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Shiitake Mushroom Shows No Detrimental Effect on Kidney Functions of Normotensive Adult Female Populations

Md. Bazlul Karim Choudhury[1], Md. Masud Hossain[2], Md. Erfan Reza[3], Mohammad Golam Mohsin[4] and Akhter Jahan Kakon
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Abstract

Kidney is one of the vital organs in the body which filters impurities out of the bloodstream. Human health concerns such as kidney disease, cancers, diabetes mellitus, and cardiovascular disease are clearly influenced by dietary intake, stressful condition and other lifestyle factors. The major attribute of mushrooms is their medicinal properties; the present study was conducted to evaluate the effect of shiitake mushroom on kidney function of normotensive adult female populations. A total 33 normotensive adult female subject aged from 25 to 66 years and free from renal impairment, diabetes mellitus and other known acute or chronic diseases was included in the study. Three grams of dried shiitake mushroom powder as capsule form was taken by the subjects in three divided doses for three months. The finding of the study showed non-significant small reduction of plasma creatinine (0.81 ± 0.02 and 0.76 ± 0.03, $p = 0.690$) and uric acid (4.43 ± 0.18 and 4.29 ± 0.16, $p = 0.812$); but statistically non-significant small elevation of plasma urea level which was within normal range. Finding of the study suggests that shiitake mushroom has no detrimental effect on kidney rather it might have positive impact on kidney functions of normotensive adult female populations.

Keywords: Shiitake mushroom, Chronic Kidney Disease (CKD), Blood urea, Blood creatinine, Blood uric acid.

INTRODUCTION

Chronic Kidney Disease (CKD) is a global health problem with a high economic cost to health systems. It is also an independent risk factor for cardiovascular disease (CVD). CKD is usually asymptomatic until later stages. CKD is associated with age-related renal function decline accelerated in hypertension, diabetes, obesity and primary renal disorders (Gansevoort *et al.*, 2013). Premature mortality, and/or decreased quality of life is the ultimate fate of CKD. While the direct cause of CKD is unknown (Zhao *et al.*, 2013), note that several studies implicate free radicals and decreased antioxidant activities in the development of CKD complications. Patients with a history of nephrolithiasis (kidney stones) are at an increased risk of CKD.

Chronic kidney disorders can be difficult and costly to treat, enhancing the appeal of natural, alternative therapeutic options. Few definitive medical treatments are available for CKD. The typical approach relies on healthy lifestyle prescriptions like refraining from smoking and tobacco use, limiting alcohol consumption, controlling blood pressure and blood sugar levels, and avoiding drugs like acetaminophen and ibuprofen. As different stressful condition may cause kidney diseases, mushrooms may have free radical scavenging activity thereby can be used for the prevention and treatment for renal injury. Mushrooms pacifically

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support kidney function by helping to regulate bodily fluid levels. Healthy kidneys are an essential component of overall wellness, supporting normal blood pressure and water balance throughout the body.

Although mushrooms are widely known for their great taste and amazing health benefits, but little is known about its effective role on kidney in Bangladesh as well as in other countries. Some researchers observed that the medicinal mushroom *Ganoderma* exerts some protective effects in rats with chronic glomerulonephritis induced by Adriamycin (ADR). *Ganoderma* reduced 24 -hours urinary protein excretion, serum creatinine and cholesterol levels and also improved the pathological changes of kidney tissue in rats (Zhong *et al.,* 2008). Reactive oxygen species (ROS) play a key role in the pathophysiological processes of renal diseases. Thus, antioxidants are expected to decrease the vulnerability of the kidney to oxidative challenges. Polyphenols, abundant in grapes, some vegetables, fruits and tea may act as ROS scavengers, iron chelators and enzyme modulators (Pietta *et al.*, 1998).

Epidermis of Poria mushroom (*Poria cocos*) is one of ancient traditional Chinese medicines (TCMs), which is usually used for the treatment of chronic kidney disease (CKD) for thousands of years in China (Zhao *et al.* 2013). Poria's ability to quell oxidative stress holds promise for patients suffering from chronic kidney disease (CKD) characterized by kidney damage and a gradual decline in renal function. CKD is more prevalent today than it was just a few decades ago, affecting an estimated 26 million Americans over the age of 20. CKD risk is elevated among people with diabetes, obesity, and hypertension, other chronic conditions all of which are also on the rise in the US (Hill *et al.* 2016).

Others have indicated that poria improves nephrotic syndrome, a constellation of symptoms including high urine protein levels, low blood protein levels, high cholesterol and triglyceride levels, and swelling. The mushroom appears to influence body fluid regulation via the inhibition of water and sodium channels, specifically by suppressing the expression of both AQP2, a protein coding gene that plays a role in urinary water reabsorption, and the epithelial sodium channel, which facilitates sodium reabsorption (Lee *et al.*, 2014). Pachyman also acts as an antinephritic. More than 20 years ago, Japanese researchers demonstrated the effectiveness of pachyman isolated from poria against original-type anti-glomerular basement membrane (anti-GBM) glomerulonephritis, a form of autoimmune disease that causes acute inflammation of the kidneys, at least in rats. They concluded that pachyman quells renal disease by inhibiting the deposition of C3, a protein that can disrupt renal function when it builds up in the kidneys (Hattori *et al.*, 1992).

Shiitake mushroom is one of the members of macrofungus family with huge potential for therapeutic applications. It is one of the five most cultivated edible mushrooms in the world. Its production is second only to button mushroom (Chang, 1999; Stamets, 2000). For hundreds of years, medicinal mushrooms are used as alternative medicine in different countries like Korea, China, Japan and eastern Russia (Lull *et al.*, 2005). Shiitake mushroom has shown to present medicinal compounds which are effective in treating various tumors and infections, among other activities which are still being studied (Wang and Zhang, 2009).

Although lot of research suggests huge beneficial effect of shiitake mushroom but there is very little work on its safety level upon the kidney function. For this region the current study was designed on normotensive adult female population. It is expected that the finding of this study will be beneficial for patient with renal injury as well as will contribute to conclude the dilemma whether shiitake mushroom is injurious for the kidney or not.

MATERIALS AND METHODS

The study was conducted at Mushroom Development Institute (MDI) Sobhanbag, Savar, Dhaka. A total of 33 normotensive adult female subjects (systolic BP ≤ 140 mmHg and/ or diastolic BP ≤ 90 mmHg) free from diabetes (fasting plasma glucose < 7 mmol/l) were included in the study. The age ranges of the subject were 25 to 66 years. After getting the written consent of the subjects they were included. Persons with renal impairment and/or other known acute or chronic diseases as well as history of addiction were excluded in the study. During the study any acute condition or medication or malabsorption were excluded. They were allowed to continue the medication which they were getting before. Age, sex, occupation, educational status, marital status, family history and drug history were recorded in a preformed data collection sheet.

The subjects were evaluated for health status at the beginning of the study. Both systolic and diastolic blood pressure was measured following standard procedure using sphygmomanometer by a trained physician. Mean of duplicate measurements was taken. Ten milliliter of fasting blood sample was collected from median cubital vein with all aseptic precautions. Collected blood was poured into fluoride and EDTA containing test tube. It was then gently shaken for proper mixing with the anticoagulants. Within short time the anticoagulant-mixed blood was centrifuged at 3000 rpm for 5 minutes. Separated plasma was transferred into two eppendorf containing 1 ml in each. Fasting plasma glucose (FPG) was estimated by glucose oxidase method, plasma urea was estimated by enzymatic, colourimetric, endpoint – Berthelot method. Plasma creatinine was estimated by alkaline picrate method and plasma uric acid was estimated by uricase colorimetric method. Analysis was done by semi auto biochemical analyzer 3000 evaluation using commercially available reagent kit.

