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Bangladesh Journal of Mushroom

Volume 7 Number 2 December 2013

Contents

1. **Akhter Jahan Kakon, Ruhul Amin, Md. Bazlul Karim Choudhury, Tasnim Farzana, Md. Nur Hossain and Nirod Chandra Sarker**- Effect of Gamma Radiation on Microbial Load of Mushroom Powder 1-6

2. **Manirul shaheen, Md. Mustafizur Rahman, Nirod Chandra Sarker, Md. Mominul Hoque, Abdus Salam Khan and Akhter Jahan Kakon**- Yield Performance of Different Strains of *Auricularia auricula* Mushroom in Winter and Summer Season in Bangladesh 7-12

3. **Nirod Chandra Sarker, Md. Bazlul Karim Choudhury, Abdus Salam Khan, Md. Mominul Hoque, Bimol Chandra Dey and Manirul shaheen**- Effect of Sterilization Practices of Contaminated Mother Culture on Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*) 13-19

4. **Sabiha Azmi Sanjuti, Nuhu Alam, Nirod Chandra Sarker, Md. Bazlul Karim Choudhury, Ruhul Amin and Akhter Jahan Kakon**- Cultural Conditions for Vegetative Growth and Random Amplification of Polymorphic DNA in Different Strains of *Pleurotus sajor-caju* 21-28

5. **Zeenia Afsary, Nuhu Alam, Nirod Chandra Sarker, Ruhul Amin and Akhter Jahan Kakon**- Morphological Characterization of Commercially Cultivated Oyster Mushrooms in Bangladesh 29-36

6. **Md. Bazlul Karim Choudhury, Md. Shahdat Hossain, Md. Masud Hossain, Akhter Jahan Kakon, Mohammed Anwarul Kabir Choudhury, Nashir Uddin Ahmed and Taufiqur Rahman**- *Pleurotus ostreatus* improves Lipid Profile of Obeys Hypertensive Non-diabetic Males 37-44

7. **Tasnim Farzana, Suman Mohajan, Md. Mominul Hoque and Nirod Chandra Sarker**- Comparison of Proximate Nutrients Composition in Local Market and Soya Mushroom Biscuits 45-51

8. **Zeenia Afsary, Nuhu Alam, Md. Bazlul Karim Choudhury, Manirul Shaheen, Tahera Binte Mujib and Nirod Chandra Sarker**- Nutrient and Mineral Content of Different spp. of Oyster Mushroom 53-58

9. **Shailendra Mohon Singha, Nuhu Alam, Nirod Chandra Sarker and Manirul Shaheen**- Influence of Physicochemical Conditions of Culture Medium on Invitro Mycelial Growth in Different Strains of *Volvariella volvaceae* 59-66

10. **Tahera Binte Mujib, Nirod Chandra Sarker, Md. Bazlul Karim Choudhury, Md. Kamrul Hassan, Abdus Salam Khan, Ruhul Amin and Akhter Jahan Kakon**- Organoleptic Evaluation of Mushroom Ketchup 67-75

Bangladesh J. Mushroom. 7(2): 1-6. 2013 (December)

Effect of Gamma Radiation on Microbial Load of Mushroom Powder

Akhter Jahan Kakon, Ruhul Amin, Md. Bazlul Karim Choudhury[1], Tasnim Farzana[2], Md. Nur Hossain[2] and Nirod Chandra Sarker
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

An experiment was conducted to study the role of gamma irradiation to eliminate the pathogenic microbes. Among the three analyzed powder samples with different irradiation treatments, reishi mushroom powder contained the highest microbial load (log_{10} value 6.301 or 2.0×10^6 cfu/g) and the lowest microbial load (log_{10} value 5.602 or 4×10^5 cfu/g) was found in oyster mushroom powder at basal condition. The load of total plate count decreased while the samples were treated with higher doses of irradiation. In reishi mushroom powder, total plate count at 2.5 kGy was log value 4.99 and it was decreased gradually at 5.0-10.0 kGy;. The same type of result obtained from oyster and shiitake mushroom powder at 7.5 kGy irradiation. In case of total coliform, fecal coliform, *E. coli* and salmonella, the reishi mushroom powder sample became safe after treated with 7.5 kGy irradiation. The total coliform (>1100 MPN/g) was found at 0 kGy in oyster mushroom powder and Salmonella was not found after treated with 5.0 kGy irradiation. The total coliform (>1100 MPN/g) was also found at 0 kGy in shiitake mushroom powder while it was not found after irradiation with all the treatments. However, conducting such type of experiments would enable us to decide as to what kind of treatment should be adopted for eliminating the pathogenic contaminants from mushroom powder as well as to ensure safety of our foods.

Key words: Gamma irradiation, Microbial load, Plate count, Salmonella, Contaminants.

INTRODUCTION

Approximately 25% of all food products are lost after harvesting due to insects, vermin, and spoilage. Currently, a significant number of chemicals are used on food products for preserving/preventing insect losses. In developing countries where handling, transportation and storage conditions are less adequate than in the United States these losses are significantly greater. In addition, forborne diseases caused by pathogenic bacteria result in an estimated 9,000 deaths each year and 24 million cases of illness annually in the United States alone. Irradiation has the potential to reduce both loss of produced food and food borne illness.

Irradiation can destroy pathogenic organisms, as well as substantially decrease spoilage organisms in animal foods. FDA approved use of irradiation in pork to control *Trichinella spiralis* in 1985. In 1990, the FDA approved irradiation to control illness-causing

[1] OSD, DG Health, Mohakhali, Dhaka-1212, Bangladesh; [2] Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dr. Kudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh.

microorganisms in poultry. FDA also approved use of irradiation to control fresh and frozen red meat in 1997. Irradiation is the only known technique to completely eliminate Escherichia coli 0157:H7 in raw meat. Irradiation also significantly reduces levels of Salmonella, Listeria and Campylobacter in raw meat products.

A potentially attractive alternative for eliminating organism is exposure to ionizing radiation. Previous papers have suggested this method is highly effective in inhibiting physical changes associated with postharvest deterioration and maintaining a fresh product appearance (Kader, 1986). Song *et al.* (2006) concluded that irradiation treatments of carrot and kale juice improve the microbiological safety with maintaining or even enhancing the anti-oxidative activity. Food processing by employing radiation is well established as a physical, non-thermal mode of food preservation (cold-pasteurization) that processes foods at or nearly at ambient temperature. Irradiation of food products causes minimal modification in the flavor, color, nutrients, taste, and other quality attributes of food. However, the levels of modification (in flavor, color nutrients, taste etc.) might vary depending on the basic raw material used, irradiation dose delivered, and on the type of radiation source employed (gamma, X-ray, UV, electron beam) (Bhat and Sridhar, 2008; Bhat *et al.*, 2007; Mexis *et al.*, 2009). Gammas are short wave length, high energy photons, and have deep penetrating power. Gamma rays come from spontaneous disintegration of radioactive nuclides (Cobalt 60 or Cesium 137) as their energy source. International agencies including IAEA, FAO and WHO concluded that irradiation of any food commodity up to a dose of 10 kGy exhibits no health risks (Diehl, 2002). However, the aim of this investigation was mainly directed to the effect of different doses of gamma-irradiation on the microbial load of mushroom powder.

MATERIALS AND METHODS

The present study was to evaluate the microbiological quality of the irradiated mushroom powder. The experiment was conducted at microbiology laboratory of National Mushroom Development and Extension Centre (NAMDEC), Sobhanbagh, Savar, Dhaka.

Collection of Samples: Samples of mushroom powder were collected from local sales centre. Radiation treatments were given at Institute of Food and Radiation Biology division in Bangladesh Atomic Energy Commission, Dhaka. Different doses of radiation treatments (kilo Gray) were 0 (control), 2.5, 5.0, 7.5 and 10. The features of collected samples are shown in Table 1.

Table 1: General description of collected samples

Sl. No.	Samples derived from	Samples condition when it was collected	
		Package	Weight
1.	Oyster Mushroom	Plastic jar	50g
2.	Reishi Mushroom	Plastic jar	50g
3	Shiitake Mushroom	Plastic jar	50g

Total viable count (TVC): Twenty five grams (25 g) of each sample was diluted in 225 ml of sterile distilled water (diluents) and mixed vigorously by shaking. 1ml of the

resultant mixture was aseptically transformed to 9 ml of distilled water in a test tube. The dilution was continued serially until the required dilution was attained. One milliliter (1 ml) of each dilution was inoculated into a sterile Plate Count Agar (PCA). This action was carried out under sterile aseptic conditions. Immediately, inoculated samples and agar medium was mixed thoroughly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar be solidified. Solidified petri dishes were kept in invert position and incubated promptly for 24 ± 2 h at 35±2^{0}C. Then total viable counts were counted.

Presumptive test of total coliform (TC), fecal coliform (FC) and *E. coli*: 25 g sample was weighed and added into 225 ml of Butterfield's phosphate-buffered water and decimal dilutions were prepared. Number of dilutions was prepared depending on anticipated coliform density. All suspensions were shaked 25 times in arc for 30 cm or vortex mix for 7 second. 1 ml of each dilution was transferred to 3 McCartney bottles containing LST broth and inverted Durham's tubes. Inoculated McCartney bottles were incubated at 37^{0}C for 24h. Gas production were examined and recorded in tubes. Gas-negative tubes were re-incubated for an additional 24 h and examined (BAM, 1998).

Confirmed test for coliforms: A loop-full of suspension was transferred into a tube of Brilliant Green Bile Broth (BGBB) from each gas positive Lauryl Sulphate Broth (LST) tube and pellicle was being avoided if present. BGBB tubes were incubated at 37^{0}C and examined for gas production at 24h. Then Most Probable Number (MPN) of coliforms was calculated based on proportion of confirmed gas positive tubes for 3 consecutive dilutions using MPN charts.

Confirmed test for fecal coliforms and *E. coli*: From each gas positive LST tube of the presumptive test, a loopful of each suspension was transferred to tube containing BGBB and EC broth. Inoculated tubes were incubated at 44^{0}C for 24 h and examined for gas production. When negative, reincubated and examined again at 48h. Observing the gas production, the number of fecal coliform and *E. coli* were calculated using MPN charts (BAM, 1998).

Isolation of Salmonella: Twenty five grams (25g) sample was weighed and homogenated in 225 ml Buffered Peptone water and incubated at 35^{0}C for 24 h. Three (03) mm loop full (10μl) of incubated broth medium was streaked on bismuth sulfite (BS) agar and xylose lysine desoxycholate (XLD) agar, and incubated plates for 24h at 37^{0}C. The presence of colonies were examined that may be Salmonella.

Identification of Salmonella: *Triple Sugar Iron (TSI) agar test:* Each presumed-Salmonella culture was inoculated into tubes of TSI with sterile needle by stabbing and streaking and again inoculated Lysine Iron Agar (LIA) slant in the same manner without flaming the loop. Salmonella gave positive reaction in TSI slants and produced H_2S as shown by the blackening of the media. The butt of the media was turned to yellow.

LIA test: In LIA slant, the butt color was purple for positive test and was considered a distinct yellow LIA butt as negative result.

Simmons citrate agar test: Containing growth from unclassified TSI agar slant was inoculated into the medium using needle by streaking slant and stabbing butt. It was incubated 96h at 37^0C. The results were considered positive when color of the medium changed from green to blue and when there was no or very little growth and no change of color, the result was considered as negative. Most cultures of Salmonella were citrate-positive.

RESULTS AND DISCUSSION

The results of total viable count (TVC), colifrom, fecal colifrom, *E.coli* and salmonella obtained from different mushroom products are summarized below:

Among the three analyzed powder samples with different irradiation treatments, reishi mushroom powder contained the highest microbial load ($\log_{10}$ value 6.301 or 2.0×10^6 cfu/g) and the lowest microbial load ($\log_{10}$ value 5.602 or 4×10^5 cfu/g) was found in Oyster mushroom powder at 0 kGy. According to the BDS 1829. 2010, the acceptable microbial load is 1000 cfu/g. The fig.1 shows three analyzed mushroom powder sample are not acceptable in quality, if it does not undergoes any further sterilization treatment.

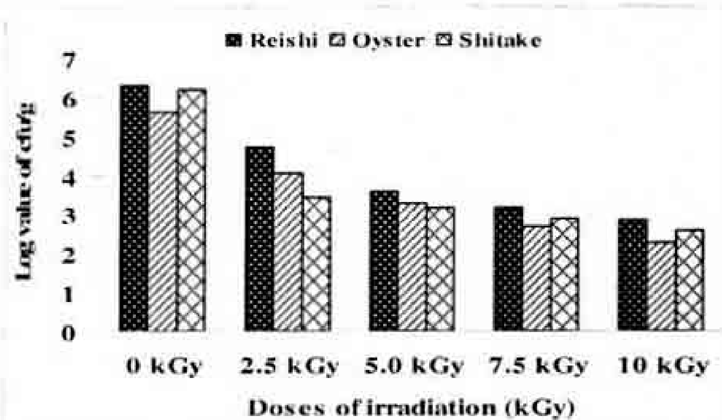

Fig. 1. Total Viable Count (Log value) of powdered mushroom samples.

The figure 1 showed that the load of total plate count decreased while the samples were treated with higher doses of irradiation. In reishi mushroom powder, total plate count at 2.5 kGy was log value 4.99 and it was decreased gradually at 5.0, 7.5 and 10.0 kGy; the figures were log value 3.602, 3.176 and 2.845 respectively. It showed that only 10 kGy irradiation dose was suitable for becoming acceptable total plate count of Reishi mushroom powder (BDS 1829, 2010). In case of oyster mushroom powder, the acceptable total plate count (log value 2.699) obtained at 7.5 kGy. Same type of result was observed (log value 2.903) in shitake mushroom powder at 7.5 kGy irradiation.

Considering total coliform, fecal coliform, *E. coli* and salmonella, the reishi mushroom powder sample became acceptable after treated with 7.5 kGy irradiation. It was

remarkable that the fecal coliform and *E. coli* were in acceptable level (<3) after all the treatments including control. Salmonella persisted up to 5.0 kGy irradiation (Table 2).

Table 2. Irradiated Reishi mushroom powder of coliform, fecal coliform, *E.coli* and salmonella

Reishi Mushroom Powder		Test Results			
Irradiation Doses	Total Plate Count (cfu/g)	Total Coliform (MPN/g)	Fecal Coliform (MPN/g)	*E.coli* (MPN/g)	*Salmonella*
Control (0 kGy)	2.0×10^6	23	<3	<3	Present
2.5 kGy	5×10^4	15	<3	<3	Present
5.0 kGy	4×10^3	9.2	<3	<3	Present
7.5 kGy	1.5×10^3	<3	<3	<3	Absent
10 kGy	7×10^2	<3	<3	<3	Absent

The total coliform >1100 MPN/g was found at 0 kGy in oyster mushroom powder. It was decreased to 3.6 MPN/g after treated at 2.5 kGy and it was absent or <3 at other treatments. The level of fecal coliform and *E. coli* were absent or <3 at all the treatments including control. Salmonella became absent after treated with 5.0 – 10 kGy irradiation.

Table 3. Irradiated oyster mushroom powder of coliform, fecal coliform, *E.coli* and salmonella

Oyster Mushroom Powder		Test Results			
Irradiation Doses	Total Plate Count (cfu/g)	Total Coliform (MPN/g)	Fecal Coliform (MPN/g)	*E.coli* (MPN/g)	*Salmonella*
Control (0 kGy)	4×10^5	>1100	<3	<3	Present
2.5 kGy	1.2×10^4	<4	<3	<3	Present
5.0 kGy	2×10^3	<4	<3	<3	Absent
7.5 kGy	5×10^2	<4	<3	<3	Absent
10 kGy	2×10^2	<4	<3	<3	Absent

Total coliform >1100 MPN/g was found at 0 kGy in shiitake mushroom powder which was declined to acceptable limit (< 4) after irradiation with all the treatments. It was noticeable that after all the treatments (including control) the presence of fecal coliform and *E. coli* were in acceptable limit (< 3). Salmonella persisted after the treatment with 2.5 kGy which became absent at 5.0 kGy and onward (Table 4).

Results of TVC demonstrated that all the powder mushroom samples contain higher cfu/g at 0 kGy. Obtained findings are inconsistent with others (Kamal *et al.*, 2010; Van-Kampen *et al.*, 1998; Gilbert *et al.*, 1996; Patricia and Azanza, 2005). On the other hand, coliform, fecal coliform, *E. coli and salmonella sp.* also found comparatively higher in mushroom powder at 0 kGy. Burton (1989) found that bacterial counts were consistently lower depending on processing and storage conditions. However, contaminated mushrooms or mushroom based products require minimal processing to consume. Conducting such type of experiments would enable us to decide as to what kind of

treatment should be adopted for eliminating the pathogenic contaminants from mushroom powder as well as to ensure safety of our foods.

Table 4. Irradiated Shiitake mushroom powder of coliform, fecal coliform, *E. coli* and salmonella

Shiitake Mushroom Powder		Test Results			
Irradiation Doses	Total Plate Count (cfu/g)	Total Coliform (MPN/g)	Fecal Coliform (MPN/g)	*E.coli* (MPN/g)	*Salmonella*
Control (0 kGy)	1.6×10^6	>1100	<3	<3	Absent
2.5 kGy	2.7×10^3	<3	<3	<3	Absent
5.0 kGy	1.5×10^3	<3	<3	<3	Absent
7.5 kGy	8×10^2	<3	<3	<3	Absent
10 kGy	4×10^2	<3	<3	<3	Absent

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Yield Performance of Different Strains of *Auricularia auricula* Mushroom in Winter and Summer Season in Bangladesh

Manirul shaheen, Md. Mustafizur Rahman[1], Nirod Chandra Sarker, Md. Mominul Hoque, Abdus Salam Khan and Akhter Jahan Kakon
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

The yield performance of different strains of ear mushroom in two seasons was investigated. The highest mycelial growth rate was observed when Au 4 cultivated in summer season while the least growth rate was recorded when Au 3 grown in winter season. Among the different strains and two season, the highest yield (248.0 g/packet) was obtained from Au 3 in winter season which was statistically similar to Au 4 (240.5 g/packet), Au-3 (237.8 g/packet) in summer season while the lowest yield (144.8 g/packet) was obtained from Au 5 in summer season. The present results revealed that in the summer season most of the strains gave higher yield in first flash compared to other. On the other hand in winter season most of the strains gave higher yield in second flash.

Keywords: *Auricularia auricula,* Season, Strain, Yield, Summer, Winter.

INTRODUCTION

Mushrooms of the genus *Auricularia* commonly known as wood ear mushrooms are edible fungi which have been domesticated for cultivation in different parts of the world (Kirk *et al.*, 2001). Currently, the genus *Auricularia* is the fourth most important cultivated mushrooms after *Agaricus*, *Lentinula* and *Pleurotus*. It is the most suitable mushroom species to cultivate in tropical and subtropical regions of the world (Zoberi, 1972 and Well, 1984). The rapid increase in domestication of this genus from the wild is attributed to its nutritional and medicinal properties (Chang and Miles, 2004). Nutritionally, its basidiocarps contain high protein levels (about 30% of dry weight) and essential elements such as vitamins, minerals and polysaccharides (Chang and Miles, 2004). The polysaccharides have been found to have antitumor, antiviral, antibacterial and antiparasitic properties (Ukai *et al.*, 1983; Yang *et al.*, 2002). Zervakis *et al.* (2001) reported that understanding the nutritional and physiological preferences of mushroom mycelia is essential to its domestication.

Auricularia mycelium growth and mushroom development are clearly influenced by environmental factors. *Auricularia* mushrooms are moderate temperature fungi. The temperature range for mycelial growth is 6-36^{0}C with the most suitable temperature 22-30^{0}C. Below 5^{0}C and above 38^{0}C, mycelial growth is restrained, but it has a strong tolerance to short-term cold and heat. The evolution and development of the fruit body

[1] Planning wing, Ministry of Agriculture, Secretariat, Segunbagicha, Dhaka.

occurs between 15-27^{0}C, but the best range is 20-24^{0}C. At low temperatures, growth and development of *Auricularia* mushrooms is slow but the mycelium is strong and the fruit body blacker and thicker, whereas at higher temperatures, the mycelium and fruitbody are weaker and thinner. At higher temperatures and air humidity, wood ear softening will appear. Air humidity of 90-95% is suitable for the formation and appearance of fruit bodies. Below 80% humidity, the fruit body dries out and becomes shriveled, and its growth and development stop. Light plays an important role in fruit body formation and the mushroom requires not only a quantity of diffused light but certain directly radiated light. The fruit body thus formed is black and strong. A light intensity of 500 lux is preferable for fruit body formation. Fruit body formation does not occur in the dark. Mycelial growth is normal under dark conditions but diffused light has a facilitating action (Luo, 1993).

So, it is very important to develop a commercial cultivation system of this mushroom. The most common practice of this mushroom cultivation in different countries is wood log cultivation. But due to the scarcity of suitable logs, the cultivation practice of this mushroom shifted from log to bag culture and is gaining popularity. In case of bag cultivation, types of substrates are most important for fructification. *Auricularia auricula* can be cultivated on the sawdust which may originate from different kinds of trees. A successful synthetic cultivation has been reported on solid substrates, utilizing sawdust and agricultural wastes as the main media components (Triratana *et al.*, 1991 and Malarvizhi *et al.*, 2003).

The present investigation was under taken to find out the yield potential of *Auricularia auricula* in winter and summer season.

MATERIALS AND METHOD

This experiment was conducted at the National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh during October to May 2013. In this experiment three different strains *viz.* Au 3, Au 4, Au 5 were selected and grown in two seasons viz. summer and winter.

Spawn packet preparation, inoculation and incubation: Sawdust spawn packets of 500g size were prepared, inoculated and incubated following the procedure that developed and explained by Sarker *et al.* (2007).

Culture condition: After completion of mycelium running, spawn packets were opened by "D" shaped cut on the shoulder at both the sides of the packet and removed the sheet. Then the packets were transferred to the culture room at 25-30^{0}C temperature and 85-95% relative humidity. Water was sprayed 4-5 times per day to maintain the temperature and relative humidity.

Experimental design, data collection and analysis: The experiment was laid out in completely randomized design (CRD) with 4 replications. Data on days required to

completion of mycelium running, days required from opening to primordia initiation, days required from opening to first harvest, days required from opening to second harvesting, yield of first flush, yield of second flush, number of effective fruiting body/packet, weight of individual fruit body (g), yield (g/ packet) were collected and analyzed following Gomez and Gomez (1984) using MSTAT-C computer programme. Means were separated by Duncan's Multiple Range Test (DMRT) using the same computer programme.

RESULTS AND DISCUSSION

Days required to completion of mycelium running (DRMR): Days required to completion of mycelium running of *Auricularia auricula* was significantly influenced by different strains in two seasons (Table 1). The highest days required to completion of mycelium running (35.25 days) was observed when Au 3 cultivated in winter season which was statistically similar to Au 4 in winter season (34.50 days). The lowest days required to completion of mycelium running (30.50 days) was observed when Au-4 cultivated in summer season. In the experiment it was observed that in summer season the mycelium running of spawn packets was faster than that of winter season. These results are in agreement with Yesmin *et al.* (2010) who observed that the days required completing mycelium running for *Auricularia polytricha* on different substrates ranged from 16.75 to 30.50 days.

Days required from opening to primordia initiation (DROPI): No significant difference was observed in DROPI among the different strains in the same season but in the different season there was a significant variation. The DROPI ranged from 22.50 to 31.50 (Table 1). The lowest DROPI (22.50 days) was found in Au 3 in winter season which was statistically similar to Au 4 in winter season (23.75 days). The highest DROPI (31.50) was found in Au 3 in summer season which was statistically similar to Au 5 in winter season (31.50 days). The results are in agreement with Yesmin *et al.* (2010) who observed that the days required from opening to primordia initiation for *Auricularia polytricha* on different substrates ranged from 25.75 to 28.50 days.

Days required from opening to first harvest (DROFH): Significant difference was observed in DROFH among the different strain in the different season. The maximum DROFH (45.75 days) was recorded in Au 5 in winter season and it was minimum (40.50 days) in Au 4 at summer season (Table 1). In this parameter it was observed that the summer season gave first flush than the winter season.

Days required from opening to second harvesting (DROSH): Significant difference was observed in DROSH among the different strain in the different season. The maximum DROSH (61.25 days) was recorded in Au 4 of winter season and it was minimum (56.50 days) in Au 4 at summer season (Table 1). In this parameter it was observed that the second flush gave faster than the time consume of first flush.

