±0.52
MC-465	16.00±0.52	40.67±0.83	34.00±0.75	14.00±0.46	13.00±0.12	26.00±0.64
S.Em.±	0.37	4.23	0.50	0.34	0.46	0.50
CD 5%	1.19	13.33	1.59	1.08	1.47	1.60

Morphological characteristics of different strains of *Agaricus bisporus* (Lange) Sing

Morphological characteristics of different strains were given in table. 2 that maximum average body weight was observed in NBS-5 (21.0 gm) followed by Delta (19.00 gm), ICAF (18.0 mm), A-15 (17.0 gm) and minimum average body weight was observed in MC-465 (26.00 mm), maximum average body length was observed in MC-465 (40.67 mm) followed by ICAF (39.00 mm), A-15 (38.0 mm), Delta (37.0 mm) and minimum average body length was observed in NBS-5 (16.00 gm), whereas maximum average pileus diameter was observed in NBS-5 (40.00 mm) followed by Delta (38.00 mm), ICAF (37.00 mm), A-15 (35.0 cm), however minimum width of pileus was observed in MC-465 (34.00 mm). Maximum average thickness of cap was observed in NBS-5 (19 mm) followed by Delta (18.0 mm), ICAF (16.50 mm), A-15 (15 mm), and minimum in MC-465 (14.0 mm) moreover average maximum weight of pileus was observed in NBS-5 (17.0 mm) followed by Delta (15.50 mm), ICAF (14.50 mm), A-15 (14.00 mm), and minimum were taken in MC-465 (13.00 mm). Maximum average stripe length was observed in MC-465 (26.0 mm) followed by A-15 (22.00 mm), ICAF (21.00 mm), NBS-5 (19.00 mm) and minimum average stripe length was observed in Delta (20.00 mm).

Thus, it can be conclude from above results that maximum average body length and stripe length was observed in MC-465 but have lowest body weight, pileus diameter, thickness and stripe length. Maximum body weight was observed in NBS- 5 strain of white button mushroom with maximum pileus diameter, thickness and pileus weight but have lowest body and stripe length. This results showed that confirmative results with finding of [19]. reported that Cultural and morphological variations of seven strains of *Agaricus bisporus* (CM-1, CM-5, CM- 10, Delta, S-130, S-140 & X-13) and a strain (NCB-13) of *Agaricus bitorquis* were studied on the basis of growth on MEA medium, synthetic compost and casing soil, and different characters of their fruiting body. Strain S-130 showed maximum growth on MEA medium, compost and casing soil whereas NCB-13 produced higher fruit body weight and stipe width. Lesser stipe length and maximum pileus diameter were observed in strain Delta. Maximum pileus thickness was observed in CM-5. [20] studied the growth behavior and yield potential of different strains (U-3, Delta, A-15, NCS-459, NCS-465 and Portbella) and found that the average fruiting body weight was observed to be significantly higher in case of strain Portbella (12.00 gm). In the growth behavior, U-3 gave the best performance followed by the other strains. Maximum average length, width of stalk and average width, length of cap was observed in Portbella followed by A-15, U-3, Delta, NCS-459 and minimum was observed in NCS-465.

CONCLUSION

Nowadays, mushrooms are popular valuable foods because they are low in calories, carbohydrates, fat, and sodium, they are also cholesterol-free. Besides, mushrooms provide important nutrients, including selenium, potassium, riboflavin, niacin, vitamin D, proteins, and fiber. All together with a long history as food source, mushrooms are important for their healing capacities and properties in traditional medicine. There are different kinds of edible mushrooms cultivated all over the world. Experimental findings of comparative evaluation yield performance of different strains of *Agaricus bisporus* (white button mushroom) in-vitro and in-vivo conditions including its performance in culture media, ph and temperatures for mycelial growth which will helps to create the conditions for researchers and farmers to grow the white button mushrooms by providing such artificial condition to obtain the high its yield and it is also concluded that MEA medium temperature 25 °C and pH 8 gives the fast growth of mycelium and appeared to better in maintenance of culture and sub-culture. Among various strains of *Agaricus bisporus*, Delta produced maximum

mushroom yield as compared to MC-465, A-15, ICAF and NBS-5 strains and maximum number of fruit bodies were produced in strains NBS-5, whereas minimum fruit body was observed in ICAF. The average fruiting body weight was observed to be significantly higher in case of strain NBS-5. In the growth behavior, Delta gave the best performance followed by the other strains. Maximum average length, width of stalk and average width, length of cap was observed in MC-465 followed by NBS-5, Delta, A-15 and minimum was observed in ICAF. This investigation will also help for selection of culture media, pH and temperature for the maintenance of genetic materials and cultivation at commercial level.

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