Fresh fruiting bodies of shiitake mushroom were harvested from culture house of MDI. Collected mushrooms were dried at moisture level 4-5% using an electric drier machine. Dried mushrooms were grinded and poured into capsule shells, so that each capsule contains 500 mg powder. Prepared capsules were ready to dispense and preserved into moisture free glass containers.

Subjects had directed to take two capsules three times daily for three months. So, each subject took three grams shiitake mushroom powder daily. After the end of three months the subjects were re-evaluated and all the laboratory investigations were repeated. Obtained results were expressed as mean ± SE. Paired Student's 't' test had done. 95% confidence limit was taken as the level of significance.

RESULTS AND DISCUSSION

In the study the mean (± SE) age (years) of the subjects were 45 ± 2.44, ranges from 25 to 66. The mean (± SE) of fasting plasma glucose (mmol/l) was 5.86 ± 0.23 ranges from 4.3 to 6.8. The mean (± SE) of systolic and diastolic blood pressure (mmHg) were 113 ± 2.64 and 72.95 ± 1.47 ranges from 105 to 140 and 60 to 85 respectively (Table 1).

Table 1. Evaluation of age, fasting plasma glucose, systolic and diastolic blood pressure, of the subjects

Parameter	N	Mean (± SE)	Range
Age (years)	33	45 ± 2.44	25 to 66
FPG (mmol/l)	33	5.86 ± 0.23	4.3 to 6.8
Systolic BP (mmHg)	33	113 ± 2.64	105 to 140
Diastolic BP (mmHg)	33	72.95 ± 1.47	60 to 85

The mean ± SE plasma urea before and 3 months after mushroom supplement was 24.74 ± 0.67 and 25.13 ± 0.77 respectively (Fig. 1). There was non-significant but small elevation of plasma urea ($p = 0.786$), which was within normal physiological range, between the two periods seen.

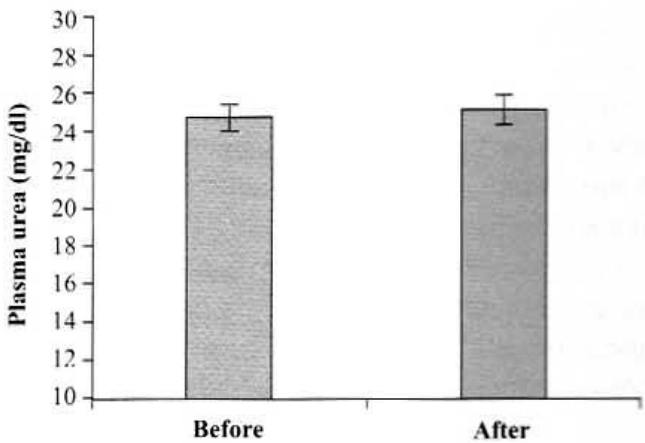

Fig. 1. Mean (± SE) plasma urea before and 3 months after mushroom supplementation.

Considering plasma creatinine (mg/dl) the mean ± SE before and three months after mushroom supplimentation was 0.81 ± 0.02 and 0.76 ± 0.03 respectively. Here also a non-significant small declining mean difference ($p = 0.69$) was observed (Fig. 2).

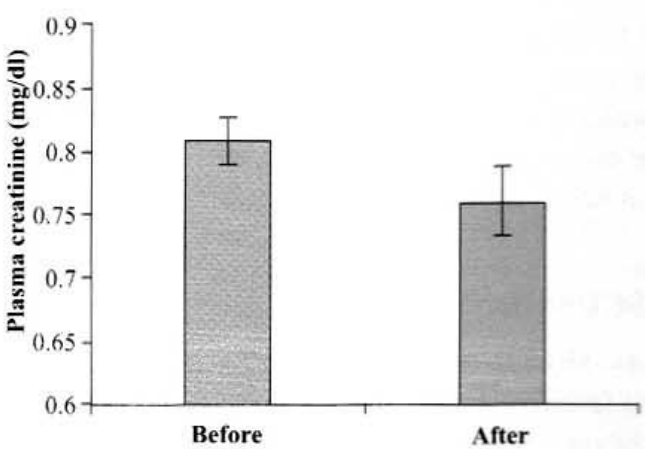

Fig. 2. Mean (± SE) plasma creatinine before and 3 months after mushroom supplementation.

The mean ± SE plasma uric acid (mg/dl) before and 3 months after mushroom treatment were 4.43 ± 0.18 and 4.29 ± 0.16 respectively. No statistically significant mean different was observed (p = 0.812), between the two periods (Fig. 3).

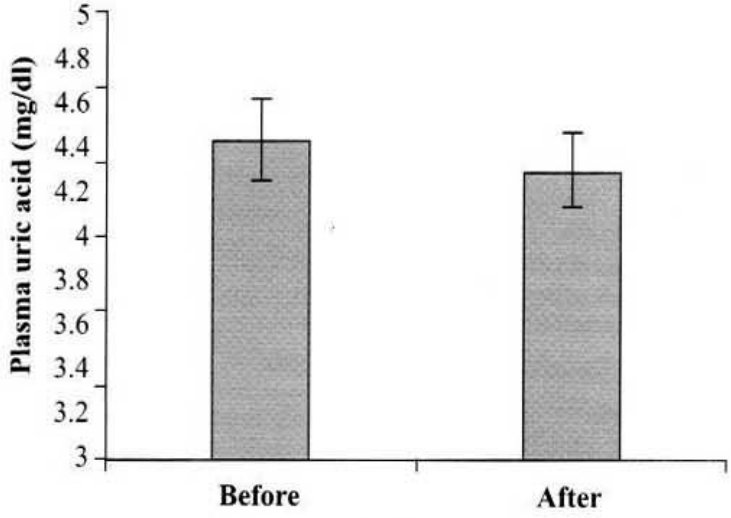

Fig. 3. Mean (± SE) plasma uric acid before and 3 months after mushroom supplementation.

It was noticeable in the study that although there is small raising tendency of plasma urea, plasma creatinine and uric acid level had small declining tendency. But none of these changes was statistically significant. As mushrooms are high quality protein rich food (Breene, 1990), it is mentionable that urea is one of the protein metabolites. So, regular consumption of shiitake mushroom naturally might be responsible for small increase in blood urea level. Again, uric acid and creatinine are also protein metabolites. So, these two parameters also normally can rise in increasing protein intake. But this study showed instead of rising, there is small reduction of uric acid and creatinine level in the blood, suggesting the ameliorative effect of shiitake mushroom on kidney functions.

One of the previous study shows *Pleurotus ostreatus* causes non significant small rising of palsma urea and creatinine and minute reduction of uric acid level of hypertensive diabetic male volunteers (Choudhury *et al.*, 2011). Current study is partially supportive of the previous one but instead of small rising here observed small reduction of plasma creatinine level. This difference may be due to variation in mushroom strain. In another study Choudhury *et al.* (2013) observed the ameliorative effect of *Pleurotus ostreatus* on kidney function of hypertensive male subjects.

In a study it was observed that Poria (one type of mushroom) appears to support kidney health in a number of ways. First, it possesses antioxidant properties. A free radical scavenger, poria was shown in one study to reduce intracellular oxidative damage. The study's authors argue that the mushroom's antioxidant activity could play a role in enhancing cell viability, implicating it as a natural agent in the treatment of many diseases marked by oxidative stress (Park *et al.*, 2009).