Table 1: Effect of seasons on growth of different strains of *Auricularia auricula*

Strain	Seasons	Days required to completion mycelium running	Days required from opening to primordia initiation	Days required to first harvest	Days required to second harvest
Au – 3		35.25a	22.50c	43.50b	59.00ab
Au – 4	Winter	34.50a	23. 75bc	42.50bc	61.25a
Au – 5		32.50b	25.25b	45.75a	61.00a
Au – 3		30.75b	31.50a	41.00cd	57.00b
Au – 4	Summer	30.50b	30.50a	40.50d	56.50b
Au – 5		31.50b	31.50a	41.00cd	57.00b
CV (%)		**3.94**	**4.81**	**2.93**	**2.95**

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

Number of effective fruiting body/packet: The number of effective fruiting body was non significant in summer season but significant in winter season (Table 2). The highest NEFB (23.25) was observed when Au 3 cultivated in summer season which was statistically similar to Au 4, Au 5 in summer season and the lowest number of fruiting bodies (14.25) was found when Au 5 in winter season. Yesmin *et al.* (2010) observed that the number of effective fruiting body of *Auricularia polytricha* on different substrates ranged from 14.25 to 25.75.

Weight of individual fruiting body (WIFB): The WIFB in different treatments ranged from 10.00g to 13.25g (Table 2). The highest WIFB (13.25g) was observed in Au 4 in winter season followed by Au 3 in winter season. The lowest WIFB (10.00g) was observed when Au 5 cultivated in summer season. This result is differed with Yesmin *et al.* (2010) who observed that the weight of individual fruiting body of *Auricularia polytricha* on different substrates ranged from 4.35 to 5.78g.

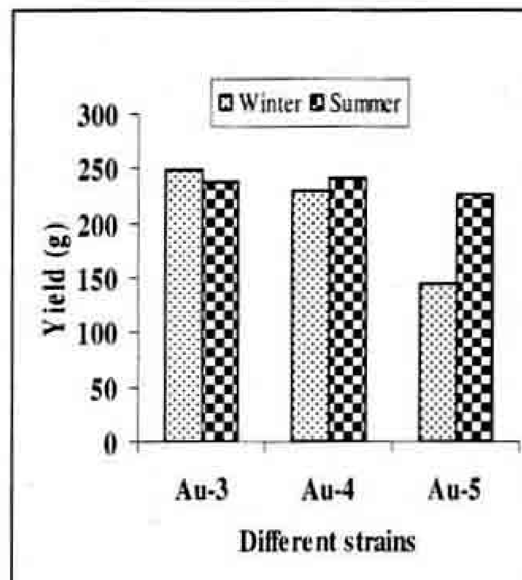

Fig. 1. Comparison between two seasons of different strains of *Auricularia auricula* on the yield.

Yield: Significant difference was observed on yield of different strains of *Auricularia auricula* mushroom in two seasons (Fig. 1). The highest yield (248.0g/packet) was obtained from Au 3 in winter season which was statistically similar to Au 4 (240.5 g/packet) and Au 3 (237.8g/packet) in summer season while the lowest yield (144.8 g/packet) was observed in Au 5 in winter season. Yesmin *et al.* (2010) observed that the range of yield 61.25 to 137.30 g/packet for *Auricularia polytricha* on different substrates.

Yield of first flush: Significant difference was observed in YFH among the different strain in the different season (Table 2). The maximum YFH (187.0g/packet) was recorded in Au 3 at summer season and it was minimum (86.50g/packet) in Au 5 at winter season. In this parameter it was observed that in the summer season most of the strain gave higher yield in their first flash but in the winter season only Au 3 gives higher yield than that of other strain.

Yield of second flush: Significant difference was observed in YSF among the different strain in the different season (Table 2). The maximum YSF (92.0g/packet) was recorded in Au 4 at winter season and it was minimum (50.75g/packet) in Au 3 at summer season. In this parameter it was observed that in the winter season most of the strain gave higher yield in their second flush. It is very important that the second flush play an important role in the winter season.

Table 2: Effect of seasons on yield and yield attributes of different strains of *Auricularia auricula*

Strain	Seasons	Number of effective fruiting body/ packet	Weight of individual fruit body (g)	Yield of first flush (g/packet)	Yield of second flush (g/packet)
Au – 3	Winter	21.00b	11.75b	165.00b	83.00b
Au – 4		17.25c	13.25a	138.00c	92.00a
Au – 5		14.25d	10.25c	86.50d	58.25c
Au – 3	Summer	23.25a	10.25c	187.00a	50.75d
Au – 4		23.00a	10.25c	182.00a	58.50c
Au – 5		22.75a	10.00c	174.30ab	51.50d
CV (%)	-	**5.33**	**6.48**	**5.72**	**6.02**

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

Dry yield (g): Significant difference was observed in dry yield of different strains of *Auricularia auricula* mushroom in two seasons (Fig. 2). The highest dry yield (37.93 g/packet) was obtained from Au 4 in summer season which was similar to Au 4 (36.27 g/packet) in winter season while the lowest yield (18.53 g/packet) was observed in Au 5 in winter season. Considering Au-3, the yields were 31.07 and 29.77 g/packet respectively in winter and summer season. These results help to describe that the dry yield of Au 4 strain showed the best performance both winter and summer seasons.

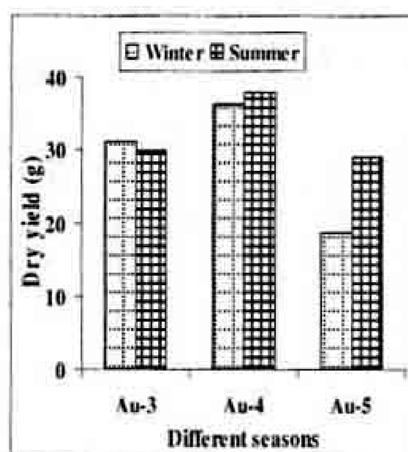

Fig. 2. Dry yield potentials of *Auricularia auricula* in winter and summer season.

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Effect of Sterilization Practices of Contaminated Mother Culture on Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*)

Nirod Chandra Sarker, Md. Bazlul Karim Choudhury[1], Abdus Salam Khan, Md. Mominul Hoque, Bimol Chandra Dey and Manirul shaheen

National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

Effect of six different sterilization practices on contaminated mother culture was investigated to find out the growth and yield of oyster mushroom (*Pleurotus oystratus*) in straw substrate during May to August, 2013. The minimum days (6.00) was recorded in treatment T_1 (autoclave + wash with clean water + autoclave) to complete mycelium growth while the maximum days (8.25) was required in T_5 (only wash with clean water + boiling 30 min). The minimum days (9.00) was found in T_1 while the maximum days (12.50) was recorded in T_6 (boiling 5 min + wash with clean water + boiling 30 min) to complete mycelium growth for spawn packet. The highest number of effective fruiting bodies (26.50) was found in T_3 (boiling 5 min + wash with clean water + autoclave) and the lowest (11.25) was in T_6. The length of stalk ranged 1.20 to 2.17cm. The highest length of stalk (2.17 cm) was found in T_5 (only wash with clean water + boiling 30 min) and the lowest (1.20 cm) was in T_6. The diameter of stalk, pileus and thickness of pileus ranged 0.62 to 1.15 cm, 5.57 to 6.80 cm and 0.50 to 0.70 cm, respectively. The highest yield and biological efficiency (206.0g and 82.40%) were recorded in T_1 followed by T_3 (180.50g and 72.20%). Treatment of contaminated mother culture with sterilization practice showed significant difference in contamination rate ranged 16.67% to 100% where the maximum contamination was found 100% in T_4 (autoclave + wash with clean water + boiling 30 min) and the minimum (16.67%) was in T_3 followed by T_1 (25.00%). Therefore, it may be concluded that autoclave + wash with clean water + autoclave is suitable for sterilization of contaminated mother culture.

Key words: *Pleurotus ostreatus*, Mother Culture, Contamination, Sterilization, Rice straw, Growth, Yield.

-10 / 20

INTRODUCTION

In many countries of the world including Bangladesh, edible mushrooms have been priced as delicacies for several years. Mushrooms have been reported to be rich in protein, glycogen, vitamins, crude fibers and essential mineral compounds (Ogundana and Fagade, 1982; Fasidi, 1996; Jonathan, 2002; Stamets, 1993). Mushroom cultivation is a popular income generating activity that can alleviate poverty, eradicate malnutrition and create employment opportunity for educated, uneducated youths, adolescents, men and women respectively. It can be a suitable job for poor people, alternative income source for all. Basically Mushroom production is labor and management-intensive which takes a considerable amount of knowledge, research, planning, and capital investment to set up a production system. In Bangladesh, there is a huge prospect of mushroom cultivation

[1] OSD, DG Health, Mohakhali, Dhaka-1212, Bangladesh.

because of its climate which is fairly suitable for high volume of mushroom production (Begum, 2008). The small & marginal farmers are generally uneducated & economically poor. The production of spawn and compost is a difficult technical job for them which also require heavy investments. The non- availability of quality spawn is a common problem of large mushroom growers. Its cultivation in Bangladesh has increased manifold, but the major constraint in the popularization of this crop are diseases and pests, which happen to be devastating and perpetuate easily from one season to another. Both *P. ostreatus* and *P. sajor-caju* may be grown year-round using simple and low-cost technology. The best yield performance of oyster mushrooms was recorded in the period from October to March and July to August when temperatures range from 18 to 26^0 C and 26 to 31^0 C, respectively. The best and most luxuriant growth occurs, generally in the winter season. Contaminants were observed during times of the year when both humidity and temperature are elevated (May to September). Fungal, bacterial and viral contamination is commonly found in mushroom production facilities (Royse, 1989).

The incidence of contamination can be decreased by the use of sterile cultivation procedures and the maintenance of environmental conditions within optimal ranges (Przybylowicz and Donoghue, 1990; Hordeau *et al.*, 1991). The present studies were planned to find out the easiest, economical and practicable methodology of sterilization practices of contamination mother culture, which may also be helpful to increase the growth and productivity of oyster mushroom. The findings will help and guide the mushroom growers, especially interested in the cultivation of oyster mushrooms. It also reveals that investors with minimum capital and labor investment get high rate of return in this business.

MATERIALS AND METHODS

Experimental site: The contaminated mother culture of oyster mushroom (*Pleurotus ostreatus*) was obtained from the National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka. The experiment was carried out from May to August 2013 at NAMDEC.

Treatments: In this experiment the contaminated mother culture of oyster mushroom (*Pleurotus ostreatus*) were used as a main factor. Six (6) different sterilization practices were used as treatments. The treatments were T_1 = Autoclave + wash with clean water + autoclave, T_2 = Only wash with clean water + Autoclave, T_3 = Boiling 5 min + wash with clean water + Autoclave, T_4 = Autoclave + wash with clean water + Boiling 30 min, T_5 = Only wash with clean water + Boiling 30 min, T_6 = Boiling 5 min + wash with clean water + Boiling 30 min.

Preparation of mother culture: After sterilization the contaminated mother culture containing paddy grain according to treatments, the packets were cooled for 24 hours and transferred into a clean room. A fully colonized master mother containing mycelium of *Pleurotus ostreatus* were mixed thoroughly with the treated mother culture at the rate of 10%. Then polypropylene bags of 7" × 10" size were filled with 300g of the above

mentioned mixture and packed tightly. The Polypropylene bags were plugged with little cotton. Then the inoculated packets were placed in the laboratory at room temperature (30 $\pm 2^0$) C). After 6 to 8 days the mother culture were fully colonized by the mycelium.

Preparation of substrate: The straw was chopped to 4-5 cm length and then poured in water at 60^0C for one hour and then drained out the water and kept it to get cool slowly. After about 22 hours the straw was spread over polythene sheet in the open place to reduce the moisture level at 65%.

Preparation of spawn packets: The polypropylene bags of 9" x 12" cm size were filled with pasteurized straw at 500g/packet. Then the packets were spawning (three layers) with treated mother culture according to treatments, and their mouths were plugged by inserting absorbent cotton with the help of plastic necks.

Experimental condition: The packets were kept in a dark room at 25^0C for incubation. When colonization of mycelium was completed, the spawn packets were taken to culture house and were opened by 'D' shaped cut on the shoulder and removed the sheet. The relative humidity and temperature of the culture house were maintained at 80-90% and 20-25^0C respectively by spraying water. Diffused light, about 200 lux and proper ventilation in culture house were maintained. After harvesting of mushroom, the residues were removed from the packet and temperature and relative humidity were maintained as before. The yield was obtained from single, double and third flush in the harvest period. Yield in g/packet was recorded by weighing all the fruiting bodies in a packet after removing the lower dirty portion. Biological efficiency was calculated according to the formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield (g)}}{\text{Total dry substrate used (g)}} \times 100$$

Data collection and statistical analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications. Data on days to complete mycelium running in mother culture, days to complete mycelium running in spawn packet, number of effective fruiting bodies, length and diameter of stalk, diameter and thickness of pileus, number of flush, yield, and biological efficiency were recorded and analyzed following Gomez and Gomez (1984) using MSTAT-C computer program. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days required to completion of mycelium running in mother culture: Appreciable variation was found in days required to completion of mycelium running in mother culture in different treatments and ranged from 6.00 to 8.25 except T_4. The highest days (8.25) required to completion mycelium running in T_5 treatment followed by T_6 and T_3.

The lowest days (6.00) required to completion of mycelium running mother was found in T_1 (Table1).

Days required to completion of mycelium running in spawn packet: The minimum days (9.00) required to completion of mycelium running in spawn packets was observed in T_1 which was statistically similar to T_2, T_3 & T_5 treatment respectively. The maximum days (12.50) required to completion of mycelium running was observed in T_6 treatment (Table1). This result is similar with Moonmoon *et al.* (2012) who reported that 15.00 days is required to complete mycelium running for *Pleurotus ostreatus* in paddy straw substrate. Mahjabin *et al.*, 2011 also reported that 13.00 days is required to complete mycelium running for *Pleurotus djamor* in hot water treated rice straw.

Number of effective fruiting body: Number of effective fruiting bodies under different treatments differed significantly (Table 1) except treatment T_4. The highest number of effective fruiting bodies (26.50) was found in T_3 followed by T_1 (23.50) which was statistically different to other treatments. The lowest number (11.25) of effective fruiting bodies was found in T_6 which were statistically differ to other treatments. This result is partially supported by Shelly *et al.* (2010) who observed that the number of fruiting body of *Pleurotus ostreatus* 30.25/packet on paddy straw substrate.

Size of fruiting body: The length of stalk ranged from 1.20 to 2.17 cm with significant difference (Table 1) except treatment T_4. The highest length of stalk was found in T_5 (2.17cm) which was statistically similar to treatment T_1 & T_3. The lowest length of stalk was found in T_6 (1.20). The diameter of stalk differed significantly and ranged from 0.57 to 1.15 cm (Table 1). The highest diameter of stalk was found in T_5 (1.15cm) while the lowest diameter of stalk (0.57cm) was found in T_3.

Table 1. Effect of sterilization practices of contaminated mother culture and it's re-use in rice straw substrate on growth and yield attributes of oyster mushroom

Treatment	Days to complete mycelium running in mother culture	Days to complete mycelium running in spawn packet	Number of effective fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
T_1	6.00b	9.00b	23.50b	2.12a	0.87ab	6.17ab	0.60bc
T_2	7.00ab	10.00b	22.75b	1.60b	0.88ab	6.80a	0.67ab
T_3	7.25a	10.50b	26.50a	2.10a	0.57ab	5.72b	0.57cd
T_4	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_5	8.25a	10.50b	18.00c	2.17a	1.15a	5.75b	0.70a
T_6	8.00a	12.50a	11.25d	1.20b	0.62ab	5.57b	0.50d
CV (%)	**13.14**	**11.74**	**11.39**	**18.54**	**26.49**	**9.60**	**10.87**

In a column do not differ significantly at 5 % level according to DMRT. T_1 = Autoclave + wash with clean water + autoclave, T_2 = Only wash with clean water + autoclave, T_3 = Boiling 5 min + wash with clean water + autoclave, T_4 = Autoclave + wash with clean water + boiling 30 min, T_5 = Only wash with clean water + boiling 30 min, T_6 = Boiling 5 min + wash with clean water + boiling 30 min.

The diameter of pileus ranged from 5.57 cm to 6.80 cm with significant difference among the treatments (Table 1) except treatment T_4. The highest diameter of pileus (6.80cm) was

found in T_2 followed by T_1 (6.17cm) and the lowest diameter of pileus (5.57 cm) was found in T_6.

The thickness of pileus in different treatments differed significantly and ranged from 0.50cm to 0.70cm (Table 1). The highest thickness was found in T_5 (0.70cm) which was statistically similar to T_2 (0.67 cm). The lowest thickness of pileus (0.50cm) was found in T_6 which was statistically similar to T_3.

Yield/ Packet (g): Significant variation was observed in yield under different treatments (Fig. 1). The highest yield (206.0g) was found in T_1 followed by T_3 (180.5g) and the lowest yield was found in T_6 (56.25g). Yield was counted in the harvest period double flush. This result is partially supported by Khan *et al.* (2012) who reported that yield of oyster mushroom in rice straw substrate ranged from 106g–534.50g and also reported yield increased with increasing the amount of rice straw. Amin *et al.* (2008) also reported yield was increased with increasing the amount of rice straw for the cultivation of oyster mushroom. Shelly *et al.* (2010) observed that the total yield of *Pleurotus ostreatus* 176.30g/packet on paddy straw substrate.

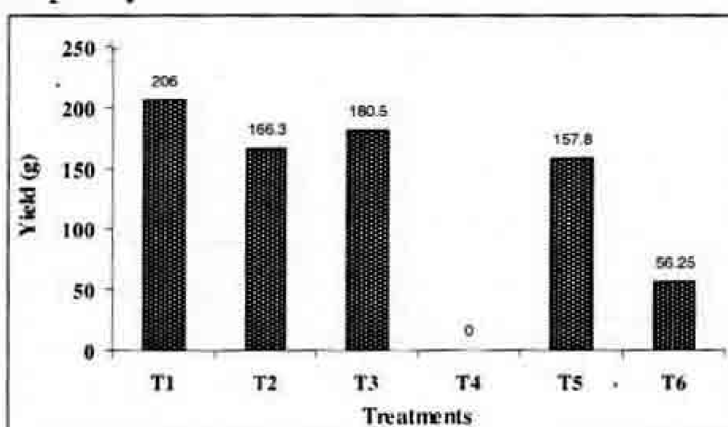

Fig. 1. Yield performance of different re-treatment mother culture.

Biological efficiency: The highest biological efficiency (82.40%) was found in T_1 followed by T_3 (72.20%) and the lowest biological efficiency was found in T_6 (26.10%) where Shelly *et al.* (2010) was observed that the biological efficiency (121.30%) of *Pleurotus ostreatus* packet on paddy straw substrate (Fig. 2).

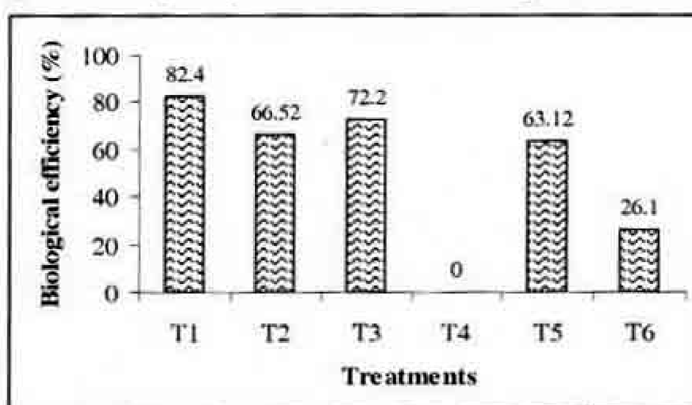

Fig. 2. Yield performance of different re-treatment mother culture.

Contamination rate in mother culture: There was a significant difference in precent contamination rate, which ranged from 16.16 to 100% (Fig. 3) by green mould and other bacteria during re-treated mother culture. The highest contamination rate (100%) was found in T_4 and the lowest contamination rate (16.16%) was found in T_3 during re-treated mother culture. A mushroom farmer must be able to identify and eradicate these microbial contaminants which could affect mushroom yield. This could be achieved by proper sterilization and incorporation of appropriate antibacterial agents into the medium used for mycelial propagation of these mushrooms. The ability of sterilization methods to eliminate substrate contaminants is shown by the presence or absence of contaminants in the substrate after sterilization, spawning and incubation. Kurtzman (2010) reported several causes of mushroom substrate contamination. Oei (1996) determined 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.

According to Balasubramanya and Kathe (1996), the microorganism species that competed with *Pleurotus* sp. after pasteurization with hot water (80^0C for 2h) were the fungi *Penicillium* sp. and *Trichoderma* sp. probably due to the partial breakdown of cellulose and hemicelluloses, thus making them available to competitors. Different sterilization methods can be used for cultivation of oyster mushroom production and its yield improvement (Khan *et al.*, 2011). Contamination by faster growing organisms is consequently a major problem since it causes decreased yields and production rates (Royse, 1989). 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 micro organisms.

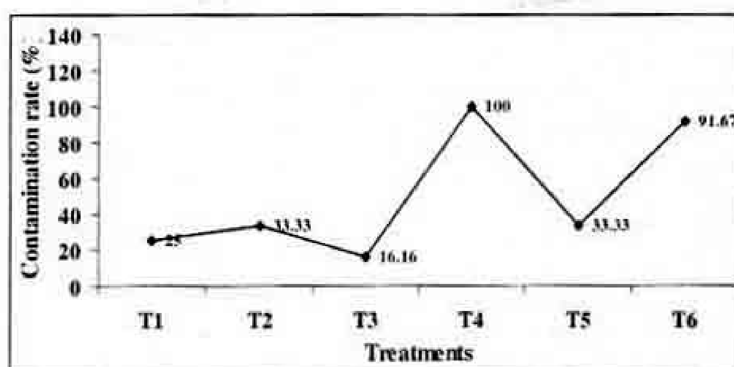

Fig. 3. Contamination rate of different re-treatment mother culture.

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Cultural Conditions for Vegetative Growth and Random Amplification of Polymorphic DNA in Different Strains of *Pleurotus sajor-caju*

Sabiha Azmi Sanjuti, Nuhu Alam, Nirod Chandra Sarker[1], Md. Bazlul Karim Choudhury[2], Ruhul Amin[1] and Akhter Jahan Kakon[1]
Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Abstract

This study was initiated to evaluate the favorable vegetative growth and to determine molecular phylogenetic relationship in five different strains of *P. sajor-caju*. Optimum temperature for the mycelial growth was obtained at 30°C. This mushroom grew well at acidic condition and pH 5.0 was the most favorable. Considering growth phenotype of mycelia, glucose peptone, was the favorable, while Hennerberg was the unfavorable media. Dextrin was the best and xylose was the less effective carbon sources. The most suitable nitrogen sources were ammonium acetate and arginine, whereas alanine was the least effective for the mycelial growth of *P. sajor-caju*. Investigation of genetic diversity is necessary to identify the strains. The strains were also analyzed by RAPD with 20 arbitrary primers. Eight primers were efficient to amplify the genomic DNA. The number of amplified bands was variable depending on the primers or the strains. The size of polymorphic fragments was obtained in the range of 0.2 to 2.0 kb.

Key words: Culture media, Mycelial growth, Physicochemical, *Pleurotus sajor-caju*, RAPD.

INTRODUCTION

Pleurotus sajor-caju is one of the popular edible mushrooms in Bangladesh due to their bright color, unique flavor and texture (Alam *et al.*, 2008; Zhang *et al.*, 1994). This mushroom grows on fallen trees and stumps of broad-leaf and commercially cultivated on saw dust, rice straw, sugarcane bagasse, cotton seed and peanut hulls (Alam *et al.*, 2007). Mushrooms are rich in dietary fiber, nutrients, and some particular compounds known to bring physiological benefit to humans, as well as to other animals (Alam *et al.*, 2010).

Cultivation of the oyster mushroom for the production of fruiting bodies has increased greatly throughout the world during the last few decades and its popularity has been increasing due to its easy cultivation and high yield potential (Chang, 1990). Rice straw appeared to be the best substrate for oyster mushroom cultivation when compared to banana leaves, maize stover, corn husks, rice husks and elephant grass. Cellulose/lignin ratios in substrates were positively correlated to mycelial growth rates and yields of *P. ostreatus* and *P. sajor-caju*. In addition, there was a positive correlation between the C/N ratios for the yield of oyster mushroom (Philippoussis *et al.*, 2001).

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[1] National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka, Bangladesh; [2] OSD, DG Health, Mohakhali, Dhaka-1212, Bangladesh.

Molecular biology has become to be a very important tool in the characterization of oyster mushrooms. Various molecular genetic tools have been introduced for the verification of mushrooms, such as RFLP, RAPD, and SSU rDNA and ITS sequence analyses. Among the molecular approaches, the random amplification of polymorphic DNA (RAPD) had been first developed to detect polymorphisms between organisms despite the absence of sequence information, to produce genetic markers, and to construct genetic maps (Williams *et al.*, 1990). RAPD is a convenient method for detecting genetic diversity assessed by amplification at low stringency with a single short primer of arbitrary sequence. Therefore, RAPD has been adapted in various fungal species and is increasingly popular due to simple, rapid, and low cost method for detecting genetic diversity (Alam *et al.*, 2009; 2010). The present study was aimed to assess the genetic diversity and physicochemical requirements for the vegetative growth in different strains of *P. sajor-caju*.

MATERIALS AND METHODS

Mushroom strains: Five cultivated strains of *P. sajor-caju* were collected from the different ecological regions of Bangladesh. Pure cultures were deposited in Mycology, Plant Pathology and Plant Protection laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka.

Temperature and pH: The optimum temperature for the mycelial growth of *P. sajor-caju* was tried to find out, six different temperatures such as 10, 15, 20, 25, 30 and 35^0C were used. A 5 mm diameter agar plug removed from 10 days of old culture and placed in the centre of PDA plate. The medium was adjusted to pH 6.0 and incubated for 10 days at the temperature 10, 15, 20, 25, 30 and 35^0C. In case of pH, the medium was adjusted to pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with the addition of 1 N NaOH or HCl before autoclave and incubated for 10 days at 25^0C temperature. The measurement of mycelial growth was performed according to the methods described by Alam *et al.* (2010).

Culture media: Ten different culture media (Czapek dox, glucose peptone, glucose tryptone, Hamada, Hennerberg, Hopkins, Lilly, mushroom complete, potato dextrose agar and yeast malt extract) were used to investigate the mycelial growth of *P. sajor-caju*. The compositions of culture media were prepared according to described method (Alam *et al.*, 2009). The media were adjusted to pH 6.0 before autoclave.

Carbon and Nitrogen sources: This experiments were performed on the basal medium (0.05 g $MgSO_4$, 0.46g KH_2PO_4, 1.0g K_2HPO_4, 120µg thiamine-HCl, 20g agar and 1 litre of distilled water) supplemented with each of 10 carbon sources (Dextrin, Fructose, Galactose, Glucose, Lactose, Maltose, Mannose, Sorbitol, Sucrose and Xylose) and ten nitrogen sources (Alanine, Ammonium acetate, Ammonium phosphate, Arginine, Calcium nitrate, Glycine, Histidine, Methionine, Potassium nitrate and Urea). To evaluate the most favorable carbon and nitrogen sources for the mycelial growth, each carbon source with 5g of peptone was added to the basal medium separately at the concentration of 0.1 M/1000 ml and mixed thoroughly. Each nitrogen source with 20g of glucose was

supplemented to the basal medium at the concentration of 0.02 M (Alam *et al.*, 2009). In both cases, the basal medium was adjusted to pH 6.0 before autoclaving.

DNA extraction: Genomic DNA was extracted according to the procedure of Lee and Taylor (1990) with some modifications as follows. Fresh mycelia were collected from the 10 days old culture on PDA medium and were frozen with liquid nitrogen. Frozen mycelia were grounded with sterilized mortar-pestle and kept in 1.5 ml micro tube. As extraction buffer, equal amount of 50 mM Tris-HCl (pH 7.5), 50 mM EDTA (pH 8.0) and 1% sarkosyl was added to the micro tube and incubated at 65^0C for 30 min. After incubation, same amount of PCI (25 ml phenol: 24 ml chloroform: 1 ml isoamyl-alcohol) was added, vortexed and centrifuged under 4^0C at 12,000 rpm for 10 minutes. After wards, only supernatant of upper part was taken in 1.5 ml micro tube, added 1000µl of 99.9% alcohol and centrifuged at 12,000 rpm for 5 minutes under 4^0C. In this case, supernatant was removed, added 500µl of 70% alcohol with precipitated DNA, vortexed and centrifuged at 12000 rpm for 5 minutes under 4^0C. Again supernatant was removed and waited until residual alcohol evaporated. Finally 500µl of sterilized distilled water was added. DNA concentration was measured using spectrophotometer (Cubero *et al.*, 1999).

RAPD analysis: Genomic DNA was amplified by the RAPD technique (Williams *et al.*, 1990) in which 20 sorts of arbitrary 10-base oligonucleotide primers (Operon Technologies Inc.) i.e. OPA-01, 5'CAGGCCCTTC3'; OPA-02, TGCCGAGCTG; OPA-03, AGTCAGCCAC; OPA-04, AATCGGGCTG; OPA-05, AGGGGTCTTG; OPA-06, GGTCCCTGAC; OPA-07, GAAACGGGTG; OPA-08, GTGACGTAGG; OPA-09, GGGTAACGCC; OPA-10, GTGATCGCAG; OPA-11, CAATCGCCGT; OPA-12, TGCGCGATAG; OPA-13, CAGCACCCAC; OPA-14, TCTGTGCTGG; OPA-15, TTCCGAACCC; OPA-16, AGCCAGCGAA; OPA-17, GACCGCTTGT; OPA-18, AGGTGACCGT; OPA-19, CAAACGTCGG; OPA-20, 5'GTTGCGATCC3' were used to produced amplified fragments. RAPD-PCR reaction was performed using a thermal cycler with an initial denaturation stage of 5 minutes at 94^0C, followed by 35 cycles of denaturation for 1 minute at 94^0C, annealing for 1 minute at 36^0C, extension for 2 minutes at 72^0C and a final extension for 7 minutes at 72^0C. RAPD products were electrophoresed on 1.4% agarose gel in 1 × TAE buffer for 1.15 hour at 100V, with a 1kb DNA ladder as a size marker and then stained while agitated in an EtBr solution (0.5% µg/ml). The stained gels were visualized and photographed using a UV transilluminator. RAPD bands were recorded as present (1) or absent (0) to generate the data matrix. The similarity coefficients (S) were calculated between isolates across bands for all primers using the formula $S = 2Nxy/ (Nx + Ny)$, where Nx and Ny are the number of bands shared by the two strains (Nei and Li, 1979).

RESULTS AND DISCUSSION

Effect of temperature and pH: A temperature range of 10-35^0C was considered to find out the most suitable one. The highest average mycelial growth (82.86 mm) was recorded at 30^0C and lowest average (16.41 mm) at 10^0C temperature. Almost similar mycelial growth was observed at the temperature of 20 and 35^0C (Table 1). In case of maximum

mycelial growth, there are no significant difference between the temperature of 25 and 35^{0}C. Therefore, experimental results indicated that optimum temperature range was 25-30^{0}C for the mycelial growth of *P. sajor-caju*. The findings of this study are comparable to the previous study of Alam *et al.* (2008) and reported that 30^{0}C is the optimum temperature for the mycelial growth of *P. adiposa*.

Table 1. Effect of temperature on the mycelial growth in different strains of *Pleurotus sajor-caju*

Strains	Mycelial growth (mm)*					
	10^{0}C	15^{0}C	20^{0}C	25^{0}C	30^{0}C	35^{0}C
PS-1	16.17±1.0	22.17±2.0	46.92±1.0	71.17±4.0	86.08±0.9	38.35±0.5
PS-2	19.50±0.4	34.58±0.9	50.25±0.4	78.67±2.6	86.00±0.5	23.56±1.2
PS-3	12.83±0.2	15.00±0.3	49.58±0.2	73.92±1.0	79.75±2.1	40.48±0.5
PS-4	15.08±0.2	15.75±0.6	52.42±0.2	70.08±1.4	76.67±2.6	39.50±0.7
PS-5	18.50±0.6	24.25±0.8	46.92±0.6	61.83±1.6	85.83±0.9	49.66±1.4
MEAN	16.41±0.5	22.55±0.9	48.6±0.48	71.13±2.1	82.86±1.4	37.8±0.86

*Mean of 3 replications

Table 2. Effect of pH on the mycelial growth in different strains of *Pleurotus sajor-caju*

Strains	Mycelial growth (mm)*					
	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0
PS-1	65.67±2.3	73.42±7.0	81.17±3.9	74.50±4.2	73.25±0.6	78.00±0.7
PS-2	54.56±5.9	77.00±1.7	78.83±4.3	73.00±5.2	67.83±4.0	62.00±2.2
PS-3	38.00±7.4	82.83±5.1	68.75±8.9	67.75±2.1	73.00±2.4	60.17±3.6
PS-4	37.42±3.2	75.83±1.7	75.42±3.9	66.75±1.2	66.25±1.5	62.25±1.9
PS-5	31.22±3.2	75.67±1.9	77.00±2.7	75.56±1.7	71.11±0.5	62.67±3.7
MEAN	45.37±8.4	76.95±3.5	76.23±4.7	71.51±2.9	70.29±1.8	65.02±2.4

*Mean of 3 replications.

pH value at the range of 4.0-9.0 was studied to find out the suitable culture conditions for the mycelial growth. The highest radial growth of mycelium was found at pH 5.0 (Table 2). There was no significance variation between the ranges of pH 5.0-6.0 on the mycelial growth. This result is agreeable with the data collection from the study of Hur (2008). He studied that the cultural characteristics and log-mediated cultivation of *P. linteus* and found that the pH value 6.0 was the best. Present results indicated that *P. sajor-caju* grew well at acidic or neutral or alkaline conditions.

Effect of culture media: Ten different culture media were used to find out the optimum mycelial growth of the selected strains of *P. sajor-caju*. Considering growth phenotype of mycelia, glucose peptone, potato dextrose agar were the favorable, while Hennerberg was the unfavorable media. Average highest and lowest mycelial growth was recorded in glucose peptone (85.65 mm) and Hennerberg (19.56 mm), respectively (Table 3). Hur (2008) reported that the excellent mycelial growth of *P. linteus* was found in mushroom complete medium which is almost similar to our findings.

Table 3. Effect of culture media on the mycelial growth in different strains of *Pleurotus sajor-caju*

Strains	Mycelial growth (mm)*									
	CZA	GLP	GLT	HAM	HEN	HOP	LIL	MUC	PDA	YEM
PS-1	39.83±2.2	84.58±2.4	76.83±2.8	74.67±0.5	24.00±0.9	27.67±1.8	46.17±2.2	76.08±2.0	81.17±4.0	58.42±1.9
PS-2	39.67±2.2	86.42±0.2	69.11±4.8	68.67±2.8	15.75±0.7	26.17±1.0	47.33±4.6	73.58±1.0	78.83±4.3	75.33±3.3
PS-3	37.08±1.8	86.67±0.3	59.67±0.9	73.25±5.4	21.17±1.0	26.17±1.5	46.17±3.2	75.25±1.3	68.75±8.9	68.17±8.0
PS-4	38.42±2.1	84.83±1.4	63.67±1.5	78.92±1.9	18.08±1.2	24.33±0.9	53.75±2.1	75.92±1.6	75.42±3.9	77.08±3.3
PS-5	37.67±1.4	85.75±1.0	68.17±3.9	79.67±3.4	18.83±1.0	24.83±0.9	45.17±2.5	73.50±3.5	77.00±2.7	61.83±1.5
Mean	38.53±1.9	85.65±1.06	69.79±2.8	75.03±2.8	19.56±1.2	25.83±1.2	47.71±2.9	74.86±1.9	76.23±4.7	68.16±3.6

*Mean of 3 replications. CZA: Czapek Dox, GLP: Glucose peptone, GLT: Glucose tryptone, HAM: Hamada, HEN: Hennerberg, HOP: Hoppkins, LIL: Lilly, MUC: Mushroom complete, PDA: Potato dextrose agar and YEM: Yeast-malt extract.

Table 4. Effect of carbon sources on the mycelial growth in different strains of *Pleurotus sajor-caju*

Strains	Mycelial growth (mm)*									
	Dex	Fru	Gal	Glu	Lac	Mal	Man	Sor	Suc	Xyl
PS-1	69.00±3.4	49.30±3.0	20.40±2.5	44.00±4.5	42.00±1.6	49.80±4.4	45.30±5.0	49.90±1.8	30.50±1.3	13.30±1.7
PS-2	80.90±1.4	58.90±5.9	27.30±1.1	52.70±4.2	33.50±0.4	71.10±3.2	54.80±1.6	38.50±5.3	62.20±3.8	9.30±1.1
PS-3	77.50±4.3	51.30±2.0	22.00±2.8	52.30±1.9	32.70±0.6	46.80±3.2	47.50±2.7	35.10±5.9	48.20±5.4	10.40±1.0
PS-4	72.30±8.7	53.50±5.9	21.50±2.2	46.30±4.4	32.90±1.4	49.10±4.2	49.70±3.3	32.70±3.0	53.50±3.7	9.90±1.2
PS-5	73.80±5.6	60.8±11.2	18.20±2.3	53.20±7.8	30.00±0.8	50.80±7.0	49.20±3.5	36.40±6.2	53.50±7.7	10.30±1.2
Mean	74.7±4.6	54.76±5.6	21.88±2.18	49.7±4.56	34.22±0.96	53.52±4.4	49.3±3.22	38.52±4.4	49.58±4.3	10.64±1.2

*Mean of 3 replications, Dex: Dextrin, Fru: Fructose, Gal: Galactose, Glu: Glucose, Lac: Lactose, Mal: Maltose, Man: Mannose, Sor: Sorbitol, Suc: Sucrose and Xyl: Xylose.

Table 5. Effect of nitrogen sources on the mycelial growth in different strains of *Pleurotus sajor-caju*

Strains	Mycelial growth (mm)*									
	Amp	Arg	Ama	Ala	Can	Gly	His	Met	Pon	Ure
PS-1	35.92±1.2	18.50±2.4	52.67±2.5	9.5±0.5	29.08±0.3	34.92±1.6	25.08±0.7	26.08±1.2	26.75±0.6	32.08±4.4
PS-2	36.58±3.0	50.25±2.7	50.00±3.1	12.5±0.2	19.17±2.3	32.83±4.0	23.08±1.0	28.25±0.2	40.50±2.4	43.08±3.3
PS-3	40.17±4.4	55.89±4.0	58.17±2.7	7.5±0.5	21.33±4.9	37.89±1.1	21.42±1.4	27.25±1.1	41.83±3.9	45.50±4.9
PS-4	37.08±4.3	52.25±3.9	53.08±4.5	17.5±1.4	22.42±2.2	38.17±1.1	20.58±0.4	25.50±0.4	31.83±3.2	51.67±2.6
PS-5	33.50±1.5	59.33±2.2	29.75±15.0	15.5±0.8	21.67±0.4	33.50±1.6	20.33±1.2	26.08±0.7	32.75±4.4	42.17±8.4
Mean	36.65±2.9	47.2±3.04	48.7±5.56	12.5±0.7	22.73±2.0	35.46±1.9	22.09±0.9	26.63±0.7	34.78±2.9	42.9±4.72

*Mean of 5 replications. Ala: Alanine, Ama: Ammonium acetate, Amp: Ammonium phosphate, Arg: Arginine, Can: Calcium nitrate, Gly: Glycine, His: Histidine, Met: Methionine, Pon: Potassium nitrate and Ure: Urea.

Effect of carbon and nitrogen sources: Among the ten different carbon sources, dextrin was found to be the best for the mycelial propagation of *P. sajor-caju* and followed by fructose, mannose and sucrose. On the other hand xylose was the most unfavorable carbon sources (Table 4). This result is similar to that of Shim *et al.* (2003). Ten different nitrogen sources were assayed to find out vegetative growth conditions of *P. sajor-caju*. Among the nitrogen sources, ammonium acetate was found to be the best and followed by arginine and urea. However, lowest mycelial growth was recorded in alanine, histidine, calcium nitrate and methionine (Table 5). Shim *et al.* (2005) reported that glycine was the

most favorable nitrogen source, which result is not similar to our findings. They also clarified that histidine was the most unfavorable nitrogen sources for the mycelial growth of *M. procera*, which is similar to our result. In general organic nitrogen sources are more effective then inorganic nitrogen sources.

Table 6. DNA bands in different strains of *Pleurotus sajor-caju* by RAPD assay on 10 base OPA primers

Primers	DNA band (kb)	Strain of *Pleurotus sajor-caju*				
		1	2	3	4	5
OPA-01	2.0	+	-	-	-	-
	1.6	+	+	+	+	+
	1.2	+	+	+	+	+
	0.8	+	+	+	+	+
	0.4	+	-	+	+	+
	0.2	-	+	-	-	-
OPA-02	1.5	+	+	-	-	-
	1.2	+	+	-	-	-
	0.8	+	+	+	+	+
	0.4	+	+	+	+	+
OPA-03	2.0	+	+	+	-	-
	1.6	+	-	+	-	+
	1.0	+	+	+	-	+
	0.8	-	+	+	+	+
	0.5	-	+	-	+	-
OPA-04	1.5	+	-	-	-	-
	1.0	+	+	+	+	-
	0.5	+	+	+	+	+
	0.2	+	+	+	+	+
OPA-07	1.3	+	+	+	-	-
	0.9	+	+	+	-	-
	0.8	+	+	+	-	-
	0.7	+	+	+	+	+
	0.5	-	-	-	+	+
	0.4	-	-	-	+	+
OPA-09	1.2	+	+	+	-	-
	0.9	+	+	+	+	+
	0.7	+	+	+	+	+
	0.4	-	-	-	+	+
OPA-11	1.5	+	+	-	-	-
	0.7	+	+	-	-	-
	0.2	-	-	+	+	+
OPA-20	1.0	-	+	-	+	+
	0.8	+	+	+	-	+
	0.6	-	+	-	-	-
	0.5	+	+	+	+	-
	0.4	-	-	+	+	+

Lane 1, PS-1; 2, PS-2; 3, PS-3; 4, PS-4; 5, PS-5, - indicate absence of DNA band, + indicate presence of DNA band.

RAPD analysis: Twenty sorts' arbitrary 10 base oligonucleotide primers were used to amplify the segments of DNA in five different strains of *P. sajor caju.* Among the tested primers, eight primers such as OPA-1, 2, 3, 4, 7, 9, 11 and 20 were found to be efficient for amplifying the genomic DNA (Table 6). These efficient primers showed significant band profiles on the tested strains and high possibilities to screening of each strain (Fig.1, 2, 3, 4, 5 and 6). RAPD-PCR generated distinct multiple products showing considerable

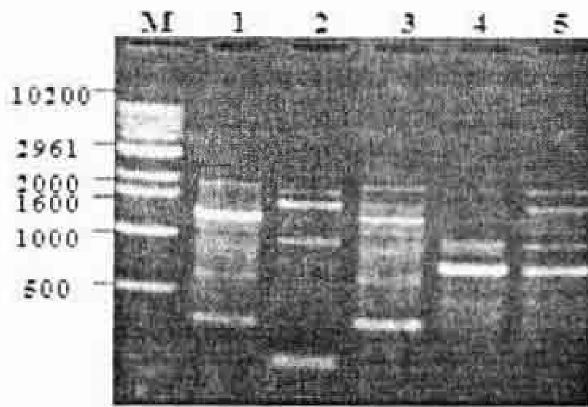

Fig. 1. RAPD profiles in different strains of *Pleurotus sajor-caju* with primer OPA-1. M, molecular size marker (1 kb DNA ladder); lane 1, PS-1; 2, PS-2; 3, PS-3; 4, PS-4; 5, PS-5.

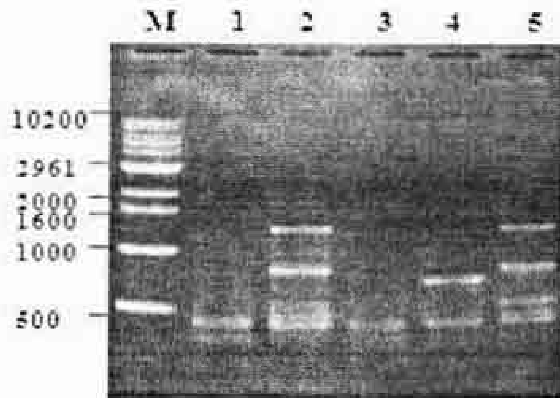

Fig. 2. RAPD profiles in different strains of *Pleurotus sajor-caju* with primer OPA-2. M, molecular size marker (1 kb DNA ladder); lane 1, PS-1; 2, PS-2; 3, PS-3; 4, PS-4; 5, PS-5.

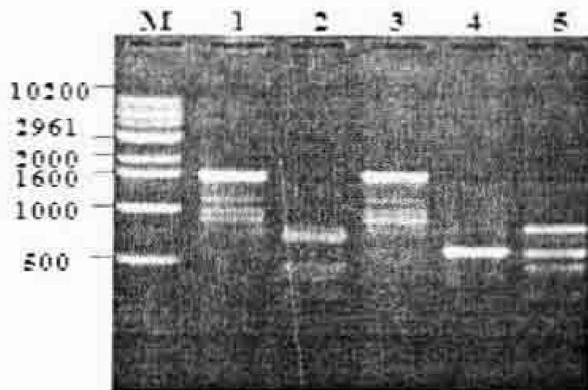

Fig. 3. RAPD profiles in different strains of *Pleurotus sajor-caju* with primer OPA-3. M, molecular size marker (1 kb DNA ladder); lane 1, PS-1; 2, PS-2; 3, PS-3; 4, PS-4; 5, PS-5.

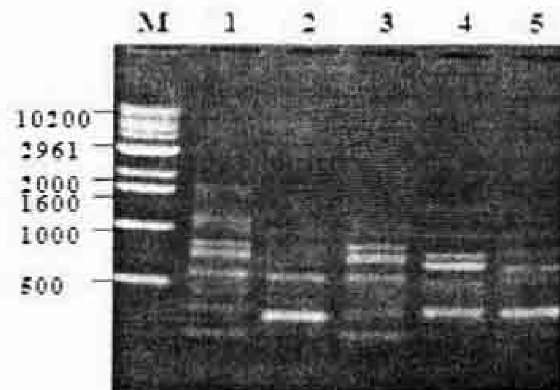

Fig. 4. RAPD profiles in different strains of *Pleurotus sajor-caju* with primer OPA-4. M, molecular size marker (1 kb DNA ladder); lane 1, PS-1; 2, PS-2; 3, PS-3; 4, PS-4; 5, PS-5.

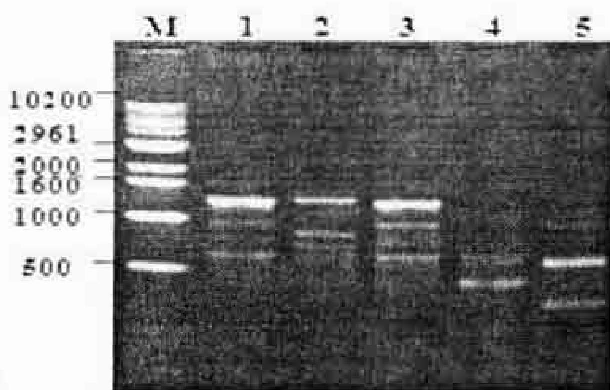

Fig. 5. RAPD profiles in different strains of *Pleurotus sajor-caju* with primer OPA-7. M, molecular size marker (1 kb DNA ladder); lane 1, PS-1; 2, PS-2; 3, PS-3; 4, PS-4; 5, PS-5.

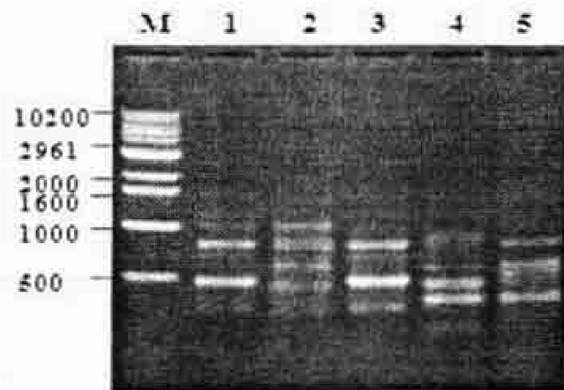

Fig. 6. RAPD profiles in different strains of *Pleurotus sajor-caju* with primer OPA-20. M, molecular size marker (1 kb DNA ladder); lane 1, PS-1; 2, PS-2; 3, PS-3; 4, PS-4; 5, PS-5.

variability among the tested strains. The number of amplified bands was variable depending on the primers or the strains. The size of these polymorphic fragments was obtained in the range of 0.2 to 2.0 kb. Polymorphism of DNA bands showed the same characters in the replication tests. Therefore, if a certain strain is tested for DNA

polymorphisms using the same primers, it could be identified whether the strain is the similar or not by consulting Table 6. Our results are comparable to the study made by Alam *et al.* (2009). RAPD primers are useful to clarify genetic relationships among strains. Strain selection and development of a molecular map of breeding interest would be realistic way to improve yield and quality of this mushroom.