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Nutritional Comparison of Different F1 (Hybrids) Generations and Their Parents

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Abstract

Pleurotus mushrooms are considered as a good source of nutrition, because of the presence of high amount of proteins, vitamins and minerals. Apart from having high nutritional value, it also possesses medicinal properties because of low fat and cholesterol content. The nutritional composition of five cross F1 (hybrids) and their parents of oyster mushrooms such as C1(Pop. PG), C2(Pop.Py), C3(Pop.PO2), C4(Pop.PO2), C5(PO2.HK51) and C8(Pop. Flo) were determined. The protein content was found highest in Py and Po96 (20.60g/100g dry sample) and lowest in C3F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus djmour*, (4.64g/100g dry sample). The carbohydrate content was found highest in C5F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus highking-51* (63.16g/100g dry sample) followed by C4F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus citrinopileatus* (55.84g/100g). The carbohydrate content was found lowest in C8F1 *ie* crossing between *Pleurotus djmour* and *Pleurotus florida* (23.52g/100g dry sample). The highest lipid content (6.30 g/100g) was estimated in C3F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus djmour* and lowest lipid content (2.23 g/100g) was estimated in C8F1 *i.e.* crossing between *Pleurotus djmour* and *Pleurotus florida*. The fiber content was found highest in C8F1 *i.e.* crossing between *Pleurotus djmour* and *Pleurotus florida* (58.89g/100g dry sample). The fiber content was found lowest in C5F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus highking-51*, (12.43 g/100g dry sample). The total ash content was found highest in C4F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus citrinopileatus*, (16.40 g/100g dry sample). The total ash content was found lowest in C2F1 *i.e.* crossing between *Pleurotus djmour* and *Pleurotus citrinopileatus*, (7.95 g/100g dry sample). Obtained findings from the study might be helpful for deciding the choice/selection of suitable genotype to fulfill the specific nutritional requirement and hence it can take part to overcome nutritional problems.

Keywords: Lipid, Fibre, Moisture, Protein, Minerals, Carbohydrate, Ash, Metabolizable energy.

INTRODUCTION

Mushrooms are being recognized as important food items from ancient times. Their usage is being increased day by day for their significant role in human health, nutrition and disease. Malnutrition and iron deficiency are serious health problems in developing countries and more than 80% of total populations are suffering from malnutrition. Mushrooms of *Pleurotus* sp. are commonly called 'oyster mushrooms'. They are the second most popular mushrooms after button mushroom all over the world (Adejoye *et al.*, 2006) and the most popular in Bangladesh. Approximately 70 species of *Pleurotus* have been recorded to date and new species are discovered more or less frequently although some of these are considered identical with previously recognized species. Oyster mushroom is a good source of non-starchy carbohydrates with high content of dietary fiber and moderate quantity of protein with important amino acids, minerals and vitamins (Croan, 2004). Cultivation of *Pleurotus* is a valuable and profitable agribusiness and is gaining rapid popularity amongst the entrepreneurs (Naraian *et al.*, 2011) because the production cost is low and easy to adopt by the marginal farmers (Banik & Nandi 2004; Pant *et al.*, 2006). More of the governments

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of developing countries also considers mushroom as a suitable component for a balanced diet as it comprised adequate amount of macro and micro nutrients. Tariqul *et al.* (2012) reported that nutrient composition of the food has to be made well known and available to the mass population for a balanced diet. However, nutritional composition is affected by many factors including 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).

Knowing the nutrient composition of the food is also important for developing food composition database. Sometimes, commercial strains of oyster mushroom grown in several consecutive subcultures resulting in yield reduction and quality deterioration (Naraian *et al.*, 2011). In order to make the cultivated commercial species economically attractive to the growers, continuous yield improvement practices are essential (Uhart *et al.*, 2008). In addition, high yielding variety can be developed through hybridization and genetic modification of the existing strain may also be the option to solve this problem. It is easy to introduce a new strain if the strains are economically feasible and nutritionally viable. In Bangladesh, mushroom cultivation has been started recently and Mushroom Development Institute (MDI) has introduced some new genotype of oyster mushroom in Bangladesh. But the yield potentiality and the nutrient composition are unknown. Therefore, more information about this genus and its species is necessary to identify good strains to ensure continuous yield improvement (Uhart *et al.*, 2008) and to screen the efficient varieties for Bangladesh. The objective of this work was to evaluate the proximate composition of oyster mushroom strains newly introduced in Bangladesh.

MATERIALS AND METHODS

Mushroom was cultivated and harvested in the culture house and the study was carried out in the'Quality Control and Quality Assurance' laboratory of Mushroom Development Institute (MDI), Savar, Dhaka from June 2014 to March 2015.

Treatments: Fruiting body of five different F1(hybrids) of oyster mushrooms and their parents such as C1(Pop.PG), C2(Pop.Py), C3(Pop.PO2), C4(Pop.PO2), C5(PO2.HK51) and C8 (Pop.Flo) were selected in this study for investigation. Mushroom was cultivated on sawdust and fruiting bodies were harvested. Then the selected fruit bodies were taken for nutritional analysis. Fresh mushroom was taken and then they were dried for the estimation of protein, lipid, crude fibre and total ash.

Determination of total protein: Five gram of grinded mushroom was taken with 50 ml of 0.1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000rpm by a table centrifuge machine (*DIGISYSTEM: DSC-200T; Taiwan*). The supernatant was collected and total protein content was measured according to the method of Lowry *et al.* (1951). For the determination of protein content from fresh mushroom, 5g was taken with 50ml phosphate buffer and homogenized with a tissue homogenizer (*Polytron: PT 1200*). Five ml of homogenized was taken with 50 ml of 0.1N NaOH and protein content was determined as mentioned above.

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 1000rpm 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 total lipid from fresh mushroom, 5g was taken with 50ml phosphate buffer and homogenized with a tissue homogenizer. Five ml of homogenized was taken with 50 ml 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 (We) in an electric balance (*KEYI: JY-2003; China*). The crucible was heated in a muffle furnace (*Nebertherm: Mod-L9/11/c6; Germany*) at 600^{0}C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

Crude fibre (g/100g sample) = [100-(moisture + fat)] × (We-Wa) / Wt. of sample (*Raghuramulu et al.,* 2003).

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 5-6 hours at 600^{0}C. It was then cooled in a desiccator 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:

Ash content (g/100g sample) = Wt. of ash × 100 / Wt. of sample taken (Raghuramulu *et al.,* 2003).

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation (Raghuramalu *et al.,* 2003):

Carbohydrate (g/100g sample) = [100 – (Moisture + Fat + Protein + Ash + Crude Fiber)]

RESULTS AND DISCUSSION

Several nutritional parameters were measured. Table 1 shows the nutritional parameters of dry mushrooms.

Protein content: The protein content was found highest in Py and Po96 (20.60g/100g dry sample) followed by PO2 (10.23g/100g). The protein content was found lowest in C3F1 *ie* crossing between *Pleurotus ostreatus* and *Pleurotus djmour*, (4.64g/100g dry sample) (Table 1). It was observed that the protein content has low in F1 genotype whereas almost

each parent content high protein. This finding was differed by the study of Khlood and Ahmad (2005), who reported that strain PG1 contained more than 5% crude protein comparing to the strain PO2. Chang *et al.* (1981) also reported that the fruiting bodies of mushrooms contain 26.6-34.1% crude protein. *Pleurotus* species is considered as a good source of protein and this protein content varies accordingly with different aspects; like genetic structure of species, composition of substrate, physical and chemical differences in the growing medium, size of the pileus and the time of the harvest. Another reason can be the presence of chitin, the nitrogen containing polysaccharide of the fungal cell walls which is present in different amounts in various mushroom species, that consequently results in different protein concentration (Manzi *et al.*,1999).