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Morphological Characterization of Commercially Cultivated Oyster Mushrooms in Bangladesh

Zeenia Afsary[1], Nuhu Alam[1], Nirod Chandra Sarker, Ruhul Amin and Akhter Jahan Kakon

National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

This study was carried out to investigate the morphological variation of the indigenous oyster mushrooms i.e. *Pleurotus ostreatus*, *Pleurotus citrinopileatus*, *Pleurotus geesteranus* and *Pleurotus sajor-caju*. Studied oyster mushrooms showed a wide range of variation both in terms of macro and micro morphology. Pileus shape was spathulate in *P. ostreatus*, while convex at first tapering to downward in *P. citrinopileatus* and *P. geesteranus* incase of *P. sajor-caju* was of coralloid appearance or oyster shaped. Pileus color of *P. ostreatus* and *P. geesteranus* were antique white, yellow to golden rod yellow and old lace in *P. citrinopileatus*, *P. sajor-caju*. Smooth pileus texture was found in all species but central was velvety in *P. sajor-caju*. Pileus margin were sinuate in *P. ostreatus*, *P. geesteranus*, while laciniate for *P. citrinopileatus* and crenulate for *P. sajor-caju*. Stipe texture was smooth in all species. Stipe color was antique white, sienna and bisque. Descendng gill attachment was found in selected species. Gill spacing was crowded in *P. geesteranus*, *P. citrinopileatus* and *P. sajor-caju*, while *P. ostreatus* showed close gill spacing. Highest diameter (12.5 cm) and thickness (1 cm) of pileus were found in *P. sajor-caju* and *P. ostreatus*, while the lowest diameter (5.5 cm) and thickness (0.5 cm) of pileus were found in *P. citrinopileatus*, *P. sajor-caju*. and *P. citrinopileatus* respectively. Highest length (4.5 cm) and diameter (1.5 cm) of stipe was recorded in *P. ostreatus* and *P. geesteranus*, while lowest length (3.3 cm) and diameter (0.4 cm) was found in *P. sajor-caju* and *P. citrinopileatus*. Leucosporae and ochrosporae spore print was observed. Arrangement of hymenophral trama was irregular in *P. sajor-caju* and *P. ostreatus*. regular in *P. citrinopileatus and P. geesteranus*. Basidia was 2-spored in *P. geesteranus* and *P. citrinopileatus*, while 3-spored in *P. ostreatus* and *P. sajor-caju*. Cylindrical basidiospore were present in *P. citrinopilleatus*, *P. geesteranus P. sajor-caju* and oblong, hyaline, inamyloid in *P. ostreatus*. Cheilocystidia present in all selected species. However, variation key was formed, which is used to identify the selected oyster mushrooms.

Key words: Macro and micro morphology, *Pleurotus ostreatus*, *Pleurotus citrinopileatus*, *Pleurotus geesteranus* and *Pleurotus sajor-caju*, Taxonomic key.

INTRODUCTION

Oyster mushroom belongs to the genus- *Pleurotus*, family-Pleurotaceae, order-Agaricales, and class- Basidiomycota (Khan *et al.*, 2008). In nature oyster mushrooms appear in cluster on dead trees from late fall to spring, and are distributed almost all round the world. Four basidiospores form at the end of each basidium on the gill of a fruiting body. Only secondary mycelia can produce fruiting body under the proper conditions. The four haploid nuclei are then made into four new basidiospores (Alam *et al.*, 2010).

[1] Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

The first domesticated species was *Pleurotus ostreatus* but later *Pleurotus citrinopileatus, Pleurotus geesteranus* and *Pleurotus sajor-caju* has gained much significant as commercial species in Bangladesh.

Basidicarps pleurotoid sessile to shortly stalked, lignicolous. Pileus normally smooth; margin at first incurved. Veil present or absent. Gills are decurrent, rarely dichotomous, edges entire. Flesh thick to very thin, fleshy to sub hygrophanous or watery mucilaginous, often becoming tough with loss of hydrature and maturity. Spores white or pale pinkish in mass, smooth, inamyloid, multi- guttulate or not, globose, ellipsoid or cylindrical, rarely spores white or pale pinkish in mass, smooth, inamyloid, multi-guttulate or not, globose, ellipsoid or cylindrical, rarely suballantoid, inamyloid, basidia short to long, cheilocystidia present or absent. Pleurocystidia present in few species (Chang and Miles, 1989). Hyphae monomitic or dimitic, rarely branched; generative hyphae more or less inflated or not, becoming thick walled in some species, clamped, except in a few species The genus Pleurotus comprises various edible mushroom species and has important medical and biotechnological properties and environmental applications (Cohen *et al.*, 2002). *Pleurotus* spp. species present high adaptability for growth and fructification within a wide variety of environmental condition. So far classical taxonomical regarding indigenous mushroom in Bangladesh is totally absent. A taxonomic key is a method used to classify and identify mushrooms. Therefore present study was to evaluate the taxonomic characterization based on macro and micro morphology of the commercially cultivated indigenous four different oyster mushrooms in Bangladesh.

MATERIALS AND METHODS

The study was carried out in Mycology, Plant Pathology and Crop Protection Laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka-1342 and National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka, Bangladesh. Fruiting bodies of four different species of oyster mushrooms such as *Pleurotus ostreatus*, *Pleurotus citrinopileatus*, *Pleurotus geesteranus* and *Pleurotus sajor-caju* (Fig. 1) were collected from NAMDEC for the comparative morphometric analysis.

Methods for collection, preservation and description of oyster mushrooms have been followed as per standard techniques (Atri *et al.*, 2012). Pileus shape, diameter, margin, texture, color, gill attachment to stipe, gill spacing, stipe length, diameter, color, texture were described macroscopically on fresh specimens. Spore color is one of the simplest aids for the identification of mushrooms with gills. To determine the color of the spores it is necessary to make a spore print. A spore print is made by cutting off the stem of a mushroom just beneath the cap and placing the cap, gills down, on a piece of paper, covering it with an inverted glass or any other vessel that will keep the air moist and quiet around the cap. Microscopic characters were studied from free hand sections mounted in 5% KOH, stained with 1% Congo red. Colors in description are based on Methuen Handbook of Colors (Kornerup and Wanscher, 1978). After observing all the morphological characters, the carpophore tissue was taken from the point of junction of

stipe with the pileus and sterilized by dipping in 1% sodium hyphocloride for few minutes. After thoroughly washing in sterilized distilled water the fungal tissue was inoculated on potato dextrose agar medium. The optimum temperature for mycelium growth was 25 ± 1^0C. Mycelial color, growth rate, odor and microscopic structures of both the aerial and the submerged mycelium were observed weekly up to 6 weeks according to Nobles (1965).

Fig. 1. Fruiting bodies of A. *Pleurotus ostreatus*; B. *Pleurotus citrinopileatus*; C. *Pleurotus geesteranus*; D. *Pleurotus sajor-caju*.

RESULTS AND DISCUSSION

Taxonomy of *Pleurotus* (Fr.) P. Kummer (1871): Basidiomes usually large, fleshy, solitary to imbricate, flabellate to dimidiate, glabrous to tomentose, white, cream, gray, pink, brown, more rarely blue, yellow or lilac. Stem short, solid, eccentric to lateral, rarely subcentral. Lamellae decurrent, sometimes anastomosing to the stem, light-colored, thin to broad, margin entire. Veil present or absent at margin of pileus or forming an annular zone on the stem. Spore print white, cream, pinkish or lilac. Spores cylindrical to subcylindrical, thin-walled, hyaline, not amyloid or dextrinoid, without germ pore. Cheilocystidia absent or poorly developed, disappearing early, thin-walled, clavate or mucronate. Subhymenium well developed hymenophoral trama irregular. Pileipellis frequently poorly developed, with parallel radial hyphae, sometimes pigmented. Hyphal system monomitic or dimitic, gelatinous tissue usually absent, clamps connection present and ligni colors.

The genus *Pleurotus* does not have clear distinctive characters as occurs with other genera. The hyphal system may be monomitic or dimitic, without binding hyphae. This character might confuse the boundaries with other related genera, such as *Lentinus* (Stankovičová, 1973). There exists a clear delimitation in *Pleurotus* between species with a monomitic hyphal system, thin- or thick-walled generative hyphae, such as *P. ostreatus* and dimitic ones, such as *P. sajor-caju*. All *Pleurotus* species have thin-walled, smooth,

cylindrical to subcylindrical spores. They may be small <8 μm long, medium 8–12 μm and >13 μm long.

Key to the species

1(a). Pileus margin laciniate or crenulate, hymenophoral trama regular, gill spacing crowded, cheilocystidia clavate 2

1(b). Pileus margin sinuate, hymenophoral trama irregular, gill spacing close, cheilocystidia lageniform ....*P. ostreatus*

2(a). Pileus color antique white,or old lace, pileus not fragile, stipe solid, basidiospores cylindrical, spore print leucosporae...3

2(b). Pileu color yellow to golden rod yellow, pileus fragile, basidiospores oblong, sporepint ochrosporae...*P. citrinopileatus*

3(a). Pileus infundibuliform, coralloid appearance, hyphal system dimitic, basidia 3- spored.. P. *sajor-caju*

3(b). Pileus convex at first tapering to downward, hyphal system monomitic, basidia 2- spored

....*P. geesteranus*

Table 1. Qualitative characters of *Pleurotus ostreatus*, *Pleurotus citrinopileatus*, *Pleurotus geesteranus* and *Pleurotus sajor-caju*

Characters	Oyster mushrooms			
	P. ostreatus	*P. citrinopileatus*	*P. geesteranus*	*P. sajor-caju*
Pileus shape	Spathulate	Convex at first tapering to downward	Convex at first tapering to downward	Oyster shaped, coralloid
Pileus color	Antique white	Yellow to golden rod yellow	Antique white	Old lace
Pileus texture	smooth	smooth	smooth	Central velvety outside smooth
Pileus margin	sinuate	laciniate	sinuate	Crenulate
Stipe color	Antique white	sienna	Antique white	Bisque
Stipe texture	smooth	smooth	smooth	Smooth
Gill attachment to stipe	descending	descending	descending	Descending
Gill spacing	close	crowded	crowded	Crowded

Table 2. Quantitative characters of *Pleurotus ostreatus*, *Pleurotus citrinopileatus*, *Pleurotus geesteranus* and *Pleurotus sajor-caju*

Name of species	Pileus diameter (cm)	Thickness of pileus (cm)	Stipe length (cm)	Stipe diameter (cm)
P. ostreatus	10.5	1	4.5	1.2
P. citrinopileatus	5.5	0.5	3.4	0.4
P. geesteranus	7.6	0.6	3.5	1.5
P. sajor-caju	12.5	0.5	3.3	0.7

***Pleurotus ostreatus* (Jackuin ex Fr.) Kummer:** Pileus 4-20 cm, slightly spathulate or kidney shaped, white, cream or grayish, in some cases dark gray when young, surface smooth, margin smooth. Stipe eccentric or lateral, 1-3 cm long, 0.5-2 cm thick firm

sometimes hairy at the base, surface smooth. Spore print leucosporae (Table 1). Flesh white, soft, spongy. Hyphal is system monomitic, generative hyphae present with thin walled and septet with clap connections. Sclerified generative hyphae also present. Basidiospores were 8.7-11.2 × 3.7 µm, cylindrical, thin-walled, hyaline, smooth, inamyloid (Table 2). Basidia were clavate, 4-spored and basidioles numerous. Pleurocystidia not observed. Cheilocystidia 12 subventricose, ventricose or subclavate and lamellar trama was irregular (Fig. 2, 3, 4 and 5).

Culture characters: Mycelium hyaline, filamentous, radial growth, well adhered to substrate. Margin was irregular, undulate. Against the light, an unequal refraction was observed, causing a relief aspect. Odor was not distinct. The mycelium did not cover the Petri plates in 6 wk. Pinkish zones were observed in some strains.

***Pleurotus citrinopileatus*:** This mushroom commonly known as golden oyster. Pileus convex at first depressed when old, tapering to downwards, pileus color yellow to golden rod yellow, pileus glabrous, 5.1-12.7 cm in diameter, pileus margin laciniate, firm, smooth, margin often splitting. Stipe usually lateral, short, sometimes elongated, usually 2.5-5.1 cm long, hollow, stipe color navajo white. Flesh white, Hymenophoral trama regular. Basidiospore was oblong, hyaline, inamyloid, cheilocystidia ventricose-rostrate. Spore print leucosporae. Basidia were 2-spored (Fig. 2, 3, 4 and 5).

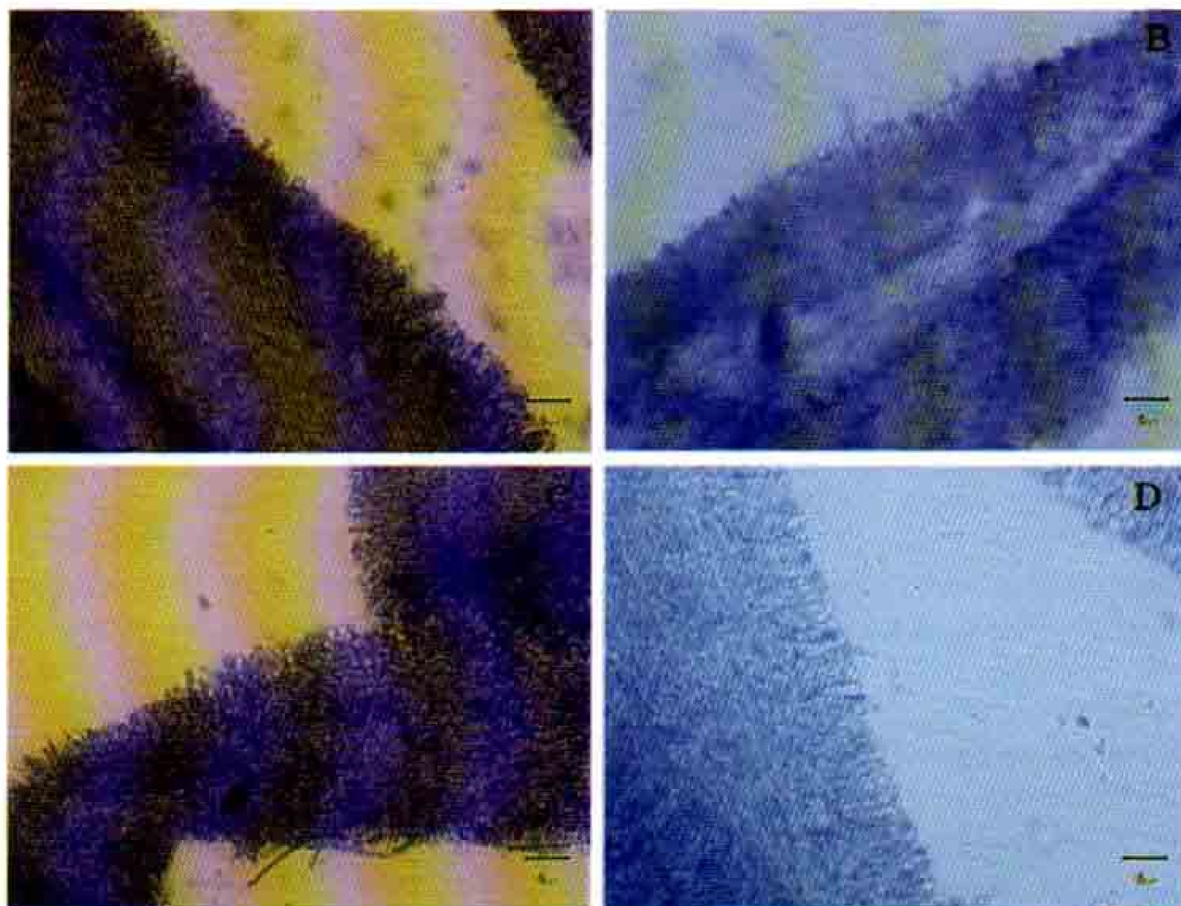

Fig 2. Section of gill presenting cystidia, basidia and arrangement of hymenophoral trama of A, *Pleurotus ostreatus*; B, *Pleurotus geesteranus*; C, *Pleurotus citrinopileatus*; D, *Pleurotus sajor-caju*.

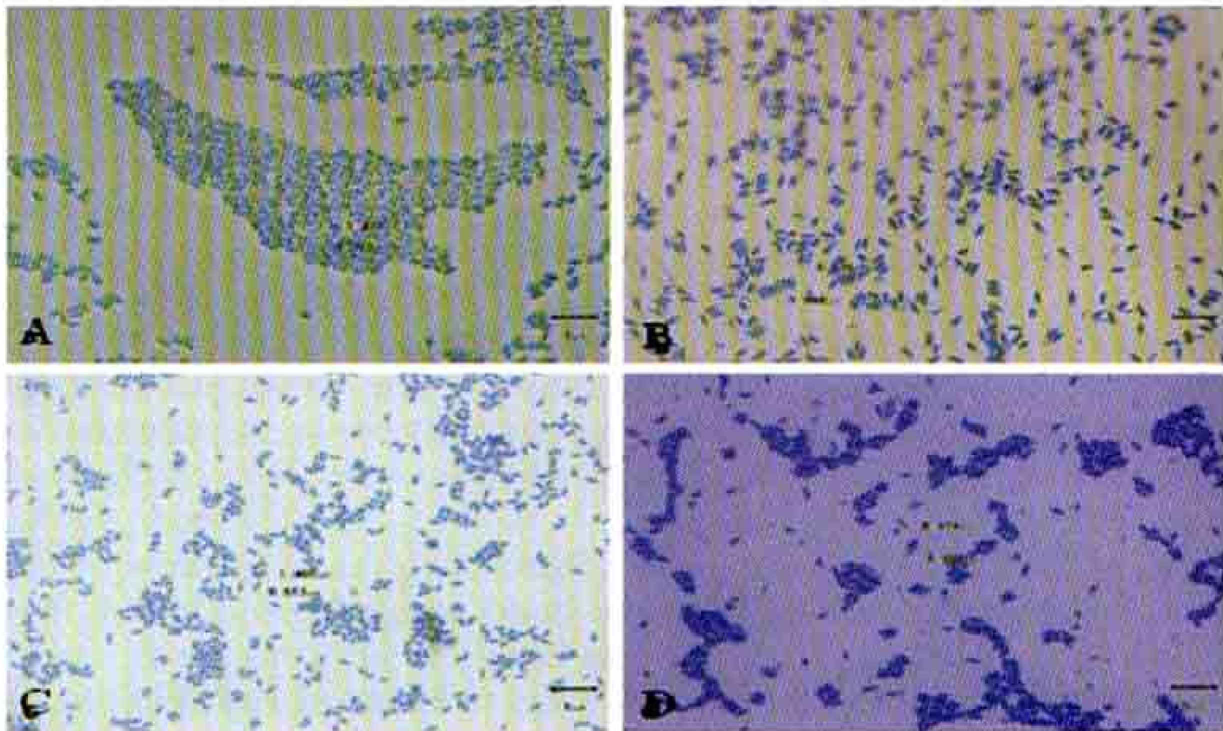

Fig. 3. Spore length and diameter of A, *Pleurotus ostreatus*; B, *Pleurotus geesteranus*; C *Pleurotus citrinopileatus*; D, *Pleurotus sajor-caju*.

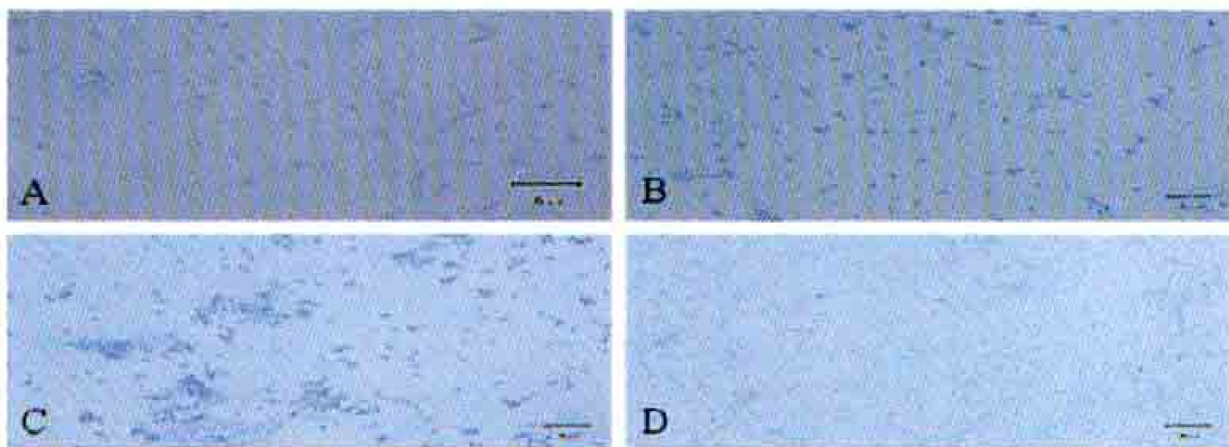

Fig. 4. Spore germination of A, *Pleurotus ostreatus*; B, *Pleurotus geesteranus*; C, *Pleurotus citrinopileatus*; D, *Pleurotus sajor-caju*.

Cultural characters: Cottony, whitish mycelium, often with tufts of dense growth, sometimes with yellowish tones, and occasionally run through with underlying rhizomorphic strain.

***Pleurotus geesteranus*:** Pileus up to 7cm in diameter, surface grey to brownish grey when young to brownish at maturity, smooth, convex at first tapering to downward. Pileus color antique white, margin sinuate the pileus surface, dry, veil absent, margin depressed, flesh up to 0.3 cm thick, white, unchanging on exposure, taste and odor mild. Lamellae yellowish white decurrent, extending down on to the stipe, subdistant (up to 0. 5m apart from each other), spore print white. Stipe was white, eccentric, up to 3 cm long, 1cm broad, almost equal throughout, solid, smooth, flesh white underneath. Sporesoblong

elliptical, cylindrical, inamyloid. Basidia 2-spored, gill edges heteromorphous. Hymenophoral trama regular. Pleurocystidia and cheilocystidia present, clavate. Pileus, with thin hyphae (Fig. 2, 3, 4 and 5).

Cultural characters: Whitish, longitudinally radial at first, sometimes rhizomorphic, soon thickening and becoming cottony in age.

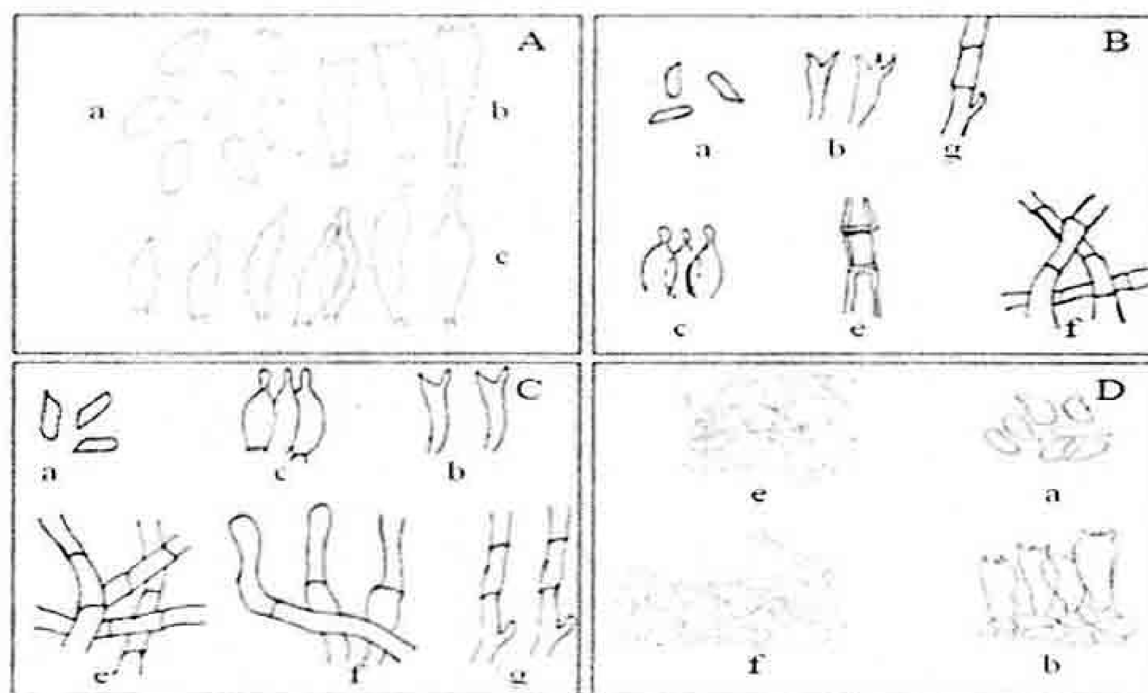

Fig. 5. Shape and arrangement of basidia, basidiospore, cheilocystidia, hyphae A. *P.ostreatus* B. *P.geesteranus* C. *P. citrinopileatus* D. *P. sajor-caju*.

***Pleurotus sajor-caju*:** Fruit body stout, fleshy white shell like, pleurotoid, caespitose. Pileus up to 14 cm in diameter, surface grey to brownish grey when young to brownish at maturity, smooth, scales absent on the pileus surface, dry; veil absent; margin depressed, flesh up to 0.3 cm thick, white, unchanging on exposure, taste and odor mild. Lamellae yellowish white decurrent, extending down on to the stipe, subdistant (up to 0.3 cm apart from each other), unequal, divided into three tiers, ventricose, gill edges smooth, spore print white. Stipe rudimentary to almost none, yellowish white, excentric up to 2 cm long, 2 cm broad, almost equal throughout, solid, smooth, hairy tomentose towards the base, flesh white under neath. Spores oblong elliptical, bean shaped, smooth, inamyloid. Basidia club shaped tetrasporic, tetra-sterigmatic sterigmata long, gill edges heteromorphous. Hymenophoral trama was irregular. Pleurocystidia was absent but cheilocystidia present. Pileus, with thin hyphae forming a scurfy layer over the pileus surface. Pileus context composed of elongated, branched, sepatate hyphae measuring in breadth, pileocystidia small Gill trama consisting of interwoven generative hyphal elements measuring 2.2-4.8 μm in width. Hyphal pegs present. Stipe cuticle context composed of clamped, septate, hyphae (Fig. 2, 3, 4 and 5).