Carbohydrate content: The carbohydrate content was found highest in C5F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus highking-51* (63.16g/100g dry sample) followed by C4F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus citrinopileatus* (55.84g/100g). The carbohydrate content was found lowest in C8F1 *i.e.* crossing between *Pleurotus djmour* and *Pleurotus florida* (23.52g/100g dry sample) (Table 1). It was observed that the carbohydrate content has low when crossing with the parent *Pleurotus djmour*. This result is partially supported by Kalac, 2009 who observed that the fruiting bodies of mushrooms contain 40.6%-53.3% carbohydrate. Carbohydrate content includes fibre, such as the structural polysaccharides beta-glucans, chitin, hemicelluloses and pectin substances. The high carbohydrate content is due to the higher level of non-fiber carbohydrates such as sugars (Reis *et.al.*, 2012). The sugars present in mushrooms are fructose, mannitol, sucrose, trehalose, glucose and myo-inositol.

Lipid content: The lipid contents varied in different genotype from 2.23– 6.30g per 100g of dried sample. The highest lipid content (6.30 g/100g) was estimated in C3F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus djmour* which was followed by Parent Py and Po96(4.60g/100g). The lowest lipid content (2.23 g/100g) was estimated in C8F1 *i.e.* crossing between *Pleurotus djmour* and *Pleurotus florida* (Table 1). This result was partially supported by Alam *et al.* (2007), who reported that lipid content (ranging from 4.3% to 4.4%) in case of *Pleurotus florida* and *Pleurotus sajor-caju* on different substrates. Mushrooms are low calorific food with very low amount of fat and cholesterol. Fat fractions of mushrooms mainly contain unsaturated fatty acids as reported by Yilmaz *et al.*, 2006 and Pedneault *et al.*, 2006. Hugaes, 1962 observed that mushrooms are rich in linolenic acid, which is an essential fatty acid. Shashirekha *et al.*, 2005 also reported that linoleic acid is the most dominant unsaturated fatty acid cultivated on both rice straw and cotton seed supplemented mushroom. Linoleic acid serves on the basis for production and transformation of mushroom flavour constituents (Rajarathnam *et al.*, 1990).

Fibre content: The fibre contents in different genotype of fruiting body were 12.43 – 58.89g per 100g of dried mushroom. The fiber content was found highest in in C8F1 *ie* crossing between *Pleurotus djmour* and *Pleurotus florida* (58.89g/100g dry sample) followed by C3F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus djmour* (52.31g/100g). The fiber content was found lowest in C5F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus highking-51*, (12.43 g/100g dry sample) (Table 1). Oyster mushroom can serve as the least fattening food because it contains no starch, low sugar content and high amount of fibre (Samuel *et al.*,2012). In general, mushroom contains 90% water and 10% dry matter.

Table 1. Nutritional composition of different F1 generations and their parents (g/100g of dried sample)

Mushroom genotype		Protein	CHO	Lipid	Fibre	Total ash
	Pop	5.40	47.69	4.12	31.23	11.56
	PG4	5.10	56.88	3.04	25.67	9.31
C1(Pop.PG)	C1F1	6.44	29.60	4.53	49.78	9.65
	Pop	5.40	47.69	4.12	31.23	11.56
	Py	20.6	39.4	4.60	19.90	10.6
C2(Pop.Py)	C2F1	6.50	31.60	2.23	51.72	7.95
	Pop	5.40	47.69	4.12	31.23	11.56
	PO2	10.23	41.59	3.60	29.82	14.76
C3(Pop.PO2)	C3F1	4.64	28.46	6.30	52.31	8.29
	PO2	10.23	41.59	3.60	29.82	14.76
	Po96	20.6	39.4	4.60	19.90	10.6
C4(Pop.PO2)	C4F1	7.24	55.84	4.50	16.02	16.40
	PO2	10.23	41.59	3.60	29.82	14.76
	HK51	5.20	54.07	2.90	28.35	9.48
C5(PO2.HK51)	C5F1	7.46	63.16	4.01	12.43	12.94
	Pop	5.40	47.69	4.12	31.23	11.56
	Flo	8.80	39.15	4.17	36.05	11.82
C8(Pop.Flo)	C8F1	5.72	23.52	2.23	58.89	9.64

Total ash content: Considering total ash the findings were varied from 7.95 – 16.40% per 100g of dried mushroom. The total ash content was found highest in C4F1 *ie* crossing between *Pleurotus ostreatus* and *Pleurotus citrinopileatus*, (16.40 g/100g dry sample) followed by C5F1 *i.e.* crossing between *Pleurotus ostreatus* and *Pleurotus highking-51*, (12.94 g/100g dry sample) and the parent *Pleurotus ostreatus* ie PO2 (14.76g/100g). The total ash content was found lowest in C2F1 *i.e.* crossing between *Pleurotus djmour* and *Pleurotus citrinopileatus*, (7.95 g/100g dry sample) (Table 1). This result was supported by Mostak *et al.* (2016) who reported that strain PG1 contained maximum (12.8%) ash percentage followed by strain HK (11.5%).

The present study suggests that oyster mushrooms differ from each other in nutritional composition although they are of same genus, however each species are nutritious with high protein and fiber value with low fat. Hence fruiting bodies of oyster mushrooms can be taken regularly as a protein supplement or as an alternative to fish and meat. Vegetarians could also eat mushrooms because it might serve as alternative protein supplements in their diet. The low lipid and high fiber contents of the oyster mushrooms make it health beneficial food items especially against heart diseases and diabetes. In some cases, nutritional parameter such as high fibre content, low carbohydrate, maximum ash content F1 genotype better than individual parent. Raising positive awareness on nutritional status and motivating rural people to include mushroom in their daily diet may improve the undernourished state in rural areas of developing countries. This may also help to protect people from diseases caused by nutrient deficiency.

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Studies on Packaging of Fresh White Button Mushroom (*Agaricus bisporus*) for Short-term Storage

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Abstract

White button mushrooms (*Agaricus bisporus*)are highly perishable in nature and they start deteriorating immediately after harvest. Hence, it needs to be consumed fresh or processed within a short span of time to avoid the post-harvest loss of this high value commodity. Freshly harvested mushrooms are packed in poly bags and distributed to local market for day-to-day retail sales. To find out the suitable packaging material for the local market in temperate region, a packaging and storage study of freshly harvested button mushroom in polyethylene bags of different gauge thickness was carried out for 3 days period. The different gauge polyethylene bags studied were of 140, 180, 240 and 300 gauge, which are available commercially and commonly as well. Pin holes were made in the polyethylene bags to facilitate the respiration of the packed mushroom. It was found that the fresh button mushroom fruit bodies could be stored in polyethylene bags of 140 gauge thickness with two pinholes based on the weight loss and whiteness reflectance values of the stored mushroom. However, when the gauge thickness was increased to 240 gauge, the polyethylene bags with four perforations yielded better results, which were on par with the 140 gauge thickness polyethylene bags with two perforations. Hence, polyethylene could be successfully used for packing of fresh mushrooms to increase their shelf life as well as to maintain the whiteness with minimum weight loss during storage.

Keywords: White button mushroom, *Agaricus bisporus*, Packaging, Post-harvest, Shelf life, Polyethylene.

INTRODUCTION

White button mushroom (*Agaricus bisporus*)is a rich source of good quality protein, having most of the essential amino acids, minerals and vitamins with low calories. Among the different edible mushrooms commercially cultivated in India, white button mushroom contributes about 85% ofthe country's production against its global share of about 31 per cent (Arumuganathan *et al.*, 2017). India contributes about 3% of the total world button mushroom production (Prakasam, 2012). Mushrooms are highly perishable and they start deteriorating immediately after harvest due to high rate of metabolic activity, which results in browning, wilting, liquefaction, loss of texture, aroma, flavour, etc, making it unsaleable. The rate of respiration of the harvested mushrooms is relatively higher in comparison to other horticultural crops and this result in a shorter postharvest life. This is primarily due to their thin and porous epidermal structure (Rai and Arumuganathan, 2008). Therefore, they cannot be stored for more than 24 hours at ambient conditions and has to be marketed in fresh form.

Due to the presence of high moisture content and delicate in texture, it cannot be stored for more than 24 hours at the ambient conditions prevailing in the tropical conditions. But, they can be stored for more than a day under temperate conditions. The production of mushroom is done throughout the year by the environmentally controlled units, but the seasonal growers come into play during the winters and the supply at the

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local market exceeds causing less profit due to fall in price and spoilage due to market surplus (Arumuganathan *et al.*, 2004). Most of the freshly harvested mushrooms produced from environment controlled units or seasonal mushroom growing structures are marketed domestically to cater the need of the local consumers for consumption in fresh form and the rest of the mushroom is processed for long-term usage. Either consumption of fresh mushroom or processing or fresh mushrooms needs to be done within a short span of time after harvest to avoid the post-harvest loss of this high value commodity. During the recent years, there has been an increased emphasis and awareness on the consumption of mushrooms, which is reflected in the price of the produce. In India, the mushroom market is largely the contribution of small and marginal farmers with limited resources, who are dependent on local market for the sale of their produce. In developed countries, the fresh mushrooms are packed in the 'state of the art' technology for the retail market, which are normally packed in modified atmosphere packaging (MAP) or controlled atmosphere packaging (Arumuganathan and Rai, 2011). These modified atmosphere packaging and controlled atmosphere packaging are very costly to adopt for the mushroom growers in India and therefore they pack the mushrooms in small quantities preferably 250 g or 500 g packs using the locally available packaging material. Therefore, some convenient, inexpensive and easily available packaging method using the locally available poly packs are required to attain a potentially inexpensive modified atmosphere to keep the mushroom remain fresh for local market. Button mushrooms are generally packed in polypropylene bags of less than 100 gauge in most cases and often it is observed that the retails packs of mushrooms contains water droplets inside which was due to respiration of mushrooms that tends to condensate inside the package and on the mushrooms. This further worsens the product quality in terms of colour and affects the shelf life of the product in a greater extent. Whiteness is the most important quality attribute in the button mushroom, besides, of course, shape and size. Consumers have high preference for whiteness of the mushroom amongst the various quality parameters of the button mushroom. The economic loss of mushroom to the growers and distributor is determined by the weight loss of the produce. Thus, maintenance of quality in terms of colour and weight during post-harvest period is very important to market the fresh produce of mushrooms profitably in local markets. Hence, these two important post-harvest parameters that decides the consumer preference and profitability of mushroom were studied to find the suitable packaging for packing mushrooms at retailer's level for local market. The present study was conducted up with an objective to investigate the effect of polyethylene bags on the two important parameters for storage of white button mushrooms up to 3 days period under ambient condition to boost the shelf life in temperate region.

MATERIALS AND METHODS

Fresh harvested white button mushroom fruit bodies byhand picking method from the first flush of the crop were obtained from the Test cum Demonstration Facility (TDF) of Directorate of Mushroom Research (DMR), Solan, Himachal Pradesh. Harvested mushrooms were sorted out for any damage or discolouration and the stems were trimmed further to get

uniform size product for conducting the present study. Trimmed mushrooms were washed thoroughly in the solution of 0.05% Potassium metabisulphite. Washing pre-treatment of mushroom was done gently to remove the adhering dirt as well as to retain the whiteness of the mushroom. Polyethylene bags of different gauge size *viz.*, 140, 180, 240 and 300 gauge were brought from Parwanoo Plastics, Parwanoo, Himachal Pradesh. Pin holes of 2 mm diameter were made on the each side of the polyethylene bags and polyethylene bags with no holes, 2 holes and 4 holes were the three different treatments selected for this study. Two hundred and fifty grams of mushrooms were weighted, packed in the polyethylene bags and sealed carefully for each treatment. The experiment was laid out with three replications each. The packed mushrooms were stored in the ambient condition for 3 days. The atmospheric temperature and relative humidity during the study period ranged from 24.3-26.2°C and 56-72 %, respectively. Two important deciding parameters at the retailers level namely whiteness of the mushroom and weight loss during the storage period were studied for the packed mushroom at daily intervals. The whiteness values of mushroom fruit bodies particularly the button mushroom cap was recorded using reflectance meter. The meter was calibrated every time and whiteness readings of mushrooms were recorded for six times for each treatment. The initial whiteness value of fresh white button mushrooms recorded at the start of the experiment was 84.15. Whiteness values were recorded daily and the reduction in percent whiteness value of mushroom was calculated using the following formula.

$$\textit{Loss of whiteness value (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

where,

W_1 = Initial whiteness value of mushroom

W_2 = Final whiteness value of mushroom

Weight of the packed mushroom were recorded daily using a high precision electronic digital balance and per cent weight loss was calculated using the following formula.

$$\textit{Weight loss (\%)} = \frac{M_1 - M_2}{M_1} \times 100$$

where,

M_1 = Initial weight of mushroom, g

M_2 = Final weight of mushroom, g.

RESULTS AND DISCUSSION

Experiments were conducted on packaging of white button mushroom in polyethylene bags and the results and data in terms of percent reduction in whiteness value of mushroom and percent weight loss of mushroom are presented in Table 1. The data presented in Table 1 for the percentage loss in whiteness value and percentage loss in weight showed that the weight as well as whiteness values decreased during the progression of the storage period.

Table. 1. Effect of thickness and number of holes in polyethylene bag on loss of whiteness value and weight loss of button mushroom

S l . No.	Thickness of polyethylene bag	No. of holes	Loss of whiteness value (%)			Weight loss (%)		
			First day	Second day	Third day	First day	Second day	Third day
1	140	0	1.70	3.37	9.55	1.75	2.13	2.65
2	140	2	1.25	5.64	6.76	3.06	3.35	4.11
3	140	4	6.77	12.52	14.52	3.51	3.77	4.87
4	180	0	2.24	4.63	5.13	3.16	3.60	4.29
5	180	2	1.01	1.26	3.26	3.21	4.98	6.19
6	180	4	9.51	12.36	12.72	4.48	5.27	9.94
7	240	0	6.17	9.66	11.53	4.80	5.02	5.77
8	240	2	1.83	2.58	5.18	5.23	5.55	6.22
9	240	4	4.06	8.2	9.23	2.99	4.24	5.24
10	300	0	7.53	10.41	14.04	4.88	5.11	5.82
11	300	2	5.62	6.06	7.35	5.36	5.60	6.34
12	300	4	4.03	5.70	6.48	3.01	4.28	5.29