Cultural charactes: Whitish, longitudinally radial at first, sometimes rhizomorphic, soon thickening and becoming cottony in age.

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Pleurotus ostreatus improves Lipid Profile of Obeys Hypertensive Non-diabetic Males

Md. Bazlul Karim Choudhury[1], Md. Shahdat Hossain[2], Md. Masud Hossain[3], Akhter Jahan Kakon, Mohammed Anwarul Kabir Choudhury[4], Nashir Uddin Ahmed[5] and Taufiqur Rahman[6]

National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

For evaluating the effect of *Pleurotus ostreatus* on lipid profile status of obeys hypertensive non-diabetic males, the study was conducted in the National Mushroom Development and Extension Center (NAMDEC), Sobhanbag, Savar, Dhaka, with the active supervision of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. Three grams of dried *Pleurotus ostreatus* powder as capsule form was taken by the subjects in three divided doses for three months. The finding of the study showed a significant (175.35 ± 9.18 and 153.92 ± 10.23, $p = 0.001$) 12.22% reduction of plasma total cholesterol (TC); (108.48 ± 8.02 and 88.22 ± 11.73, $p = 0.020$) 18.67% reduction of plasma low density lipoprotein (LDL-C) after the end of three months mushroom treatment. Also there was a non significant small reduction (159 ± 15.04 and 156 ± 12.27, $p = 0.837$) of plasma triglyceride (TG) and small elevation (35 ± 2.33 and 37.42 ± 2.46, $p = 0.447$) of plasma high density lipoprotein (HDL-C). These findings suggest that consumption of *Pleurotus ostreatus* may able to improve lipid profile status of obeys male person suffering from hypertension.

Key words: Cholesterol, TG, HDL-C, LDL-C, Obeys, *Pleurotus ostreatus*.

INTRODUCTION

At present in remarkable chronic health problems in the world are diabetes, cardiovascular disease, hypertension and obesity creating vigorous pressure on health sector. Cholesterol is a waxy steroid of fat that is produced in the liver or intestines. It is required to build and maintain membranes. Through the interaction with the phospholipid fatty acid chains, cholesterol increases membrane packing, which reduces membrane fluidity (Sadava *et al.*, 2011). Elevated levels of the lipoprotein fractions, LDL-C, IDL-C (intermediate density lipoprotein) and VLDL-C are regarded as atherogenic. Levels of these fractions, rather than the total cholesterol level, correlate with the extent and progress of atherosclerosis (Van der Steeg *et al.*, 2008).

Total cholesterol is defined as the sum of HDL-C, LDL-C, and VLDL-C (very low density lipoprotein). In this structural role, cholesterol reduces the permeability of the

[1] OSD, DG Health, Mohakhali, Dhaka-1212, Bangladesh; [2] Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka, Bangladesh; [3] Upazila Health Complex, Araihazar, Narayanganj; [4] Department of Chemistry, Govt. Yasin College, Faridpur, Bangladesh; [5] Bolora USC, Harirampur, Manikganj; [6] Department of agriculture extension, Puthia, Rajshahi.

plasma membrane to neutral solutes (Yeagle, 1991), protons and sodium ions (Haines, 2001). Although cholesterol is important and necessary for mammals, high levels of cholesterol in the blood have been linked to damage to arteries and are potentially linked to diseases such as those associated with the cardiovascular system (Ascherio *et al.*, 1996). On the other hand HDL-C particles are thought to transport cholesterol back to the liver for excretion or to other tissues that use cholesterol to synthesize hormones in a process known as reverse cholesterol transport (Lewis and Rader, 2005). Having large numbers of large HDL-C particles correlates with better health outcomes (Gordon *et al.*, 1989).

Hypertension, dyslipidemia and diabetes mellitus runs parallelly in relation to mortality and morbidity. But there is no easy procedure to overcome them. Now a day, the use of complementary and alternative medicine (CAM) is increasing rapidly. The World Health Organization classifies 65-80% of the world's health care services as 'traditional medicine' (Jonas, 1997). Mushrooms are tremendous food supplements which comprise a wide range of fungal world. They have been in use not only for consumption, but also for medicinal purposes, since ages. They contain a wide variety of bioactive molecules including terpenoids, steroids, phenols, nucleotides and their derivates, glycoproteins, and polysaccharides (Borchers *et al.*, 1999; Mizuno *et al.*, 1995).

Mushrooms of *Pleurotus* species are very much effective in reducing harmful plasma and liver lipids (Opletal *et al.*, 1997; Jayakumar *et al.*, 2006) and improving the levels of different cellular enzymes (*Alam et al.*, 2007). It is generally known that lowering of plasma cholesterol levels reduces the risk of atherosclerosis, improves liver condition and diabetes. *Pleurotus sajo-caju* exhibits hypotensive effects through its active ingredients, which affect the renin-angiotensin system (Chang, 1996); *Trichloma mongolcium* produces vasorelaxation because of its lectin content (Wang *et al.*, 1996); *Pleurotus ostreatus* possesses antitumour activity (Yoshioka *et al.*, 1985), hepatoprotective activity (Choudhury *et al.*, 2009 and Choudhury *et al.*, 2010), antihypertensive activity (Choudhury *et al.*, 2008) and hypoglycaemic effects in experimentally induced diabetes (Chorvathova *et al.*, 1993); *Lentinus edodes* (Shiitake) and *Grifola frondosa* (Mitake) mushrooms have antihypertensive effects in spontaneously hypertensive rats (Kabir *et al.*, 1987) and *Agaricus bisporous* decreases serum LDL-C by increasing the expression of LDL-C receptor mRNA levels and LDL-C receptor activity (Fukushima *et al.*, 2000).

Considering these situations mushrooms can play an important role in improving the nutrition and disease status of the population. Thus, the objective of the present study was to determine the beneficial effects of *Pleurotus ostreatus* on lipid profile of obeys hypertensive non-diabetic males.

MATERIALS AND METHODS

The study was conducted in the National Mushroom Development and Extension Centre (NAMDEC), Sobhanbag, Savar, Dhaka in association with the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka.

A total 14 obeys non-diabetic male subjects suffering from hypertension were included in the study. They were aged (years) from 27 to 67. The subjects were clarified about the study and after getting their written consent showing willingness to participate in the study they were included. Patients suffering from acute illness, malabsorption and any kind of addicted person except smoking were excluded. If any drug previously getting by the subjects, it was continued.

At the beginning of study, subjects were evaluated for health status. The details history was taken from the subjects which included age, sex, occupation, educational status, marital status, family history and drug history. Eight to ten hours fasting blood sample was collected for analysis of TC, TG, HDL-C and LDL-C. Mushroom capsules were supplied to take two capsules three times daily. Each capsule contains 500 mg *Pleurotus ostreatus* powder, so that each subject took 3 gms mushroom powder daily. Just after ending of three months the subjects were evaluated and all the investigation procedures were repeated.

The fresh fruiting body of *Pleurotus ostreatus* was collected from the culture house of NAMDEC. They were dried using an electric drier at moisture level 4-5%. Then grinded and pour into capsule shell which contains 500 mg powder. Prepared capsules were preserved into moisture free glass containers for dispense.

Obeys subjects were considered those whose Body mass index were > 25. Systolic BP $\geq$ 140 mmHg and/or Diastolic BP $\geq$ 90 mmHg were considered as hypertension. Fasting plasma glucose < 7 mmol/L were considered as non-diabetes.

Ten ml of blood sample was collected from median cubital vein with all aseptic precaution from the subjects. It was immediately poured into test tube containing fluoride and EDTA. The test tube then gently shaken so that anti coagulant and fluoride mix with the blood properly. Then it was centrifuged by 3000 rpm for 5 minutes. Plasma was separated which were transferred into two eppendorf containing 1 ml in each. All the tests were carried out as early as possible. Total cholesterol (TC), Triglycerige (TG) and HDL-C were estimated by semi-auto biochemical analyzer (3000 evaluation) using the available reagent kit. Low density lipoprotein (LDL-C) was calculated using the Friedewald *et al.* equation. LDL = [total cholesterol] – [HDL-C] – [TG/5].

Anthropometric measurements were taken by height in cm and weight in kg with the use of a manual machine. Participants were shoeless and wore light clothing. Body Mass Index (BMI) was calculated by taking subject's weight and height (BMI = weight in kg / Height in m^2). Blood pressure (systolic and diastolic) of subjects was measured by sphygmomanometer.

The recorded characteristics of the subjects were analyzed by standard statistical methods using computer software, SPSS package programme. Results were expressed as mean $\pm$ SE. Paired Student's 't' test was used to see the level of significance. 95% confidence limit was taken as level of significance.

RESULTS AND DISCUSSION

Adult male subjects aged (mean ± SE) 43.64 ± 3.40, ranges from 27 – 61 years free from diabetes with the fasting plasma glucose (mean ± SE) 6.16 ± 0.20 ranges from 4.5 – 6.9 mmol/L were included in the study. The mean of systolic and diastolic blood pressure (mmHg) were 132.85 ± 3.69 and 92.14 ± 1.13 ranges from 120 to 160 and 85 to 100 respectively. BMI (Body mass index) of the subjects (mean ± SE) was 28.21 ± 0.61, ranges from 25.51-31.83 (Table 1).

Table 1. Age, fasting plasma glucose, systolic, diastolic blood pressure and BMI of the subjects

Parameter	N	Mean (± SE)	Range
Age (years)	14	43.64 ± 3.40	27 to 61
FPG (mmol/l)	14	6.16 ± 0.20	4.5 to 6.9
Systolic BP (mmHg)	14	132.85 ± 3.69	120 to 160
Diastolic BP (mmHg)	14	92.14 ± 1.13	85 to 100
BMI	14	28.21 ± 0.61	25.51 to 31.83

The mean ± SE plasma cholesterol (mg/dl) before and after three months mushroom treatment was 175.35 ± 9.18 and 153.92 ± 10.23 respectively. A statistically significant mean difference of cholesterol (p = 0.001) was observed. The reduction of Cholesterol between pre and post treatment state was 12.22%. This finding indicates three month mushroom treatment has significant reducing effect on serum cholesterol level. (Fig. 1).

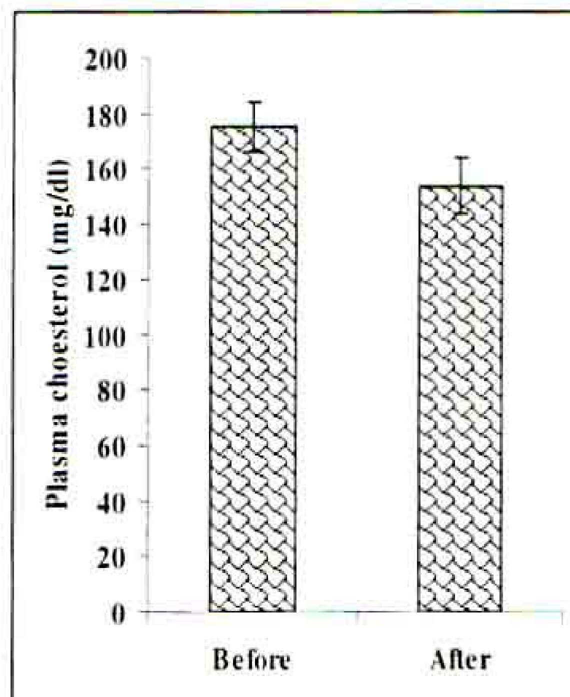

Fig. 1. Evaluation of mean (± SE) plasma cholesterol level before and 3 months after mushroom supplementation.

The mean ± SE plasma TG before and after treatment was 159.00 ± 15.04 and 156.00 ± 12.27 (mg/dl) respectively. A non-significant mean difference was observed (p = 0.837) in pre and post treatment state (1.88 reduction), indicating three months supplementation of mushroom capsule has no significant reducing effect on serum TG level of the subjects (Fig. 2).

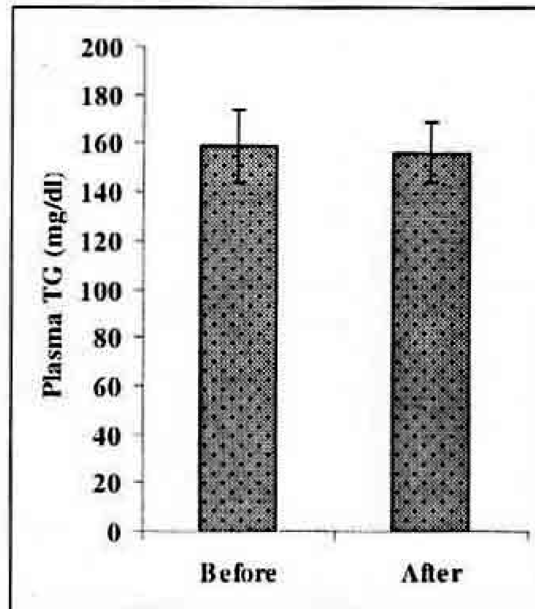

Fig. 2. Evaluation of mean (± SE) plasma TG level before and 3 months after mushroom supplementation.

The mean ± SE serum HDL-C (mg/dl) before and after treatment was 35.00 ± 2.33 and 37.42 ± 2.46 respectively. A non-significant mean difference (6.91% raised) of HDL-C (p = 0.447) was observed in pre and post treatment state indicating supplementation of mushroom as capsule form has no significant rising effect of serum HDL-C level, which is termed as good cholesterol (Fig. 3).

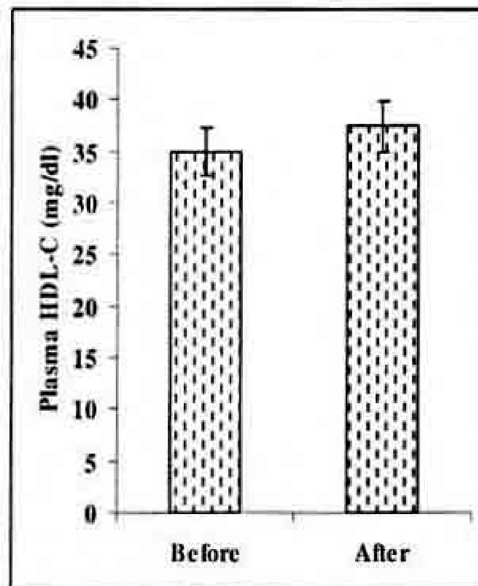

Fig. 3. Evaluation of mean (± SE) plasma HDL-C level before and 3 months after mushroom supplementation.

The mean ± SE serum LDL-C before and after mushroom treatment was 108.48 ± 8.02 and 88.22 ± 11.73 (mg/dl) respectively. A significant mean difference of LDL-C ($p = 0.020$) observed in pre and post Ramadan state (18.67% reduction) indicating supplementation of mushroom significantly reduced serum LDL-C level (Fig. 4).

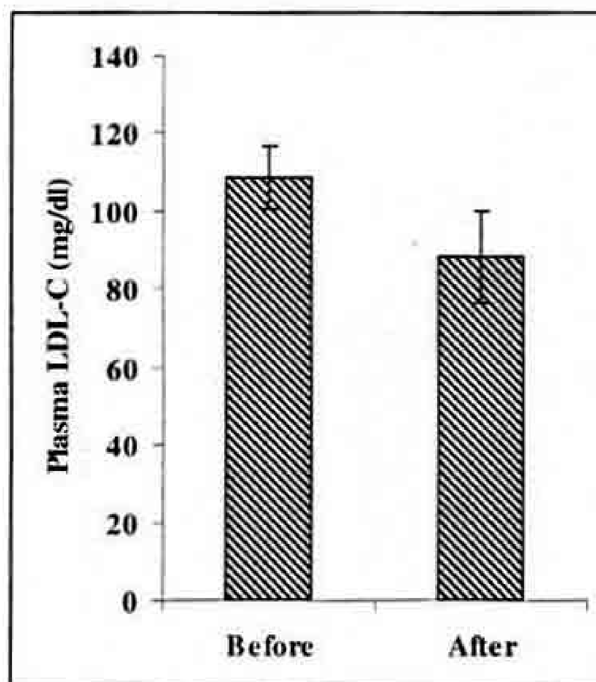

Fig. 4. Evaluation of mean (± SE) plasma LDL-C level before and 3 months after mushroom supplementation.

Findings of the study suggests supplementation of 3 grams of *Pleurotus ostreatus* as capsule form for 3 month significantly reduced serum TC and bed cholesterol LDL-C but there is non-significant minute reduction of TG, also there was non-significant small elevation of good cholesterol HDL-C. Although lots of study conducted in different corner of the World with *Pleurotus ostreatus* but most of them were limited in animal subjects. In a study Bobek *et al.* (1997) observed a significant reduction of cholesterol in serum (31-46%) and liver (25-30%) in Wister rats fed a diet containing 5% *Pleurotus ostreatus* for 52 weeks. These observations were supported by the findings of Hossain *et al.* (2003). They suggested that 5% *Pleurotus ostreatus* supplementation provides health benefits, at least partially, by acting on the atherogenic lipid profile in the hypercholesterolaemic condition.

In a previous study this author (Choudhury *et al.*, 2010) observed a significant reduction of serum TC and LDL-C but no improvement of serum TG and HDL-C by supplementation of *Pleurotus ostreatus* at the Ramadan period of female subjects. In case of males Choudhury *et al.* (2012) observed in his another study that supplement of 50 grams/day of fried *Pleurotus ostreatus* as *Ifter* item for 1 month significantly reduced serum TC, TG and bed cholesterol LDL-C, also there was significant elevation of good cholesterol HDL-C in comparison to non-mushroom supplemented control subjects. The current finding is partially supported by the previous studies. This study is also consistent

with Bobek *et al.* (1997) and Hossain *et al.* (2003) which gives the guidelines of hypolipidemic effects of oyster mushroom.

Findings of the study suggests that regular consumption of edible mushroom *Pleurotus ostreatus* is able to improve blood lipid profile of obeys hypertensive non-diabetic males.

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Comparison of Proximate Nutrients Composition in Local Market and Soya Mushroom Biscuits

Tasnim Farzana, Suman Mohajan, Md. Mominul Hoque[1] and Nirod Chandra Sarker[11]

Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dr. Kudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh

Abstract

The nutritional value of a newly developed soya-mushroom biscuit, prepared from the composite mixture of oyster mushroom (*Pleurotus ostreatus*) powder, soya-flour and wheat flour, was compared with that of the available local market biscuits. The proximate composition of this fortified soya-mushroom biscuit (sample X) and those of others (sample A to sample U) were determined according to the standard AOAC methods. Proximate composition found such as moisture (0.7 to 4.97%), protein (5.14 to 16.64%), ash (0.7 to 1.64%), crude fibre (0.07 to 0.70%), crude fat (7.66 to 34.36 %) and carbohydrate (50.39 to 79.20%). Developed biscuit had the highest content of protein (16.64 ± 0.04)%, ash (1.64 ± 0.05)%, and crude fibre (0.70 ± 0.03)% as compared to those from local market. The result shows that combination of oyster mushroom (*Pleurotus ostreatus*) powder and soya-flour for production of biscuit can enhance the nutritional status of the consumers.

Key words: Oyster mushroom, Soya-flour, Wheat flour, Biscuit.

INTRODUCTION

In developing countries like Bangladesh, with the increasing urbanization, the demand of processed food is also increasing rapidly. Among the processed foods, bakery products, particularly biscuits gain wide popularity in rural as well as urban areas among all the age groups. Biscuits possess several attractive features including low cost, wider consumption among other processed foods, easy availability, wide-ranging taste, and good eating quality and relatively long shelf-life (Gandhi *et al.*, 2001 and Ayo and Olawale, 2003). The principal ingredients that are used in making of biscuits are wheat flour, sugar, fat, water and salt. The quality of the biscuit depends on the nature and quantity of ingredients that are used. Several researchers have depicted the effect of major ingredients in biscuit dough systems and on the final product (Maache-Rezzoug *et al.*, 1998; Chevallier *et al.*, 2000a and Chevallier *et al.*, 2000b). Biscuits are high in carbohydrates, fat and calorie but low in protein, fiber, vitamin, and mineral which make it unhealthy for daily use. Because of its acceptability in all age group, longer shelf life, better taste and its position as snacks it is considered as a good product for protein fortification and other nutritional improvement (Serrem *et al.*, 2011). Biscuits have only about 6 to 7% protein (Agarwal, 1990). Protein Malnutrition is widely recognized as a major health problem in developing countries. In Bangladesh, a poverty-stricken country, it is also a persistent problem. According to the estimate of World Bank, Bangladesh is ranked 1st in the world of the

[1] National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh.

number of children suffering from malnutrition. In Bangladesh, 26% of the populations are undernourished and 46% of the children suffers from moderate to severe underweight problem. 43% of children under 5 years old are stunted. One in five pre school age children are vitamin A deficient and one in two are anemic. Women also suffer most from malnutrition. The use of protein-rich sources has been proposed a possible solution to this problem. In this case, soyabean and mushroom will be good alternative sources.

Mushroom is considered to be as a complete and safest food, suitable for all age groups. Mushroom is a unique plant food in that they are very low in carbohydrates because they cannot photosynthesis sugars, making them ideal for diabetic patents. Mushrooms contain very little fat and are low in energy (calories). They contain significant quantities of several B vitamins, including thiamin (B_1), riboflavin (B_2), pyridoxine (B_6), niacin, folate and some vitamin C. It also helps regulate blood sugar levels. Numerous scientific studies have indicated that the biologically active compounds contained in mushrooms may help to prevent the occurrence and aid the treatment of chronic diseases including heart disease and various cancers (Knoop, 1997).

Soybean, a staple food in many Asian countries, contains valuable constituents, including protein, isoflavones, saponins and phytosterols (Allison *et al.*, 2003). Soybeans are considered to be a source of complete protein (Henkel, 2000). Moreover, the essential amino acid content in soybean exceeds the amino acid requirements of children and adults, which confirms the protein quality of this legume (Maribel *et al.*, 2008). Soyabeans are low in saturated fats and are cholesterol free. They have high iron and calcium content, which help in reducing the chances of heart attack and stroke (Divi *et al.*, 1997). Consumption of soyabean may also reduce the risk of colon cancer, possibly due to the presence of sphingolipids (Symolon *et al.*, 2004).

In view of this consideration the present work was designed to fortify wheat flour with soybean and mushroom for biscuit production and to compare its nutrition quality with locally available biscuits to get a better insight in this issue.

MATERIALS AND METHOD

The study was carried out in the laboratory of Quality Control Research Section of Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Bangladesh.

Sample collection*:* 21 biscuits samples were collected from local market. They were designed as Sample A to U.

Raw materials: Soyabean was collected from Bangladesh Agricultural Research Institute. Oyster mushroom (*Pleurotus ostreatus*) was collected from National Mushroom Development and Extension Centre, Savar, Dhaka. Other ingredients were collected from local market.

Preparation of raw materials: Soyabean, after being soaked for 12 hours, were dehulled, dried and finally blended through blender. Mushroom was dried properly and made into fine powder through blender.

Preparation of biscuit: The process of manufacture is very well standardized and easy. Various ingredients like wheat flour/maida, soya-flour, mushroom powder, corn starch, soda, salt, preservatives, sugar, and oil etc. were thoroughly mixed with the help of water and properly kneaded dough is set on biscuit moulds and then baked in an oven. On completion of baking, biscuits are cooled, weighed and packed (Fig. 1).