It could be observed from the Table 1. that the percentage reduction in whiteness after first day of storage was least in case of polyethylene bags of 180 gauge thickness with two holes (1.01%) and it was followed by polyethylene bags with 2 holes of 140 gauge (1.70%).It is also evident from the table that at the end of the third of storage, the polyethylene bags of 140 gauge thickness with four holes recorded highest loss in whiteness value as 14.52 % and it was followed by polyethylene bags with 300 gauge thickness. As observed in Table 1, the percent loss in colour value of the button mushroom was declining over the period of storage irrespective of the gauge thickness of packaging material. The main reason for the decreased whiteness is due to the increase in the degree of browning by various enzyme activities and non-enzymatic browning. The improved colour might also be due to lower microbial growth resulting from low O_2 (Hotchkiss and Banco, 1992). The browning of mushroom cap and stalks was more in the bags with no holes when compared to bags with holes. This might be due to the fact that the enzymatic and non-enzymatic activities were upsurge in the ambient condition that in turn induced more browning and ultimately resulted in increased brownness of the mushroom. The results are in accordance with the earlier studies of Gormley (1975), Burton (1988) and Briones *et al.* (1992) who had assessed the colour of button mushroom and observed similar decreased in the luminance parameter during storage and was correlated with the market value of mushrooms. De la Plaza *et al.* (1995) also observed the increase in browning of mushrooms packed in polyethylene at 18^0C temperature and this could be due to the increase of oxidative enzyme activities at high temperature. It was also observed that the 240 and 300 gauge polyethylene bags with four holes registered less reduction in whiteness compared to no holes. This could be because increase in thickness of the bags will change the water vapour and oxygen permeability of the films, which in turn affect the whiteness values of the packed mushroom.

It is also observed from the Table 1 that the maximum weight loss was observed in the mushrooms packed with 180 gauge polyethylene bags with four holes (9.94 %) and it was followed by 300 gauge polyethylene bags with two holes (6.34 %). The minimum weight loss was recorded for mushrooms packed with 140 gauge polyethylene bags with no holes (2.65 %) and it was followed by same gauge polyethylene bags with two holes (4.11 %).

The physiological weight loss might be the result of respiration and evaporation rather than transpiration and it depends on the relative humidity of the storage atmosphere. Mushrooms stored with more holes registered more weight loss and this might be due to the fact that the mushroom with more holes respire more because of more available oxygen and that could led to reduction in weight by moisture loss. The weight loss from the mushroom surface is affected by the loss of carbon upon formation of CO_2 during respiration (Saxena and Rai, 1988). Mushrooms lack a protective epidermal structure to prevent excessive moisture and therefore have very high transpiration rates (San Antonio and Flegg, 1964; Roy *et al.*, 1995). The increased weight loss is also attributable to growth related metabolism of the mushrooms and the utilization of intracellular nutrients (Tano*et al.*, 1999). Water loss is also affected by storage temperature, humidity, air movement and area of evaporation (Burton, 1988). Temperature affects the evaporation directly by increasing the movement and escape of water molecules and low humidity associated with high temperature, will compound the effect by increasing water vapour pressure deficit.

CONCLUSION

It can be concluded from the study that white button mushroom could be stored under ambient condition in temperate region for three days using polyethylene bags. It can be also be concluded that polyethylene bags of minimum gauge thickness with two holes are suitable for packing and storing mushrooms. But in case of bags with higher gauge thickness more number of holes are needed for successful storage. Polyethylene bags of 140 or 180 gauge thickness required two holes for storing button mushrooms for three days and if the storage is done in polyethylene bags of 240 gauge or 300 gauge, then four holes are required. Hence, it can be concluded that packing and storing button mushrooms in polyethylene bags depends upon the thickness. Higher the thickness of polyethylene bags increased the number for perforations needed for its safe storage.

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RAPD Fingerprinting in Four Strains of Golden Oyster Mushroom Available at MDI

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Abstract

Genetic diversity in four different strains of golden oyster mushroom was studied based on their random amplification of polymorphic DNA. Fruiting body of four different strains of golden oyster mushroom i.e. PY-1, PY-2, PO96-1, PO96-2 were collected from MDI culture house. All the five RAPD primers amplified a total of 120 bands from the four strains of mushroom ranged from 380 to 3000bp. Primer OPA-07 amplified a specific band and rest of all the primers amplified polymorphic bands. All of the primer except OPA-07 (96.77%) showed 100% polymorphism. The highest linkage distance (7.00) was recorded between strain pairs PY-2 and PO96-1. Genetic relationships among the strains at the average distance of 5.00, showed two major clusters C_1 and C_2. Major cluster C_1 comprised PY-1 and PY-2. At the linkage distance of 3.00, PY-1 and PY-2 strains were subdivided from each other. On the other hand, major cluster C_2 belonged to PO96-1 and PO96-2. These strains were also differentiating from each other at the linkage distance of 2.00. The current study demonstrated that the RAPD analysis is useful for characterization, genetic diversity and identifying relationships among the strains of *Pleurotus citrinopileatus*. Present study also revealed that the strains were mostly similar with each other except some variation.

Keywords: RAPD, Fingerprint, Golden oyster, Strain, Genetic diversity.

INTRODUCTION

The determination of genotypic identity is also important to make suitable biological analysis about population, structure and evolutionary ways within and among species (Mahmood, *et al.*, 2009). Genetic diversity is a commonly used expression to describe the heritable variation found within biological entities and can be measured at the individual, population, and species level. Without genetic diversity, a population cannot evolve and it cannot adapt to environmental changes. Given the importance of genetic diversity for both short-term adaptation to environmental changes and long-term impact on species and communities, the preservation of genetic diversity has been a high priority in many conservation programs. Genetic diversity can also be used to monitor or infer other historical or demographic processes that are essential for making wise decisions related to biodiversity management at the species level and above (Kadhila, 2010). Various molecular markers have been introduced to detect polymorphism between organisms, to produce genetic markers, and to construct genetic maps (Williams *et al.*, 1990). The aim of the present experiment was carried out to determine the relationship and genetic diversity among the strains of golden oyster mushroom.

MATERIALS AND METODS

Fruiting body of four different strains of golden oyster mushroom i.e. PY-1, PY-2, PO96-1, PO96-2 were collected from MDI Savar, Dhaka during the period from January to June in 2015.

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DNA extraction: Filamentous fungi have strong cell walls which are often difficult to rupture in traditional method. In the present investigation, modified method of Aljanabi *et.al.* (1999) has been used to isolate the total genomic DNA from mushroom. DNA of four different strains was extracted from 0.2-0.3 g fruiting body of each strain. It was grinded in extraction buffer (200 mM Tris-HCI-pH 8.5,250 mM NaCI, 25mM EDTA, 20% CTAB,0.5% SDS) with a mortar pestle. The lysates were incubated at 65°C for 40 min in water bath and centrifuge 30 mins at 10,000 rpm. DNA was precipitated from the supernatant by adding equal volumes of isopropanal and resultant pellet was washed with 70% ethanol. The DNA palette was air dried and dissolved in 50µl TE buffer. DNA quantification was performed and a dilution of 50ng/µl was used in downstream application.

Precautions of DNA isolation: The following precaution should maintain during DNA extraction-

- While working with chemicals and while sampling was wear hand gloves. It is recommended to wear gloves all over the time during extraction procedure to prevent RNA contamination from the body.
- While preparing the stock solutions the pH measurement of solutions was done very carefully. The pH meter calibration was essential before measuring the pH of the solution.
- Sodium sulfite was added into the extraction buffer just before use for proper functioning.
- All glassware, micropipette tips, centrifuge tubes, glass pipettes, distilled water and buffer solutions were autoclaved to keep away from DNA contamination. Scissors, forceps, mortar and pestle etc. were sterilized with absolute ethanol.
- During grinding, longer grinding time was maintained because it can result partial degradation of DNA.
- Serial number of eppendorf tubes was maintained.
- Eppendorf tubes were kept on ice cold rack during isolation process.

RAPD analysis: Genomic DNA was amplified by the RAPD technique (Williams *et al.*,1990) in which six sorts of arbitrary 10-base of oligonucleotide primers (Operon technologies Inc.) such as OPA-03 (5` AGTCAGCCAC 3`); OPA-07 (sequence 5`GAAACGGGTG 3`); OPA-10 (sequence 5` GTGATCGCAG 3`); OPD-10 (sequence 5` TGTCTGGGTG 3`); OPC-12 (sequence 5` TGTCATCCCC 3`). RAPD-PCR recation was performed using a thermal cycler with an initial denaturation stage of 5 min at 94°C, followed by 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 36°C, extension for 2 min at 72°C and a final extension for 10 min at 72°C.

Gel electrophoresis and RAPD data scoring: RAPD products were electrophoresed on 1.4% agarose gel in lX TBE buffer for 1.15hr at 100 v with lkb DNA ladder as a size marker and then stained while agitated in an EtBr solution (0.5% µg/ml). The stained gels were visualized under a UV transilluminator and photographed using Bio-Rad gel documentation system. The amplification product generated by each RAPD primer were scored as 'l' or 'o' for presence or absence of specific allele respectively. To estimate the similarity and genetics distance among different species, cluster analysis based on Nei's unweighted pair-group with arithmetic average (UPGMA) was performed using the statistica software and a dendrogram was constructed.

RESULTS AND DISCUSSION

DNA isolation and quantification are the prerequisite for DNA fingerprinting and genetic diversity study of varieties based on molecular markers. An easy and efficient method for DNA isolation of

mushroom was adopted using available chemicals and simple equipments. The isolated DNA of four mushroom strains (PY-1, PY-2, PO96-1, PO96-2) were successfully used for DNA fingerprinting using the five RAPD markers (OPA-03, OPA-07, OPA-10, OPD-10, OPC-12).

Isolation of DNA: In the present investigation, DNA isolation protocol of MDI was used to isolate total genomic DNA from mushroom. The method was considered based on available chemicals, simple equipments without loss of quality and quantity of DNA for lateral use. Centrifugations at controlled temperature, use of extraction buffer during grinding and amount of chemicals are important considerations for DNA isolation. In this method, grinding was performed using mortar and pestle at room temperature. Separation of debris and other component of tissues were done using micro-centrifuge at 10000 rpm for 30 minutes at room temperature. The purity and quantity of the DNA extracted from the mushroom were evaluated by using 260/280nm UV absorption ratios; in most cases, the absorption ratio of 260/280nm was between 1.81 and 2.05 (Table 1). However the absorption ratios fluctuated even among DNA solutions prepared from the same sample.

Quantification of DNA: The isolated DNA was pure that was confirmed by spectrophotometric method. Spectrophotometer is commonly used in laboratories for the measurement of DNA purity. The DNA purity was measured by dividing the absorbance at 260nm by the absorbance at 280nm. The ratio of absorbance reading at 260nm (A260) was divided to the absorbance reading at 280nm (A280) i.e., A260/A280 ratio was used for the determination of DNA purity. The A260/A280 ratio higher than 2.0 generally indicates RNA contamination. For A260/A280 ratios lower than 1.8 normally indicates protein contamination during extraction process. Good quality DNA should give the A260/A280 ratio in the range of 1.8 –2.0. Aljanabi *et al.* (1999) reported the range of A260/A280 ratio from 1.76-1.96 for good quality DNA of sugarcane. In this investigation, the ranges of A260/A280 ratios among mushroom strains were from 1.80–2.05 (Table 1) which indicates the good quality of DNA. The amount of DNA recovered was enough for perform PCR amplification of Random Amplified Polymorphic DNA. The amount of recovered DNA was ranged from 700-3600 ng/µl. The highest amount of DNA was recovered from the strains PY-1 (3600 ng/µl) and the lowest amount was obtained from the variety PY-2 (700 ng/µl). The amount of isolated DNA from PO96-1 and PO69-2 strains were 1000 ng/µl and 2100 ng/µl respectively.

Table 1. The ratios of spectrophotometric absorption readings were calculated at 260 nm and 280 nm of different samples for quantification of DNA which were given below

Mushroom strains	Absorbance Readings at 260 nm	Absorbance Readings at 280 nm	A260/A280 Ratio	Concentration of DNA ng/µl
PY-1	0.072	0.035	2.05	3600
PY-2	0.014	0.007	2.00	700
PO96-1	0.020	0.011	1.81	1000
PO96-2	0.042	0.023	1.83	2100

DNA Fingerprinting through RAPD markers: DNA fingerprinting of four mushroom strains were performed using five RAPD markers (OPA-03, OPA-07, OPA-10, OPD-10 and OPC-12). Among the five RAPD primers initially tested, OPA-07, OPA-10 and OPD-10 primers produced comparatively maximum number of high intensity bands with minimal smearing. According to RAPD data the findings were as follows:

Band size: The sizes of the amplified bands in the four mushroom strains ranged from 380 to 3000bp (Table 2). Among the five RAPD primers, OPA-03 revealed band sizes that ranged from 380 bp to 1400 bp, primer OPA-07 ranged from 625bp-3000bp, primers OPA-10 ranged from 400 bp to 2250 bp (Fig. 1a); primer OPD-10 ranged from 400 bp to 1600 bp and primer OPC-12 ranged from 390 bp to 2900 bp (Fig. 1b).

Table 2. RAPD primers with corresponding DNA bands scored and their size ranges in four different strains of Golden oyster mushroom which were given below

Primer	Size ranges (bp)	Total number of bands scored	Number of polymorphic bands	Number of monomorphic bands	Polymorpihism (%)
OPA-03	380-1400	21	21	0	100.00
OPA-07	625-3000	31	30	1	96.77
OPA-10	400-2250	24	24	0	100.00
OPD-10	400-1600	28	28	0	100.00
OPC-12	390-2900	16	16	0	100.00
Total		120	119	01	

Number of bands: All the five RAPD primer amplified a total of 120 bands from the four strains of mushroom using the Thermal Cycler (Finzymes) and 1% Agarose gel (Table 2). The primer OPA-07 amplified the highest number of bands (31), primer OPD-10 amplified a total of 28 bands, primer OPA-10 amlified a total of 24 bands, primer OPA-03 amplified a total of 21 bands and primer OPC-12 amplified the lowest number of bands (16). Primer OPA-07 amplified a specific monomorphic band and rest of all the primers amplified polymorphic bands. All of the primer except OPA-07 (96.77 %) showed 100% polymorphism (Fig. 1a and Fig. 1b).

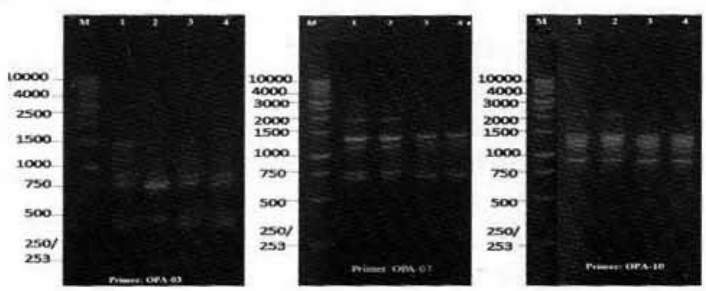

Fig. 1a. DNA fingerprinting of four strains of *Pleurotus citrinopileatus* mushroom based on RAPD primer such as OPA-03, OPA-07 and OPA10 through 1.4% agarose gel electrophoresis. (M = 1kb DNA Ladder, 1 = PY-1, 2 = PY-2, 3 =PO96-1, 4 = PO96-2).