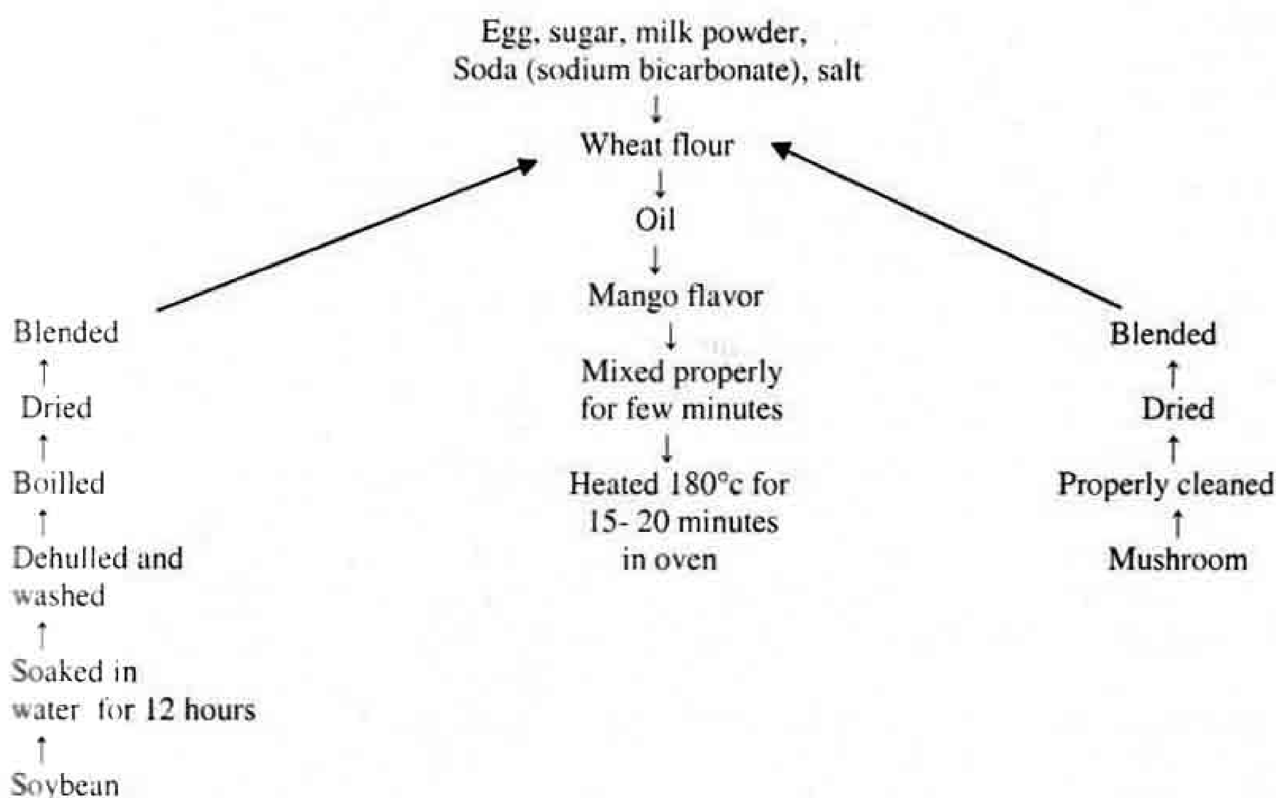

Fig. 1. Flow chart for the preparation of soya-mushroom biscuit.

Sensonry analysis: Organoleptic test of the products was done by 9 point hedonic scale scorecard, especially prepared for the purpose. A 10 member trained panelists from staff of Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Bangladesh. Each attribute was scored based on its intensity scaled on a 9- point hedonic scale (1 = disliked extremely, 2 = disliked very much, 3 = disliked moderately, 4 = disliked slightly, 5 = neither liked or disliked, 6 = liked slightly, 7 = like moderately, 8 = liked very much, 9 = liked very extremely) for colour, flavor, texture, softness and taste.

Proximate analysis: The proximate composition (i.e. moisture, ash, protein, fat, fibre, carbohydrate) and total energy of soya-mushroom biscuits were determined according to the standard analytical methods (AOAC, 1995). At least four samples of each category were analyzed.

Determination of moisture: Moisture content was determined by drying a sample in an oven at 105^0C for 5 hours, the weight loss incurred was calculated as:

% moisture = (Weight loss on drying/Weight of the sample) × 100.

Determination of crude protein: Crude protein content of the samples was determined using the Kjeldahl method. The method consists of three basic steps: 1. digestion of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia; 2. distillation of the ammonia into a trapping solution; and 3. quantification of the ammonia by titration with a standard solution. According to this method, % crude protein content of the samples = % nitrogen x 6.25.

Determination of total ash: To determine ash content, a dried and ground sample was ignite in a furnace at 600^0C for 4 hours to oxidize all organic matter. Crucibles were first dried for about 2 hours at 100^0C in an oven and placed in a desiccator. They were cooled and about 2.0g of sample was weighed into the crucible. The samples were then placed in a furnace at 600^0C for four hours. Percentage ash content was determined by weighing the resulting inorganic residue.

Weight of ash g = [{(Weight of the crucible + ash) - (Weight of the crucible)}/Weight of the sample] × 100

Determination of fat: Fat content was determined using Soxhlet extraction method. In this method, fat was determined by extracting the dried materials (food samples) with a light petroleum fraction in a continuous extraction apparatus. The solvent was distilled off and extract was dried and weighed.

Determination of crude fibre: Moisture and fat free sample was boiled with 0.255N H_2SO_4 and 0.313N NaOH consecutively for 30 minutes under a reflux condenser and each time the sample was washed with boiling water properly to remove acid and alkali residue. The sample was then transferred in a crucible, dried overnight at 100^0C and weighed (W_1) in an analytical balance. The crucible was heated in a muffle furnace at 600^0C for 20 minutes, cooled and weighed again (W_2). The difference in the weights ($W_1 - W_2$) represents the weight of crude fibre.

$$\text{Crude fibre (g/100g)} = \frac{(W_1-W_2) \times 100}{\text{Weight of the dried sample}}$$

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation:

Total carbohydrate (g/100g of sample) = {100 - (moisture + ash + protein + fat + crude fibre)}.

Determination of energy content: Metabolizable energy was calculated following the formula below:

Energy (kcal/100g) = (4 X Carbohydrate) + (4 X Protein) + (9 × Fat) (Joshi, 2002).

Statistical analyses: Data analyses were performed using Statistical Package for the Social Sciences (SPSS version 12.0). Values were expressed as percentage and mean ± SD.

RESULTS AND DISCUSSION

The nutritional analysis of the soya mushroom biscuit compared with those of the traditional ones are presented in the table 1. The content of the moisture, ash, protein, fat, fibre and carbohydrate per 100g of the newly developed biscuit were 2.97 (± 0.05), 1.64 (± 0.05), 16.64 (± 0.04), 18.10 (± 0.06), 0.70 (± 0.03), 59.95(± 0.04) respectively. Energy content of the developed product was 469.26 Kcal per 100g.

Table. 1 Nutritional value of local available biscuits compared with newly developed soya-mushroom biscuit

Products	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Energy Kcal/100g
Sample X	2.97(±0.05)	1.64 (±0.05)	16.64 (±0.04)	18.10(±0.06)	0.70 (±0.03)	59.95(±0.04)	469.26
Sample A	2.06 (±0.04)	0.74 (±0.04)	6.73 (±0.05)	14.67(±0.05)	0.06 (±0.02)	75.74 (±0.03)	461.90
Sample B	1.91(±0.05)	1.31 (±0.03)	6.51 (±0.06)	18.20(±0.02)	0.08 (±0.01)	71.99 (±0.05)	477.80
Sample C	2.34 (±0.06)	0.76 (±0.05)	6.42 (±0.03)	18.10(±0.04)	0.07 (±0.03)	72.31 (±0.04)	477.82
Sample D	2.46(±0.07)	1.32 (±0.06)	5.14 (±0.02)	17.13(±0.07)	0.25(±0.05)	73.70 (±0.02)	469.53
Sample E	2.12(±0.05)	0.70 (±0.04)	6.12(±0.04)	15.90(±0.05)	0.09 (±0.04)	75.07 (±0.07)	467.86
Sample F	2.52 (±0.03)	0.82 (±0.02)	6.27(±0.02)	16.23(±0.06)	0.06 (±0.02)	74.10 (±0.02)	467.55
Sample G	1.62(±0.02)	0.70 (±0.03)	6.72(±0.05)	22.45(±0.03)	0.30 (±0.06)	68.21 (±0.05)	501.77
Sample H	3.58 (±0.05)	0.84 (±0.04)	6.84(±0.07)	9.93(±0.04)	0.40 (±0.05)	78.41 (±0.03)	430.37
Sample I	4.06 (±0.06)	0.73 (±0.06)	7.92(±0.08)	14.54(±0.06)	0.06 (±0.01)	72.69 (±0.04)	453.30
Sample J	1.73 (±0.04)	1.10 (±0.05)	10.16(±0.05)	27.72(±0.05)	0.10 (±0.05)	59.19 (±0.06)	526.88
Sample K	2.04 (±0.04)	1.31 (±0.04)	10.27 (±0.06)	17.58(±0.05)	0.07 (±0.03)	68.73 (±0.05)	474.22
Sample L	2.80 (±0.03)	1.02 (±0.02)	10.40(±0.04)	27.70(±0.04)	0.10 (±0.04)	57.98 (±0.04)	522.88
Sample M	0.88 (±0.02)	0.74 (±0.05)	6.80(±0.03)	17.74(±0.03)	0.07 (±0.02)	73.77 (±0.07)	481.94
Sample N	0.70 (±0.04)	0.76 (±0.04)	6.81(±0.04)	24.34(±0.02)	0.09 (±0.05)	67.30 (±0.05)	515.50
Sample O	2.84 (±0.01)	0.94 (±0.02)	8.00(±0.07)	21.95(±0.04)	0.10 (±0.05)	66.17 (±0.06)	494.23
Sample P	3.62 (±0.04)	0.77 (±0.03)	8.55(±0.05)	7.66(±0.06)	0.20(±0.06)	79.20(±0.05)	419.94
Sample Q	1.31 (±0.07)	0.98 (±0.05)	8.75(±0.06)	24.89(±0.02)	0.35 (±0.02)	63.72(±0.03)	513.89
Sample R	1.77 (±0.05)	0.81 (±0.04)	10.30(±0.03)	30.98(±0.03)	0.11 (±0.05)	56.03(±0.04)	544.14
Sample S	3.06 (±0.05)	1.29 (±0.06)	10.80(±0.02)	34.36(±0.02)	0.10 (±0.02)	50.39(±0.05)	554.00
Sample T	4.97 (±0.04)	0.78 (±0.03)	6.22(±0.08)	29.70(±0.05)	0.49 (±0.01)	57.84(±0.06)	523.54
Sample U	2.98 (±0.07)	1.19 (±0.05)	5.41(±0.07)	19.80(±0.04)	0.38 (±0.03)	70.24(±0.05)	480.80

Sample A to sample U = Local available biscuits, Sample X = Developed soya-mushroom biscuit.

The crude protein ranged from 5.14 to 16.64 % in which samples from developed biscuit had the highest while local marketed samples had the least protein. In case of crude fat, it was ranged from 7.66 to 34.36 %. While considering ash content, the ranged was found

from 0.7 to1.64 % in which samples from developed biscuit had the highest while local marketed samples had the least mineral content. In case of crude fibre, it was ranged from 0.07 to 0.70 % in which samples from developed biscuit had the highest while local marketed samples had the least fibre content. The carbohydrate content ranged from 50.39 to 79.20 in which samples from developed biscuit had 59.95 (Table. 1).

Sensory attributes such as taste, flavor, color, appearance, texture, and overall acceptability were evaluated on 9-point hedonic scale. The mean scores for these attributes for soya- mushroom based biscuits are given in Table 2. It was observed that this developed biscuit is highly acceptable regarding sensory characteristics.

Table 2: Mean score of sensory evaluation for soya- mushroom based biscuits

Quality Factors	Like extremely (9)	Like very much (8)	Like moderately (7)	Like slightly (6)	Neither like nor dislike (5)	Dislike slightly (4)	Dislike moderately (3)	Dislike very much (2)	Dislike extremely (1)
Color		√							
Texture		√							
Flavor	√								
Softness		√							
Taste	√								
Overall acceptance		√							

Considering the presented results, it can be concluded that addition of soya flour and mushroom to wheat flour enhanced the nutritional composition of the biscuit. Alabi and Anuonye, 2007 and Shahzadi *et al.*, 2005 reported that legumes with high protein content are widely used as composite floor in the production of bakery products. It is also known that mixtures of legume and cereal are complementary resulting in balance if the essential amino acids needs of the body. It is clear that incorporating soya and mushroom will result in serving of better protein enriched baking food to the ever increasing number of consumers. As the prepared biscuit contains significant amount of protein, it may be an alternative source of protein for the people suffering from protein energy malnutrition. The high ash and fibre content of our developed biscuit is due to incorporation of soya flour and mushrooms which have high fibre and minerals. Increased fibre content helps to prevent constipation by increasing digestibility of foods. It also prevents obesity, heart disease, diabetes and cancer. Minerals not only impart hardness to bones and teeth but also function broadly in metabolism e.g. as electrolytes controlling the movement of water in and out of cells, as components of enzyme systems, and as constituents of many organic molecules. They are most important factors in maintaining all physiological processes, are constituents of the teeth, bones, tissues, blood, muscle, and nerve cells.

Since soya flour and mushroom are free from cholesterol and other cardiac arrest inducing substances, nutritional health hazards associated with consumption of animal fats are not only minimized but also scarce resources are saved from hospital expenses. The customer may however, pay slightly more due to the food enrichment that is advantageous.

In sum, this newly developed biscuit will provide nutritional support towards malnourished population of different age groups and at the same time, this novel strive will open a new panorama for the local entrepreneurs and aid in developing new food industries that will contribute significantly in our national economy.

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Nutrient and Mineral Content of Different spp. of Oyster Mushroom

Zeenia Afsary[1], Nuhu Alam[1], Md. Bazlul Karim Choudhury[2], Manirul Shaheen, Tahera Binte Mujib and Nirod Chandra Sarker
National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

This study was carried out to determine the nutritional values of edible oyster mushrooms- *Pleurotus ostreatus, Pleurotus djamor, Pleurotus citrinopileatus, Pleurotus geesteranus, Pleurotus eryngii* and *Pleurotus cystidiosus* that are very popular cultivated oyster mushrooms in Bangladesh. Among the six different species of *Pleurotus*, the protein content was maximum in *Pleurotus ostreatus* and it was minimum in *P. citrinopileatus* while lipid was the highest in *P. eryngii and P. cystidiosus*. On the other hand, fibre and ash were maximum in *P. cystidiosus*. On the contrary, carbohydrate was the maximum in *P. djmour*. Oyster mushrooms were rich in protein (20.6 to 25.1%) and fibres (18.1 to 22.5%), while lipids contained are lower (4.1 to 6.5%). The carbohydrate contents ranged from 35.1 to 43.5% on the basis of dry weight. Oyster mushrooms were also rich in mineral contents and total ash content ranged from 8.1 to 13.2%. The moisture content of mushroom varies from 87 to 92%. The results of this study suggested that oyster mushrooms are rich in nutritional value.

Key words: Carbohydrate, Lipid, Minerals, Oyster mushroom, Protein.

INTRODUCTION

Genus *Pleurotus* is a commercially important edible fungus, commonly known as oyster mushrooms. It has very good abilities to grow at a wide range of temperatures utilizing various lignocelluloses, so that is becoming more popular throughout the world (Alam *et al.*, 2007). Oyster mushrooms are a good source of dietary fibre and other valuable nutrients (Alam *et al.*, 2008) and contain a number of biologically active compounds with therapeutic activities such as modulation of the immune system, hypoglycemic and antithrombotic activities, decreasing blood lipid concentrations, prevention of high blood pressure and atherosclerosis (Alam *et al.*, 2009). Nutritional value of edible mushrooms is a complex task. Moreover nutritional composition is affected by many factors; these include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis, time interval between harvest and measurement methods (Benjamin, 1995). True nutritional value can only be established by conducting properly designed feeding studies with human studies undertaken. However, in the absence of definite nutritive studies, chemical composition can serve as a good relative measure for comparison of other food stuffs of nutritive value (Kaul, 2001).

[1] Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh; [2] OSD, DG Health, Mohakhali, Dhaka-1212, Bangladesh.

Mushrooms are rich in protein, minerals and vitamins and they contain an abundance of essential amino acids (Sadler, 2003). The people of Bangladesh are still not very aware of nutritional and medicinal importance of mushrooms, but the popularity of mushroom as a food supplement is increasing day by day, so it is necessary to detect nutritional status of commercially cultivated mushroom. Therefore, present investigation were carried out to find the nutritional values of commercially cultivated oyster mushrooms to build up the awareness on beneficial effects of edible oyster mushrooms to the consumer level.

MATERIALS AND METHODS

This study was carried out quality control and quality assurance (QCQA) laboratory of National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka-1342, Bangladesh. Six different oyster mushrooms i.e. *Pleurotus ostreatus*, *Pleurotus djamor*, *Pleurotus citrinopileatus*, *Pleurotus geesteranus*, *Pleurotus eryngii* and *Pleurotus cystidiosus* were obtained from NAMDEC.

Moisture determination: Five gram of fresh mushroom was weighed into a weighed moisture box (A&D company ltd. N 92; P1011656; Japan) and dried in oven at 100-105°C and cooled in dessicator. The process of heating and cooling was repeated till a constant weight was achieved. The moisture content was calculated as following equation (Raghuramulu *et al.*, 2003):

Moisture (%) = initial weight- final weight/ weight of sample × 100.

Determination of total protein: Five gram of grinded mushroom was taken with 50ml of 1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000g by a table centrifuge machine (DIGISYSTEM: DSC-200T; Taiwan). The supernatant was collected and total protein content was measured according to the Biuret method (Burtis and Ashwood, 2006) with a diagnostic kit (Total Protein: Colourimetric test- Biuret method// Crescent Diagnostics, Saudi Arabia). According to this method, the peptide bonds present in proteins react with Cu^{++} ions in alkaline solutions to form a colored complex of which the absorbance was measured spectrophotometrically at 540nm which was directly proportional to proteins contents.

Determination of total lipid: Total lipid was determined by slight modified method of Folch *et al.* (1957). Five gram of grinded mushroom was suspended in 50ml of chloroform: methanol (2:1 v/v) mixture then mixed thoroughly and let stand for 3 days. The solution was filtrated and centrifuged at 1000g by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid. For the determination of lipid content in fresh mushroom, 5ml of homogenized fresh mushroom sample was taken with 50ml of chloroform: methanol (2:1 v/v) mixture and lipid content was determined as mentioned above.

Determination of crude fibre: Ten gram of moisture and fat-free sample was taken in a beaker and 200ml of boiling 0.255N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200ml of boiling 0.313N NaOH added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100^{0}C and weighed (W_1) in an electric balance (KEY1: JY-2003; China). The crucible was heated in a muffle furnace (Nebertherm: Mod-L9/11/c6; Germany) at 600^{0}C for 8 hours, cooled and weighed again (W_2). The difference in the weights (W_1-W_2) represents the weight of crude fiber (Raghuramulu *et al.*, 2003).

Crude fibre (g/100g) = [100-(moisture + fat)] × (W_1-W_2)/ Weight of sample.

Determination of total ash: One gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 8 hours at 600^{0}C. It was then cooled in a dessicator and weighed. To ensure completion of ashing, the crucible was then heated in the muffle furnace for 1h, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or grayish white in color. Then total ash was calculated as following equation (Raghuramulu *et al.*, 2003):

Ash content (g/100g) = Weight of ash/Weight of sample taken × 100.

Determination of total carbohydrate: The content of the total carbohydrate was determined by phenol-sulphuric acid method (Sadasivam and Manickam, 2005). Carbohydrates were first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose was dehydrated to hydroxymethyl furfural. This formed a green colored product with phenol and sulphuric acid and had absorption maximum at 490nm, which was determined.

Mineral analysis: Total ash was taken for the analysis of mineral contents. 2ml of concentrate HNO_3 was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution to remove turbidity. The solution was then transferred into a volumetric flask and total volume was made 50ml by adding deionized water. This was then used to analyze the contents of calcium (Ca), iron (Fe), phosphorus (P), manganese (Mn), magnesium (Mg), zinc (Zn) and Selenium (Se) by flame and graphite method with atomic absorption spectrophotometer (PERKIN ELMER: AS 80) (Sawhney and Singh, 2005).

Statistical analysis: The statistical programs used were Microsoft Excel and Statistical Program for Social Science (SPSS 11.5). All parameters for inter group differences were

analyzed by one-way ANOVA and then post hoc comparisons, LSD (least significant difference) and DMRT (Duncan's multiple range test) at $P \leq 0.5$ level.

RESULTS AND DISCUSSION

Moisture content of fresh mushroom and total protein, lipid, fibre, ash and carbohydrate contents of dry oyster mushrooms i.e. *Pleurotus ostreatus*, *Pleurotus djamor*, *Pleurotus citrinopileatus*, *Pleurotus geesteranus*, *Pleurotus eryngii* and *Pleurotus cystidiosus* have been presented in Table 1. Among the selected oyster mushroom minimum (87.5%) and maximum (92.2%) moisture contents were found in *P. eryngii* and *P. citrinopileatus*, respectively. Moisture content, by itself may not be of any nutritional significance but it influences the nutritional value of mushrooms. Moisture is a variable component in the proximate analysis of the mushrooms and is significantly affected by environmental factors such as temperature and relative humidity during growth and storage as well as by the relative amount of metabolic water which may be produced or utilized during storage (Crisan and Sands, 1978). Crisan and Sands (1978) also reported that moisture content of fresh mushrooms varies between 85% and 95%.

Table.1. Nutrient contents in six different oyster mushrooms

Oyster mushrooms	Moisture (%)	Protein (g/100g)	Lipid (g/100g)	Fibre (g/100g)	Ash (g/100g)	Carbohydrate (g/100g)
P. ostreatus	87.7 ± 4.2^{de}	25.1 ± 1.8^{a}	4.4 ± 0.4^{b}	18.1 ± 2.2^{c}	10.4 ± 1.0^{b}	41.4 ± 6.4^{b}
P. djamor	89.4 ± 7.1^{b}	22.8 ± 0.8^{b}	4.3 ± 0.2^{b}	18.2 ± 1.3^{c}	10.3 ± 0.8^{b}	43.5 ± 8.2^{a}
P. citrinopileatus	92.2 ± 5.8^{a}	20.6 ± 0.6^{c}	4.6 ± 0.5^{b}	19.9 ± 1.4^{b}	10.6 ± 0.8^{b}	39.4 ± 5.2^{b}
P. geesteranus	88.3 ± 5.2^{d}	20.8 ± 1.2^{c}	4.1 ± 0.2^{b}	20.0 ± 2.3^{b}	8.1±0.5c	40.6 ± 6.8^{b}
P .eryngii	87.5 ± 6.2^{c}	24.8 ± 1.6^{a}	6.5 ± 0.6^{a}	22.4 ± 1.8^{a}	10.6 ± 1.0^{b}	35.5 ± 4.5^{d}
P. cystidiosus	88.8 ± 7.2^{bc}	22.3 ± 1.4^{b}	6.4 ± 0.4^{a}	22.5 ± 1.6^{a}	13.2 ± 1.4^{a}	35.1 ± 2.8^{d}

Results show mean ± SD of three trials. Values in the same column that do not share a common superscript is significant at the $P<0.05$(one way ANOVA then LSD post hoc comparison.

Protein, lipid, fiber, ash and carbohydrate contents were measured from the selected dried oyster mushrooms. The maximum protein (25.1%), lipid (6.5%), fiber (22.5%), ash (13.2%) and carbohydrate (43.5%) were recorded in *P. ostreatus*, *P. eryngii*, *P. cystidiosus* and *P. djamor* respectively. Protein contents of *P. ostreatus* and *P. eryngii* were significantly similar, while it was the lowest (20.6%) in *P. citrinopileatus*. Among the tested mushrooms lipid contents were low, while fiber, ash and carbohydrate contents were rich. Alam *et al.* (2008) reported similar results on *P. sajor-caju*, *P. florida*, and *Calocybe indica*. Protein is the most critical component in the nutritional value and protein deficiency is the most serious human nutritional problem (Kaul, 1983). Protein content of mushrooms has been reported to vary from species to species and even flush to flush within same species and also with cultivation substrate (Rai, 1995). The average fat content of mushrooms is 4-6% of the dry weight. All classes of lipid compounds are represented including relatively large amounts of the essential fatty acids, especially linoleic acid (Rai, 1995). Carbohydrates are the main components of mushrooms apart

from water. Total carbohydrate content of *Agaricus bisporus* is about 60%. In *Pleurotus* sp. carbohydrate content is reported in the range of 35 to 51% (Rai, 1995).

Important minerals i.e. calcium (Ca), iron (Fe), zinc (Zn) and selenium (Se) were detected from 100g of dried in six different selected oyster mushrooms. The results of calcium, iron, zinc and selenium content in different species of oyster mushrooms i.e. *P. ostreatus*, *P. djamor*, *P. citrinopileatus*, *P. geesteranus*, *P. eryngii* and *P. cystidiosus* have been presented in Fig. 1, Fig. 2, Fig. 3 and Fig. 4. The maximum Ca (37.45 mg/100g), Fe (45.25 mg/100g), Zn (22.82 mg/100g) and Se (0.45 mg/100g) were recorded in *P. eryngii*, *P. ostreatus* and *P. geesteranus*, respectively. The lowest amount of Ca (21.37 mg/100g), Fe (31.14 mg/100g), Zn (15.93 mg/100g) and Se (0.15 mg/100g) were recorded in *P. ostreatus*, *P. citrinopileatus*, *P. cystidiosus* and *P. djamor*, respectively. These results are in agreement with the reports of Alam *et al.* (2008) on mineral content. The results suggested that commercially cultivated oyster mushrooms were rich in protein, edible fiber and minerals but low in lipid content. These results also indicate that the studied mushrooms had good nutritive value for human nutrition.

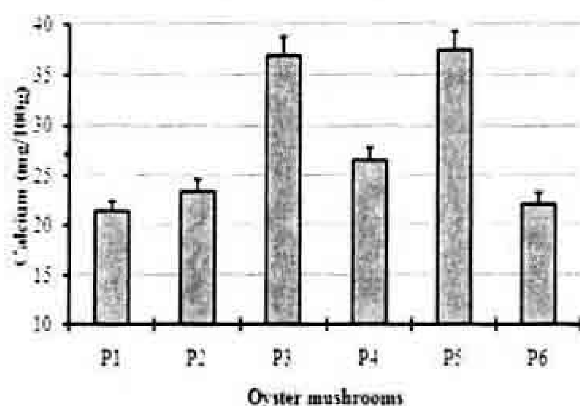

Fig. 1. Calcium contents in different species of oyster mushrooms.

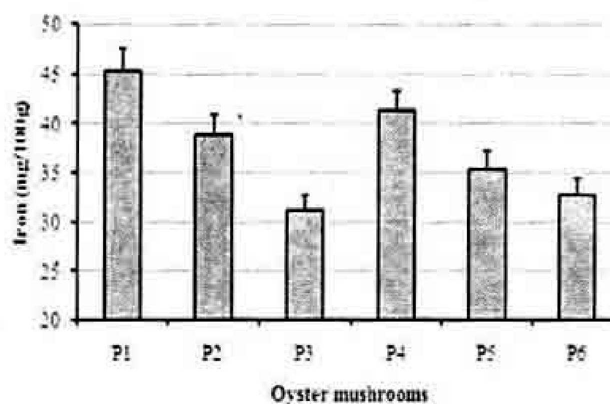

Fig. 2. Iron contents in different species of oyster mushrooms.

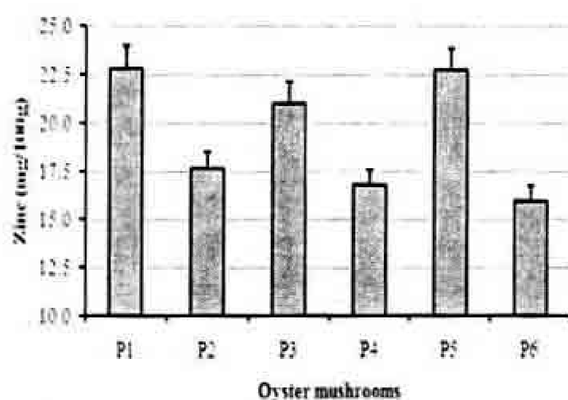

Fig. 3. Zinc contents in different species of oyster mushrooms.

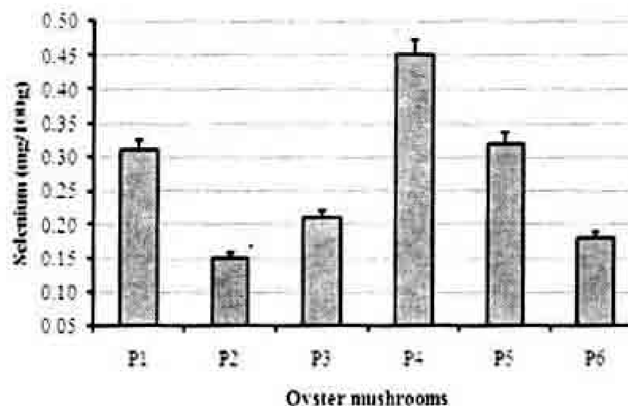

Fig. 4. Selenium contents in different species of oyster mushrooms.

Results are mean ± SD of three trials. Data was analyzed by one way ANOVA and then post hoc LSD test. P_1, *Pleurotus ostreatus*; P_2, *Pleurotus djamor*; P_3, *Pleurotus citrinopileatus*; P_4, *Pleurotus geesteranus*; P_5, *Pleurotus eryngii*; P_6, *Pleurotus cystidiosus*.

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Influence of Physicochemical Conditions of Culture Medium on Invitro Mycelial Growth in Different Strains of *Volvariella volvaceae*

Shailendra Mohon Singha[1], Nuhu Alam[2], Nirod Chandra Sarker and Manirul Shaheen

National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

A study was conducted to evaluate physicochmeical conditions of culture media on invitro mycelium growth conditions of paddy straw mushroom (*Volvariella volvaceae*). Eight strains of commercially cultivated strains of the mushroom fungus were collected from different regions of Bangladesh. Suitable temperature and pH for mycelial growth were obtained at 25^0C and pH 6. Present results indicate that *V. volvaceae* grew well at acidic condition. Lilly and Yeast-malt extract were the favorable culture media for the vegetative growth of paddy straw mushroom. Fructose was the best carbon source for mycelial growth. This was closely followed by maltose, mannose and galactose, which were not significantly different from each other. Lactose showed lowest mycelial growth of *V. volvaceae*. The average highest nitrogen source was found in methionine, whereas histidine was the less effective for the mycelial growth of *V. volvaceae*.

Key words: Cultural condition, Mycelia growth, Physicochemicals, *Volvariella volvaceae*.

INTRODUCTION

Volvariella volvaceae, commonly known as paddy straw mushroom, is a commercially important cultivated fungal species. It belongs to the division Basidiomycota, order Agaricales, and family Pluteaceae. It is one of the well known edible mushrooms especially in China, Thailand, Vietnam, Taiwan and Indian sub continent (Sarker *et al.*, 2012). It is very much preferable for its attractive fruiting bodies as well as unique taste. The mushroom has abilities to grow at a wide range of temperature regimes and humidity utilizing various kinds of lignocelluloses substrates (Singha *et al.*, 2013). The best grown temperature range is 25-35^0C and relative humidity of 60-70%. It has a high biological efficiency (60-70%) under optimum cultural conditions (Quimio, 1976). No other vegetable or cultivated mushroom species can be served as a table dish within a short time from its planting but *V. volvaceae* can do this as it comes to harvest on 10th day of incubation (Thirbhuvanmala *et al.*, 2012).

The mycelium growth depends on several factors such as growing media, pH, temperature, nutrient elements and some other environmental factors (Alam *et al.*, 2009;

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[1] Sreemangal Govt. College, Moulovibazar, Bangladesh; [2] Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Alam *et al.*, 2010). Growth medium is the most important factor because it supplies necessary nutrient for the growth of mycelium. Mycelium production of most mushrooms is affected by pH and temperature. Mushroom can be grown on different carbon sources and usually, potato dextrose agar, malt extract agar, malt extract yeast agar and potato dextrose yeast agar media are used as culture media for mycelial growth (Alam *et al.*, 2008). Generally, it is recognized that growing mycelia in a defined medium is a rapid and alternative method to obtain fungal biomass for further use. Moreover, it is also well known that the mycelium cultivation of mushrooms is enhanced by different environmental and nutritional factors as well as propagation of mycelia is an earlier and essential step to cultivate fruiting bodies of mushrooms. The present study was undertaken to elucidate the effect of different environmental and nutritional conditions on the vegetative growth of *V. volvaceae*.

MATERIALS AND METHODS

Mushroom Strains: Eight different strains of *Volvariella volvaceae* such as VV-1, VV-2, VV-3, VV-4, VV-5, VV-6, VV-7 and VV-8 were obtained from the National Mushroom Development and Extension Centre (NAMDEC), Department of Agricultural Extension, Ministry of Agriculture, Savar, Dhaka-1340, Bangladesh. This experiment was carried out at the laboratory of NAMDEC and Mycology, Plant Pathology and Crop Protection Laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka. Strains of *V. volvaceae* were transferred to potato dextrose agar plates and incubated at 25^0C temperature in the dark condition until they showed a full growth and then kept at 4^0C for further use.

Temperature and pH: Temperature 15^0C, 20^0C, 25^0C, 30^0C and 35^0C were used to find the suitable mycelia growth of *V. volvaceae*. A 5 mm diameter agar plug was removed from 10 days old cultures and placed in the center of a PDA plate. The medium was adjusted to pH 6 and incubated for 10 days at 15^0C, 20^0C, 25^0C, 30^0C and 35^0C. To determine the optimum pH, the medium was adjusted to pH 5, 6, 7, 8 and 9 in addition of NaOH or HCl before autoclave and incubated at 25^0C for 10 days. Radial growth of mycelia on each Petri dish was measured at 3 directions such as A to B, C to D and E to F (Fig. 1). Average value of mycelial growth of each Petri dish was calculated out of those 3 measurements for each of the three replicates used.

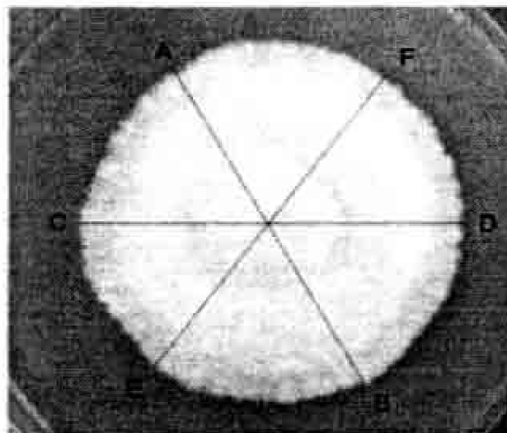

Fig.1. Method of measuring mycelial growth on Petri dish.

The following formula was used to calculate the mycelial growth of *V. volvaceae*.
Average mycelial growth on

1st Petri dish (AB+CD+EF) /3 = R_1
2nd Petri dish (AB+CD+EF) /3 = R_2
3rd Petri dish (AB+CD+EF) /3 = R_3
Average mycelial growth of each strain = $(R_1 + R_2 + R_3)/3$

Culture media: Nine different culture media i.e. Czapek dox, Glucose peptone, Glucose tryptone, Hamada, Hennerberg, Lilly, Mushroom complete, Potato dextrose agar and Yeast malt extract were prepared to investigate the mycelial growth of the selected strains. Culture media and their constituents have been presented in Table 1. The media were adjusted to pH 6 before autoclave. All culture media were inoculated with inoculums similarly. After 10 days of incubation at 25^0C temperature, mycelial growth was measured as same manner.

Table 1. Culture media and their constituents used in this study

Ingredients	Ingredients of different culture media (g/l)								
	CZA	GLP	GLT	HAM	HEN	LIL	MUC	PDA	YEM
Agar	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Asparagine	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Dextrose	0.0	0.0	0.0	10.0	0.0	0.0	0.0	20.0	10.0
Ebiose	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0
Hyponex	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0
Glucose	0.0	10.0	5.0	0.0	50.0	0.0	0.0	0.0	0.0
Malt-extract	0.0	15.0	0.0	0.0	0.0	-	20.0	0.0	3.0
Maltose	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Peptone	0.0	10.0	0.0	0.0	0.0	0.0	2.0	0.0	5.0
Potato	0.0	0.0	0.0	0.0	0.0	0.0	0.0	200.0	0.0
Sucrose	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Triptone	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0
Yeast-extract	0.0	10.0	3.0	3.0	0.0	0.0	2.0	0.0	3.0
$NaNO_3$	3.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0
K_2HPO_4	1.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
$MgSO_4$	0.5	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0
KCl	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$FeSO_4$	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$CaCl_2$	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
KH_2PO_4	0.0	0.0	0.0	0.0	1.0	1.0	0.5	0.0	0.0
KNO_3	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0

CZA: Czapek Dox, GLP: Glucose peptone, GLT: Glucose tryptone, HAM: Hamada, HEN: Hennerberg, LIL: Lilly, MUC: Mushroom complete, PDA: Potato dextrose agar and YEM: Yeast-malt extract.

Carbon and Nitrogen sources: This experiments were performed on the basal medium (0.05g $MgSO_4$, 0.46g KH_2PO_4, 1.0g K_2HPO_4, 120μg thiamine-HCl, 20g agar and 1 litter of distilled water) supplemented with each of 10 carbon sources (dextrin, fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, sucrose and xylose) and 9 nitrogen sources (alanine, ammonium acetate, ammonium phosphate, arginine, glycine, histidine, methionine, potassium nitrate and urea). To screen the most favorable carbon sources for the mycelial growth, each carbon source with 5g of peptone was added to

the basal medium separately at the concentration of 0.1M and mixed thoroughly. Each nitrogen source with 20g of glucose was supplemented to the basal medium at the concentration of 0.02M. In both cases, the basal medium was adjusted to pH 6 before autoclaving. To measure the colony diameter on the medium, all plates were incubated for 10 days at 25^0C temperature. Radial growth of mycelia was measured as described above.

RESULT AND DISCUSSION

A result of the tested temperatures, 25^0C was the optimal for the mycelial growth of eight different strains of *V. volvaceae*. Maximum mycelial growth (83.3 mm) was recorded at 25^0C in the strain of VV-5. Among the strains the highest average mycelial growth was found in 77.5 mm at 25^0C and followed by 66.6, 62.3, 47.9 and 43.7 mm, respectively, at the temperature of 30, 20, 35 and 15^0C, respectively. The lowest growth of mycelia was found either at temperature of 15 or 35^0C, while, medium growth was measured at the temperature of 30^0C and 20^0C (Table 2.). Lee *et al.* (1999) and Shim *et al.* (2003) reported that the mycelial growth of *Paecilomyces fumosoroseus* had been expedited gradually in proportion to the rise of temperature and was the most suitable at 25^0C. It could be suggested that with some exceptions 25^0C is the optimum temperature for the mycelial growth of mushrooms, therefore, this result is similar. The findings of this study are comparable to the previous study of Alam *et al.* (2008) which reported that 25^0C is the optimum temperature for the mycelial growth of *Pholiota adipose*.

Table 2. Effect of temperature on invitro mycelial growth in different strains of *Volvariella volvaceae*

Strain of *Volvariella volvaceae*	Radial colony diame (mm)*				
	15^0C	20^0C	25^0C	30^0C	35^0C
VV-1	48.6± 2.1	60.0±2.3	76.3±6.4	61.0±1.6	28.0±1.5
VV-2	52.3±2.8	74.6±7.5	82.6±8.2	66.0±2.4	32.6±2.1
VV-3	34.3±3.1	58.6±1.5	70.3±6.5	61.0±3.1	49.0±2.2
VV-4	73.3±3.8	76.0±3.0	80.6±7.5	76.6±2.9	34.0±1.8
VV-5	31.0±2.1	59.0±2.5	83.3±5.4	67.3±2.4	59.3±1.7
VV-6	34.0±2.2	48.6±2.7	74.0±6.2	68.6±2.6	62.3±2.4
VV-7	36.0±1.9	60.0±3.1	78.6±4.6	64.0±4.2	62.0±2.6
VV-8	40.3±2.5	61.6±3.4	74.6±5.2	68.3±3.5	56.3±1.8
Mean	**43.7±2.6**	**62.3±3.3**	**77.5±6.3**	**66.6±2.8**	**47.9±2.0**

*Mean of 3 replications.

Most suitable pH values for the favorable mycelial growth of eight different strains of *V. volvaceae* have been studied in the range of pH 5 to 9 and among the strains best was pH 6 except VV-1, VV-2 and VV-3 (Table 3). In the case of VV-7, almost similar mycelial growth was observed at pH 5 to 9. The other pH values also showed good mycelial growth and average results of pH 5 and 6 was better than pH 9 for the mycelial growth of the different strains of *V. volvaceae*. This result is agreeable with the data collection from the study of

Hur (2008). He studied the cultural characteristics and log-mediated cultivation of *P. linteus* and found that the pH value 6 was the best for the mycelial growth. Chi *et al.* (1996) also reported that mycelial growth of *P. linteus* was the best at pH 6.

Table 3. Effect of pH on the mycelial growth in different strains of *Volvariella volvaceae*

Strain	Mycelial growth (mm)*				
	pH 5	pH 6	pH 7	pH 8	pH 9
VV-1	71.6±1.8	75.0±2.4	67.3±1.8	65.3±1.8	58.0±1.5
VV-2	60.3±2.2	74.0±2.8	45.3±2.8	61.6±1.6	46.0±1.2
VV-3	55.6±1.4	62.3±1.8	86.6±5.2	42.6±1.4	39.3±0.8
VV-4	60.0±2.6	81.0±4.6	70.0±4.2	50.3±1.8	45.6±2.2
VV-5	86.3±5.4	86.0±5.4	73.3±1.8	85.3±5.6	78.6±2.8
VV-6	69.6±2.9	80.3±4.2	73.3±3.2	63.3±1.6	58.0±1.8
VV-7	76.6±3.2	82.3±2.4	84.6±5.4	73.0±2.8	72.3±3.4
VV-8	66.6±2.4	84.6±4.2	83.3±4.8	56.6±1.7	54.6±1.7
Mean	**68.3±2.7**	**78.2±3.5**	**73.0±3.7**	**62.3±2.3**	**56.6±1.9**

*Mean of 3 replications.

Nine different culture media were used to evaluate the optimal mycelial growth of eight different strains of *V. volvaceae*. Lilly and Yeast-malt extract were the most suitable and rest of the culture media was moderately unfavorable for the mycelial growth of *V. volvaceae*. Relatively, the highest mycelial growth was observed in the strains of VV-1 (80.3 mm), VV-2 (82.0 mm), VV-4 (80.3 mm), and VV-5 (81.0 mm), respectively, in the culture medium of Lilly and maximum growth was recorded in the strain of VV-3 (87.0 mm) in yeast-malt extract and mushroom complete culture media (Table 4). Hur (2008) reported the excellent mycelial growth of *P. linteus* was found in mushroom complete medium which is similar to our finding. Adejoye *et al.* (2006) reported that yeast-malt extract enhance the mycelial growth of *Pleurotus florida*. In case of mycelial growth of Hennerberg, it shows slightly slower growth than that of in PDA and Hamada. It may be due to the ineffective utilization of microelements by the mushrooms. Alam *et al.* (2009) reported that glucose peptone, yeast malt extract and mushroom complete media were the most favorable, while Hennerberg and Hoppkins media were the least favorable for the vegetative growth of *P. eryngii*. Average highest (72.4 mm) and lowest (53.3 mm) mycelial growth was recorded in lily and Hennerberg media respectively which was resemble to this findings.

Results on the effect of ten different carbon sources for the suitable mycelial growth of selected strains of *V. volvaceae* have been presented in Table 5. It was observed that fructose was the best carbon source for mycelial growth of *V. volvaceae*. This was closely followed by maltose, mannose and galactose which were not significantly different with each other. On the other hand xylose, glucose, sucrose, sorbitol, and dextrose showed moderate mycelial growth, while lactose showed lowest mycelial growth in different strains of *V. volvaceae*. Griffin (1994) reported that fructose and mannose are the most favorable carbon sources for the mycelial growth of fungi, which commonly utilized sugars after glucose. Shim *et al.* (2005) proved that maltose, dextrin, sucrose and mannose were the effective, where lactose was less effective for the mycelial

growth of *M. procera*. Chandra and Purkayastha (1977) reported that most of the tropical edible mushrooms were in favor of utilizing glucose than other carbon sources. The preference of glucose over other carbon compounds may be due to the fast metabolization of glucose by the fungi to produce cellular energy easily. Dextrin was found to be the best for mycelial propagation of *P. eryngii*, followed by fructose, mannose and maltose Alam *et al*. (2009).

Table 4. Effect of culture media on the mycelial growth in different strains of *Volvariella volvaceae*

Strain	Mycelial growth (mm)*								
	CZA	GLP	GLT	HAM	HEN	LIL	MUC	PDA	YEM
VV-1	76.6±1.8	50.3±1.8	45.6±2.8	47.3±4.2	39.3±0.8	80.3±6.8	50.0±2.5	52.3±1.8	58.6±1.8
VV-2	71.6±2.5	69.6±1.6	66.3±3.5	72.3±2.6	65.0±4.2	82.0±4.6	75.6±3.2	55.3±1.6	77.0±3.5
VV-3	77.0±1.8	82.0±6.4	80.6±6.8	77.0±3.4	67.0±3.4	79.6±3.9	87.0±4.8	58.3±2.2	87.0±4.7
VV-4	64.0±2.2	66.3±2.4	70.6±4.6	75.0±4.6	48.6±2.4	80.3±8.2	67.0±3.4	54.6±2.4	71.3±6.2
VV-5	71.3±3.4	75.3±2.3	75.0±5.2	75.0±3.5	67.3±3.2	81.0±7.2	75.0±5.2	63.3±3.2	81.6±8.2
VV-6	20.3±1.2	38.3±1.8	24.6±1.6	42.3±1.8	27.0±0.4	23.3±0.6	24.0±0.8	36.6±1.2	41.0±1.8
VV-7	71.6±6.5	56.6±2.4	75.3±3.5	72.6±1.6	54.0±1.6	74.0±6.4	62.3±2.4	71.6±5.4	75.6±3.5
VV-8	69.3±2.8	52.3±3.2	70.3±2.8	59.0±1.2	58.3±1.8	79.0±4.5	56.3±2.2	73.3±4.8	68.3±2.8
Mean	**65.2±2.8**	**61.3±2.7**	**63.5±3.9**	**65.1±2.9**	**53.3±2.2**	**72.4±5.3**	**62.2±3.1**	**58.2±2.8**	**70.1±4.1**

*Mean of 3 replications. CZA: Czapek Dox, GLP: Glucose peptone, GLT: Glucose tryptone, HAM: Hamada, HEN: Hennerberg, LIL: Lilly, MUC: Mushroom complete, PDA: Potato dextrose agar and YEM: Yeast-malt extract.

Table 5. Effect of carbon sources on the mycelial growth in different strains of *Volvariella volvaceae*

Strain	Mycelial growth (mm)*									
	Dex	Fru	Gal	Glu	Lac	Mal	Man	Sor	Suc	Xyl
VV-1	54.0±2.5	77.0±3.4	71.3±5.2	78.0±4.5	72.3±4.6	73.6±4.5	79.6±4.6	75.3±2.7	75.0±1.8	60.6±2.8
VV-2	78.6±3.5	82.3±4.6	79.3±4.2	77.0±4.3	38.6±1.6	79.6±6.5	82.6±5.2	80.6±3.2	75.3±2.4	70.6±6.4
VV-3	86.3±4.8	85.3±6.2	83.0±1.5	78.0±5.2	43.0±2.5	73.0±3.4	76.3±4.4	70.3±4.5	68.3±3.2	86.6±2.5
VV-4	57.3±1.7	83.6±5.4	74.0±3.2	76.3±3.6	53.3±1.8	76.6±3.8	81.6±3.4	66.6±6.2	69.3±4.8	79.3±3.4
VV-5	80.6±7.3	84.0±4.6	86.3±2.8	80.6±2.6	72.6±3.8	87.0±4.8	84.0±1.8	78.6±1.6	82.3±1.8	86.0±4.2
VV-6	76.6±4.2	80.6±4.3	73.6±6.1	64.3±2.8	47.0±2.4	85.0±4.2	76.0±2.8	67.6±1.8	71.0±1.5	57.3±5.2
VV-7	77.6±3.5	84.0±5.4	71.3±4.5	79.6±6.4	44.0±1.4	87.0±3.2	78.6±1.7	74.3±2.4	75.0±2.4	87.0±7.6
VV-8	71.0±4.6	75.6±3.8	78.0±4.5	59.3±2.4	62.0±3.2	78.3±2.4	69.3±2.2	71.3±2.7	70.6±2.6	66.0±1.8
Mean	**72.8±4.0**	**81.6±4.7**	**77.1±4.0**	**74.1±4.0**	**54.1±2.7**	**80.0±4.1**	**78.5±3.3**	**73.1±3.1**	**73.4±2.6**	**74.2±4.2**

*Mean of 3 replications. Dex: Dextrin, Fru: Fructose, Gal: Galactose, Glu: Glucose, Lac: Lactose, Mal: Maltose, Man: Mannose, Sor: Sorbitol, Suc: Sucrose and Xyl: Xylose.

Effect of nitrogen sources on the mycelial growth of eight different strains of *V. volvaceae* have been presented in Table 6. Among the tested strains, the average highest mycelial growth (64.5 mm) was found in methionine and followed by 61.7, 60.5, 60.0, 59.3, 59.0, 52.3, 52.3, and 35.7 mm, respectively, in the nitrogen sources of arginine, alanine, potassium nitrate, ammonium acetate, glycine, ammonium phosphate, urea and histidine. The

results indicated that histidine was less effective for the mycelial growth and development of all the selected strains. The finding in this study is partially comparable to the previous studies (Alam *et al.*, 2008, 2009; Hur, 2008; Shim *et al.*, 2005; Lee and Han, 2005). Shim *et al.* (2005) clarified that alanine and glycine were the most suitable, while histidine, arginine and ammonium oxalate were most unfavorable nitrogen sources for the mycelial growth of *M. procera*.

Table 6. Effect of nitrogen sources on the mycelial growth in different strains of *Volvariella volvaceae*

Strain	Mycelial growth (mm)*								
	Ala	Ama	Amp	Arg	Gly	His	Met	Pon	Ure
VV-1	63.0±2.6	42.0±2.4	39.3±1.8	60.0±3.8	51.3±2.2	19.0±1.2	63.0±3.2	57.0±1.8	53.3±1.8
VV-2	51.6±1.8	59.0±3.2	54.3±2.4	66.6±2.4	60.6±3.2	22.3±1.8	68.0±3.4	68.0±2.5	56.3±2.2
VV-3	71.6±3.4	61.0±3.6	52.6±2.1	64.6±2.2	73.3±4.2	52.6±3.5	60.6±2.8	69.3±2.4	51.6±2.4
VV-4	63.6±2.6	70.3±4.2	62.3±3.2	68.0±2.8	73.0±2.4	55.6±2.8	73.0±5.2	71.6±3.5	65.3±3.2
VV-5	82.3±4.8	69.3±4.0	70.0±3.5	61.3±3.2	76.0±4.8	52.6±2.6	74.6±4.2	62.0±2.8	69.0±2.7
VV-6	32.3±1.2	34.3±1.5	22.0±1.5	37.3±1.5	12.0±0.8	16.3±0.6	46.3±3.2	38.0±1.4	20.6±1.8
VV-7	56.6±2.4	72.0±3.5	59.0±1.8	69.0±2.4	56.3±2.4	50.6±2.5	66.3±3.4	59.3±2.4	59.6±3.8
VV-8	63.3±3.4	66.3±4.2	59.0±1.9	66.6±2.8	69.3±2.3	16.3±0.8	64.0±4.2	54.6±2.6	43.0±2.4
Mean	**60.5±2.8**	**59.3±3.3**	**52.3±2.3**	**61.7±2.6**	**59.0±2.3**	**35.7±2.0**	**64.5±3.7**	**60.0±2.4**	**52.3±2.5**

*Mean of 3 replications. Ala: Alanine. Ama: Ammonium acetate. Amp: Ammonium phosphate. Arg: Arginine. Gly: Glycine. His: Histidine. Met: Methionine. Pon: Potassium nitrate and Urea: Ure.

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Organoleptic Evaluation of Mushroom Ketchup

Tahera Binte Mujib, Nirod Chandra Sarker, Md. Bazlul Karim Choudhury[1], Md. Kamrul Hassan, Abdus Salam Khan, Ruhul Amin and Akhter Jahan Kakon

National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh

Abstract

Mushroom is an emerging high value crop with great opportunities for enterprise diversification and income generation. It is consumed as a delicacy and appreciated for its good taste, ample nutrition and enticing flavor. For developing a standard mushroom ketch up the organoleptic study was carried out in the National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka. The present study was conducted to prepare the mushroom ketch up as influenced by different treatments. The treatments were T-1 (oyster mushroom), T-2 (oyster mushroom), T-3 (shiitake mushroom), T-4 (shiitake mushroom), T-5 (oyster mushroom + tomato), T-6 (oyster mushroom + tomato), T-7 (shiitake mushroom + tomato), T-8 (shiitake mushroom + tomato). Other common and equal amount of ingredients were vinegar 15ml, onion paste 100g, ginger paste 30g, cumin seed paste 10g black pepper (powder) 1g, arrarote 2g. Another common and different amount of ingredients were common salt, sugar, sodium benzoate, garlic paste, red chili powder. All the samples were stored in sterilized glass jars and were subjected to sensory evaluation fortnightly for three months of storage. Basic sensory methods for food evaluation to measure degree of likeness were adopted for this research. Using a structured questionnaire, 15 male and female panelists independently assessed the samples for colour, appearance, flavor, texture, taste and overall acceptability. Considering all the parameters it was observed from the study (mean ± SD) that T-7 obtained the highest score (3.00±0.62, 80.83%) followed by T-5 the value of which were 2.80±0.75, 80.09%. The average lowest score in the study was 2.26±0.87, 61.30% that was obtained by treatment T-6. Obtained other scores were T-1 (2.27±0.82, 65.09%), T-2 (2.23±0.63, 61.94%); T-3 (2.52±0.74, 71.20%), T-4 (2.44±0.74, 68.52%); and T-8 (2.55±0.59, 67.69%). It might be concluded that for preparing mushroom ketch up the ingredients of all kinds of treatments T-7 i.e. shiitake mushroom and tomato enriched ketchup is highly acceptable (score 3.00±0.62, 80.83%) but regular collection of shiitake mushroom and tomato is difficult. On the other hand preparation of mushroom ketchup using T-6 i.e. oyster mushroom and tomato enriched ketchup is not so much suitable which obtained the lowest score (2.26 ± 0.87, 61.30%) in this organoleptic study.

Key Words: Mushroom, Tomato, Taste, Ketchup, Organoleptic.

INTRODUCTION

Mushrooms are important vegetables all over the world. The postharvest loss of mushrooms is very high due to high water content, seasonal gluts and lack of adequate transport and storage facilities. Hence, promotion of small-scale processing facilities in the production catchments would greatly reduce postharvest loss, make year round availability and contribute to the important of nutrition security in the country. Use of

[1] OSD, DG Health, Mohakhali, Dhaka-1212, Bangladesh.

undertaken to find out the optimal formulation of mushroom ketchup, and to study the storage-life of the developed mushroom ketchup.

MATERIALS AND METHODS

The experiment was conducted in the Quality Control and Quality Assurance (QCQA) laboratory of National Mushroom Development and Extension Centre, Savar, Dhaka during November to December 2013. Different chemicals and materials were used from the laboratory stock.

Quality assessment: Once the ketchups are prepared, their quality will be assessed periodically in order to determine the best combination of ketchups by organoleptic test. The quality parameters included appearance, colour, texture, flavor, test, acceptability, etc. The nutritional quality: such as protein, fat, fibre and mineral as well as microbial status such as presence or absence of moulds, total viable count, total coliform, fecal coliform, *E. coli*, *Salmonella*, etc. were assessed.

Sample collection: Fresh oyster (*Pleurotus high-king* (HK-51) and shiitake *(Lentinus edodes)* mushroom was collected from the culture house of National Mushroom Development and Extension Centre, Savar. Dhaka and other ingredients were collected from local market.

Raw Materials: Tomato, Sugar, Vinegar, Sodium benzoate, Arrarote, Common salt (NaCl), Red chili, Black pepper, Cumin seed, Onion, Garlic, Ginger ingredients were collected from local market. The different treatments used in preparation of mushroom ketchup are given below (Table 1). They were designed as sample combination T1 to T8.

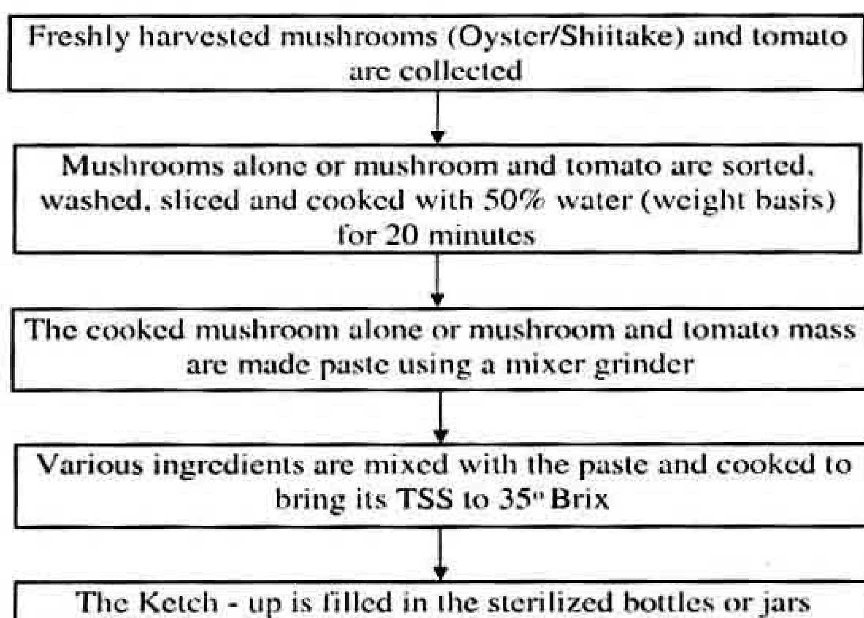

Fig. 1. Flow chart showing the steps of making mushroom ketchup.

Procedure: For making ketchup harvested oyster and shiitake mushrooms were sorted, washed, sliced and cooked with 50% water weight basis for 20 minutes. The cooked mushroom masses are made paste using a mixer grinder. The paste was placed in a stainless steel vessel with spices bag (containing selective spices in specific amount). Various ingredients shown in Table1 are mixed with the paste according to treatments and cooked to bring its TSS to 35^0 Brix. After that heating was continued to require consistency. The finished product was poured into sterilized bottle and sealed. The bottles were treated at 85^0C for 30 minutes and then cooled in air and stored at room temperature (30 ± 2^0C) and also at refrigerator (4 ± 1^0C) (Fig. 1).

Table 1. Composition of different treatments

Amount of Different Ingredients (approx) gram	Treatments							
	T1	T-2	T-3	T-4	T-5	T-6	T-7	T-8
Oyster mushroom (g)	500	500	---	---	300	300	---	---
Shiitake mushroom (g)	---	---	500	500	---	---	300	300
Tomato (g)	--	--	--	--	200	200	200	200
Common salt (g)	80	40	15	15	40	20	20	20
Sugar (g)	250	250	250	250	250	300	250	200
Vinegar (ml)	15	15	15	15	15	15	15	15
Sodium benzoate (g)	0.50	0.70	0.50	0.50	0.50	0.70	0.50	0.70
Onion paste (g)	100	100	100	100	100	100	100	100
Garlic paste (g)	5	10	5	5	5	10	5	10
Ginger paste (g)	30	30	30	30	30	30	30	30
Cumin seed paste (g)	10	10.	10.	10	10	10	10	10
Blackpepper (powder) (g)	1	1	1	1	1	1	1	1
Red chili powder (g)	10	8	5	5	8	5	5	5
Arrarote (g)	2	2	2	2	2	2	2	2
Total (g)	1000	1000	1000	1000	1000	1000	1000	1000

Serving order: Eight samples were served to each consumer in monadic order. The samples were served cooled. The first sample served was removed before the consumer received the second sample. Serving order was randomized so that approximately 50% of the consumers evaluated the "enhanced" sample first and 50% of the consumers evaluated the "non-enhanced" stored sample first.

Sensory/organoleptic evaluation: To carry out sensory evaluation of the two mushroom strains, a panel of evaluators was sought by putting up notices for volunteers. It attracted a panel of 15 both male and female untrained panelists but well educated (majority tertiary and above) and well conversant with agricultural products. The panelists were aged 30 to 55 years. Using a well structured questionnaire, the panelists independently assessed the samples for appearance, taste, flavour, colour, mouth feel (texture) and overall acceptability. Organoleptic scores were 4 = Excellent, 3 = good, 2 = Fair and 1= poor, which were supposed to show the degree of likeness. Panelists indicated their rating for

each sample by choosing the appropriate numerical score. The evaluation was carried out before lunch (Table 2).

Table 2. Mean score of sensory evaluation for mushroom ketch-up

Overall appearance	Colour	Texture	Flavour	Taste	Acceptability
4=Excellent√	4=Excellent	4=Excellent	4=Excellent	4=Excellent	4=Highlyacceptable
3=Good	3=Good√	3=Good	3=Good	3=Good√	3=Acceptable√
2=Fair	2=Fair	2=Fair√	2=Slightlyodorous	2=Fair	2=Less Acceptable
1=poor	1=poor	1=poor	1=Bad odour√	1=poor	1=Not Acceptable

Microbiological Examination

Total viable count (TVC): Twenty five grams (25g) of each sample was diluted in 225 ml of sterile distilled water (diluents) and mixed vigorously by shaking. 1ml of the resultant mixture was aseptically transformed to 9 ml of sterile water in a test tube. The dilution was continued serially until the required dilution was attained. One milliliter (1 ml) of each dilution was inoculated into a sterile Plate Count Agar (PCA). This action was carried out under sterile aseptic conditions. Immediately, inoculated samples and agar medium was mixed thoroughly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar be solidified. Solidified petri dishes were kept in invert position and incubated promptly for 24 ± 2h at 35 ± 2^0C. Then total viable counts were counted.

Presumptive test of total coliform (TC), fecal coliform (FC) and *E. coli*: 25g sample was weighed and added into 225ml of Butterfield's phosphate-buffered water and decimal dilutions were prepared. Number of dilutions was prepared depending on anticipated coliform density. All suspensions were shaked 25 times in arc for 30 cm or vortex mix for 7 second. 1ml of each dilution was transferred to 3 McCartney bottle containing LST broth and inverted Durham's tubes. Inoculated McCartney bottles were incubate at 35 ± 2^0C for 24 - 48 ± 2h. Gas production were examined and recorded in tubes. Gas-negative tubes were re-incubated for an additional 24h and examined (BAM, 1998).

Confirmed test for coliforms: A loopful of suspension was transferred into a tube of BGBB broth from each gas positive LST tube and pellicle was being avoided if present. BGBB tubes were incubated at 35 ± 2^0C and examined for gas production at 24 - 48 ± 2 hour. Then most probable number (MPN) of coliforms was calculated based on proportion of confirmed gas positive tubes for 3 consecutive dilutions using MPN charts.

Confirmed test for fecal coliforms and *E. coli*: From each gas positive LST tube of the presumptive test, a loopful of each suspension was transferred to tube containing BGBB and EC broth. Inoculated tubes were incubated at 44°C for 24 ± 2 h and examined for gas production. If negative, reincubated and examined again at 48 ± 2h. Observing the gas production, the number of fecal coliform and *E. coli* were calculated using MPN charts (BAM, 1998).

Qualitative detection of Salmonella Species

Isolation of Salmonella: 25g sample was weighed and homogenated in 225ml Buffered Peptone water and incubated at 35^0C for 24 h. Three (03) mm loopful (10μl) of incubated broth medium was streaked on bismuth sulfite (BS) agar and xylose lysine desoxycholate (XLD) agar, and Incubated plates for 24 ± 2h at 35^0C. The presence of colonies were examined that may be Salmonella.

Following test was done to identify Salmonella: *Triple Sugar Iron (TSI) agar test:* Each presumed-Salmonella culture was inoculated into tubes of TSI with sterile needle by stabbing and streaking and again inoculated LIA slant in the same manner without flaming the loop. Salmonella gave positive reaction in TSI slants and produced H_2S as shown by the blackening of the media. The butt of the slant would turn to yellow.

Lysine Iron agar test: In LIA slant, the butt color was purple for positive test and was considered a distinct yellow LIA butt as negative result.

Simmons citrate agar: Containing growth from unclassified TSI agar slant was inoculated into the medium using needle by streaking slant and stabbing butt. It was Incubated 96 ± 2h at 35^0C. The results were read as follows: Positive - presence of growth, usually accompanied by color change from green to blue. Most cultures of Salmonella were citrate-positive. Negative - no growth or very little growth and no color change.

Statistical analysis: The consumer acceptability of the developed fry was evaluated by a taste-testing panel. The panelist scores were analyzed with SPSS software.

RESULTS AND DISCUSSION

Obtained mushroom ketchup was shown in Fig. 2. The organoleptic evaluation of mushroom ketchup was influenced by different pretreatments (Table 4). Higher scores for appearance (78.33%), colour (81.67%), texture (78.33%), flavour (130.56%), taste (80%) and acceptability (71.67%). The results of organoleptic evaluation of mushroom ketchups were:

Overall appearance: Results showed that the score for appearance maximum was recorded in treatment T-7 i.e. shiitake mushroom and tomato enriched ketchup. The mean (± SD) of which was 3.13±0.64 and obtained score was 78.33%. The minimum score was recorded in sample T-2 i.e. oyster mushroom enriched ketchup. The mean (± SD) of both of them was 2.27±0.70 and obtained score was 56.67% (Table 3 and Table 4).

Colour: Results showed that the score for color maximum was recorded in treatment T-7 i.e. shiitake mushroom and tomato enriched ketchup. The mean (± SD) of which was 3.27±0.59 and obtained score was 78.33%. The minimum score was recorded in sample

T-4 i.e. shiitake mushroom enriched ketch up. The mean (± SD) of which was 2.00 ± 0.93 and obtained score was 50% (Table 3 and Table 4).

Texture: The score texture maximum was observed in treatment T-5 i.e. oyster mushroom and tomato enriched ketchup. In this treatment there are some variations in other common ingredients such as common salt, sugar, garlic paste, sodium benzoate, red chili powder etc. than T-6. The mean (± SD) of which was 3.13 ± 0.64 and obtained score was 78.33%. The minimum score was recorded in sample T-6 i.e. oyster mushroom and tomato enriched ketchup. The mean (± SD) of which was 2.07±0.88 and obtained score was 51.67% (Table 3 and Table 4).

Flavor: Results showed that the score for flavor maximum was recorded in sample T-5 i.e. oyster mushroom and tomato enriched ketch up. In this treatment there are some variations in other common ingredients such as common salt, sugar, garlic paste, sodium benzoate, red chilli powder etc. than T-6. The mean (± SD) of which was 2.80 ± 0.77 and obtained score was 130.56%. The minimum score was recorded in sample T-6 i.e. oyster mushroom and tomato enriched ketch up. The mean (± SD) of which was 2.27 ± 0.96 and obtained score was 86.11% (Table 3 and Table 4).

Taste: Results showed that the score for taste maximum was recorded in sample T-7 i.e. shiitake mushroom and tomato enriched ketchup. The mean (± SD) of which was 3.20 ± 0.68 and obtained score was 80%.The minimum score was recorded in sample T-1 oyster mushroom enriched ketchup. The mean (± SD) of which was1.40±0.63 and obtained score was 35% (Table 3 and Table 4).

Acceptability: Results showed that the score for overall acceptability maximum was recorded in sample T-7 and T-5. The mean (± SD) of which was 2.87 ± 0.74 and obtained score was 71.67%. The minimum score was recorded in sample T-1 i.e. oyster mushroom enriched ketchup. The mean (± SD) of which was 1.93 ± 0.70 and obtained score was 48.33% (Table 3 and Table 4).

Fig. 2. Prepared mushroom ketchup.

Considering all the parameters it was observed from the study (mean ± SD) that T-7 obtained the highest score (3.00 ± 0.62, 80.83%) followed by T-5 the value of which were 2.80 ± 0.75, 80.09%. The average lowest score in the study was 2.26 ± 0.87, 61.30% that was obtained by treatment T-6. Obtained other scores were T-1 (2.27±0.82, 65.90); T-2 (2.23±0.63, 61.94%); T-3 (2.52 ± 0.74, 71.20%), T-4 (2.44 ± 0.74, 68.52%) and T-8 (2.55 ±0.59, 67.69%) It might be concluded that for preparing mushroom ketch up the ingrediants of all kinds of treatments T-7 i.e. shiitake mushroom and tomato enriched ketch up is highly acceptable (score 3.00 ± 0.62, 80.83%) but regular collection of shiitake mushroom and tomato is difficult. On the other hand preparation of mushroom ketch up using T-1 i.e. oyster mushroom enriched ketch up is not so much suitable which obtained the lowest score (2.27 ± 0.82, 65.09%) in this organoleptic study.

Table 3. Sensory evaluation of mushroom ketch-up prepared from various formulations.

Treatments	Overall appearance (%)	Colour (%)	Texture (%)	Flavor (%)	Taste (%)	Acceptability (%)	Total score (%)	Remarks
T-1	63.33	75.00	63.33	105.56	35.00	48.33	65.09	Hot, salt.
T-2	56.67	60.00	60.00	100.00	43.33	51.67	61.94	Hot, salt, masalla.
T-3	63.33	53.33	68.33	113.89	61.67	66.67	71.20	Hot, salt, garlic, sugar
T-4	70.00	50.00	66.67	111.11	51.67	61.67	68.52	Hot, salt, color
T-5	66.67	76.67	78.33	130.56	56.67	71.67	80.09	Hot, salt
T-6	61.67	58.33	51.67	86.11	55.00	55.00	61.30	Hot, salt, liquid.
T-7	78.33	81.67	65.00	108.33	80.00	71.67	80.83	Hot, salt, sugar
T-8	68.33	68.33	56.67	94.44	61.67	56.67	67.69	Hot, liquid

Table. 4 Obtained score of the individual mushroom ketch-up mean (± SD)

Treatments	Overall appearance	Colour	Texture	Flavor	Taste	Acceptability	Total
T-1	2.53±0.99	3.00±0.85	2.53±0.99	2.20±0.77	1.40±0.63	1.93±0.70	2.27±0.82
T-2	2.27±0.70	2.40±0.63	2.40±0.63	2.53±0.52	1.73±0.59	2.07±0.70	2.23±0.63
T-3	2.53±0.83	2.13±0.83	2.73±0.59	2.60±0.83	2.47±0.74	2.67±0.62	2.52±0.74
T-4	2.80±0.68	2.00±0.93	2.67±0.72	2.60±0.51	2.07±0.96	2.47±0.64	2.44±0.74
T-5	2.67±1.05	3.07±0.59	3.13±0.64	2.80±0.77	2.27±0.70	2.87±0.74	2.80±0.75
T-6	2.47±0.92	2.33±0.82	2.07±0.88	2.27±0.96	2.20±0.77	2.20±0.86	2.26±0.87
T-7	3.13±0.64	3.27±0.59	2.60±0.63	2.93±0.46	3.20±0.68	2.87±0.74	3.00±0.62
T-8	2.73±0.59	2.73±0.59	2.27±0.59	2.80±0.41	2.47±0.74	2.27±0.59	2.55±0.59

Microbiological observation: The results of total viable count (TVC), colifrom, fecal colifrom, *E. coli* and salmonella obtained from mushroom ketchup are summarized below:

Among the eight treatments with different ingredients treatment T-8 contain the highest (49.81%) moisture content while the lowest (16.60%) moisture in T-5 treatment (Table 5). Considering total coliform, fecal coliform, *E. coli* and salmonella, all mushroom ketchup became acceptable after storage 1-2 month in refrigerator. It was remarkable that the total coliform, fecal coliform and *E. coli* were in acceptable level (< 3) while

salmonella absent in all treatments. Results of TVC demonstrated that T-3 treatments contain higher (55 cfu/g) followed by T-6 and the lowest (20 cfu/g) in T-5 treatment.

Table 5: Microbiological analysis of mushroom ketchup

Treatments	Moisture content (%)	Total viable count TVC/TPC (cfu/g)	Total coliform (mpn/g)	Fecal coliform (mpn/g)	E.coli (mpn/g)	Salmonella
T-1	28.02	45	<3	<3	<3	Absent
T-2	36.40	25	<3	<3	<3	Absent
T-3	43.66	55	<3	<3	<3	Absent
T-4	32.17	35	<3	<3	<3	Absent
T-5	16.60	20	<3	<3	<3	Absent
T-6	46.54	50	<3	<3	<3	Absent
T-7	46.61	40	<3	<3	<3	Absent
T-8	49.81	30	<3	<3	<3	Absent

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Contents

1. **Akhter Jahan Kakon, Ruhul Amin, Md. Bazlul Karim Choudhury, Tasnim Farzana, Md. Nur Hossain and Nirod Chandra Sarker**- Effect of Gamma Radiation on Microbial Load of Mushroom Powder 1-6

2. **Manirul shaheen, Md. Mustafizur Rahman, Nirod Chandra Sarker, Md. Mominul Hoque, Abdus Salam Khan and Akhter Jahan Kakon**- Yield Performance of Different Strains of *Auricularia auricula* Mushroom in Winter and Summer Season in Bangladesh 7-12

3. **Nirod Chandra Sarker, Md. Bazlul Karim Choudhury, Abdus Salam Khan, Md. Mominul Hoque, Bimol Chandra Dey and Manirul shaheen**- Effect of Sterilization Practices of Contaminated Mother Culture on Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*) 13-19

4. **Sabiha Azmi Sanjuti, Nuhu Alam, Nirod Chandra Sarker, Md. Bazlul Karim Choudhury, Ruhul Amin and Akhter Jahan Kakon**- Cultural Conditions for Vegetative Growth and Random Amplification of Polymorphic DNA in Different Strains of *Pleurotus sajor-caju* 21-28

5. **Zeenia Afsary, Nuhu Alam, Nirod Chandra Sarker, Ruhul Amin and Akhter Jahan Kakon**- Morphological Characterization of Commercially Cultivated Oyster Mushrooms in Bangladesh 29-36

6. **Md. Bazlul Karim Choudhury, Md. Shahdat Hossain, Md. Masud Hossain, Akhter Jahan Kakon, Mohammed Anwarul Kabir Choudhury, Nashir Uddin Ahmed and Taufiqur Rahman**- *Pleurotus ostreatus* improves Lipid Profile of Obeys Hypertensive Non-diabetic Males 37-44

7. **Tasnim Farzana, Suman Mohajan, Md. Mominul Hoque and Nirod Chandra Sarker**- Comparison of Proximate Nutrients Composition in Local Market and Soya Mushroom Biscuits 45-51

8. **Zeenia Afsary, Nuhu Alam, Md. Bazlul Karim Choudhury, Manirul Shaheen, Tahera Binte Mujib and Nirod Chandra Sarker**- Nutrient and Mineral Content of Different spp. of Oyster Mushroom 53-58

9. **Shailendra Mohon Singha, Nuhu Alam, Nirod Chandra Sarker and Manirul Shaheen**- Influence of Physicochemical Conditions of Culture Medium on Invitro Mycelial Growth in Different Strains of *Volvariella volvaceae* 59-66

10. **Tahera Binte Mujib, Nirod Chandra Sarker, Md. Bazlul Karim Choudhury, Md. Kamrul Hassan, Abdus Salam Khan, Ruhul Amin and Akhter Jahan Kakon**- Organoleptic Evaluation of Mushroom Ketchup 67-75