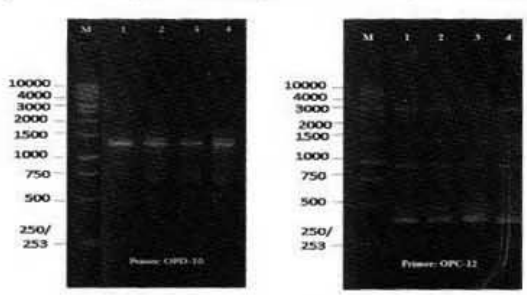

Fig. 1b. DNA Fingerprinting of four strains of *Pleurotus citrinopileatus* mushroom based on RAPD primer such as OPD-10 and OPC-12 through 1.4% agarose gel electrophoresis. (M = 1kb DNA Ladder, 1 = PY-1, 2 = PY-2, 3 =PO96-1, 4 = PO96-2).

Genetic distances: The values of pair-wise comparisons of genetic distances analyzed by using computer software "Statistica" between strains were computed from combined data for the five primers, ranged from 2.00 to 7.00 (Table 3). The highest linkage distance (7.00) was recorded between strain pairs PY-2 and PO96-1. The linkage distance (5.00) was recorded between strain PY-2 and PO96-2. The linkage distance (4.00) was recorded between strain PY-1 and PO96-2. The linkage distance (3.00) was recorded between strain PY-1 and PY-2. The lowest linkage distance (2.00) was recorded between strain pairs PO96-1 and PO96-2.

Table 3. Summary of linkage distances for different pairs of four Golden oyster mushroom strains for RAPD markers

Strains	PY-1	PY-2	PO96-1	PO96-2
PY-1	0.00	3.00	4.00	4.00
PY-2	3.00	0.00	7.00	5.00
PO96-1	4.00	7.00	0.00	2.00
PO96-2	4.00	5.00	2.00	0.00

Cluster analysis (Tree Diagram): Genetic relationships among the strains at the average distance of 5.00, showed two major clusters C_1 and C_2 presented in the Fig. 2. Major cluster C_1 comprised PY-1 and PY-2. At the linkage distance of 3.00, PY-1 and PY-2 strains were subdivided from each other. On the other hand, major cluster C_2 belonged to PO96-1 and PO96-2. These strains were also differentiating from each other at the linkage distance of 2.00.

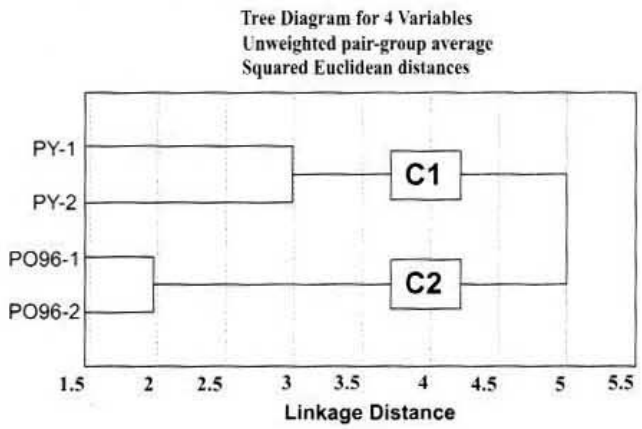

Fig. 2. Cluster analysis by Unweighted pair group method of arithmetic means average (UPGMA) of four Golden oyster mushroom strains based on five RAPD markers.

The basic DNA sequence of an organism can be presumed to be insensitive to short term environmental change and thus should provide a more stable alternative for strain/ species identification. Therefore, the random and genomic wide nature of the RAPD technique is best to indicate over all genetic relatedness/dissimilarity than the morphological analysis (Alam, 2011; Ravash *et al.*, 2009). The different primers produced different number of bands in PCR. This variation in the number of bands may be due to the sequence of primer, availability of annealing sites in the genome and template quality (Alam *et al.*, 2009; Kernodle *et al.*, 1993). The 100% polymorphism produced by four RAPD primers except OPA-07 may be due to the base substitution, insertion and deletion or collection of genetic material from different sources (Chopra, 2005 and Jusuf, 2010). The variation in the number of bands may be due to the sequence of primer, availability of annealing sites in the genome

and template quality (Kernodle *et al.*, 1993). The method can be studying genetic intra-population polymorphism and segregation, population history and geography, hybridization at the range of boundaries of allopathic population. For intra specific relationship and phylogeny reconstruction using RAPD markers most researchers scored the presence and absence of bands, calculated pair-wise genetic distance between taxa and UPGMA for construction of phylogenic tree. Wiklie *et al.* (1993) postulated that cluster analysis is a standard method for analyzing the relatedness of individuals from measured data and has the advantage over some other methods. The result will help to a) further molecular research among them, b) conserved them in our local environment, c) better understand their ecological impact and understand their genomic characteristics.

Study also revealed that, RAPD analysis can be very useful tool for mushroom grower for identification, classification, variety development and maintenance of good quality spawn.

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Effect of Different Amount of Substrates on Growth and Yield of *Ganoderma lucidum* (GL-4) Mushroom

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Abstract

Different amount of sawdust which was supplemented with rice bran and filled with different size of polypropylene bags. The filled bags were used for cultivation of reishi mushroom and the growth; yield as well as yield related attributes were compared. The variety GL-4 required maximum days (42.0) to complete mycelium running when the bags were filled with 1200g substrates which was statistically similar to 1000g substrate. Maximum days (12.50) required to form premature stage, antler stage (20.50 days), conk formation stage (32.50 days) and first harvest (47.50 days) when the bags were filled with 1000g substrates. Minimum days (20.0) required to complete mycelium running, premature stage (9.92 days), antler stage (14.33 days), conk formation stage (18.92 days) and first harvest (38.41 days) when the bag filled with 500 g substrates.

Keywords: Sawdust, Inoculation, Mother culture.

INTRODUCTION

Commercial cultivation of *Ganoderma* has been introduced worldwide, especially in tropical Asian countries to meet the gradually increasing demand of the mushroom as natural medicine. Usually in Bangladesh the summer season is considered as the best time for the cultivation of this mushroom (Kakon *et al.,* 2012; Uddin *et al.,* 2011). Reishi (*Ganoderma lucidum*), medicinal mushroom has become the object of intensive research because of its multiple health benefits with apparent absence of side effects (Mizuno *et al.,* 1985; Kim *et al.,* 1986). It contains various pharmacologically active compounds, including over 119 different triterpenes and several types of polysaccharides (Hsieh and Yang, 2004). The specific health benefits of reishi mushroom include anti-tumor, anti-inflammatory, anti-microbial, hepato-protective, hypotensive, anti-diabetic, antioxidant, modulation of the immune system and bacteriostatic. Powders, dietary supplements, concentrated tablets, syrup and tea are commercial herbal products derived from *G. lucidum* (Wachtel-Galor *et al.,* 2011).

G. lucidum can be grown with large variety of substrates, including lignocellulosic components (cellulose, hemicellulose, and lignin) that provide soluble inorganic and organic materials. One strategy for improving yield may include supplementation of the substrate with nitrogen-based supplements such as millet, gram, rye, corn, etc. (Gurung *et al.,* 2012; Park, 2012; Staments, 2000). Supplementation is the process of adding a nitrogen rich nutrient to the mushroom substrate in order to increase the potential yield. This works by providing the mycelium with a larger nutritional base in which to support stronger mycelium and produce larger, healthier fruits. Another strategy for improving yield may include increasing amount of substrate in bags. These bags are generally available in

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a variety of sizes. The size of bag obviously depends on how much substrate to put in bag. Mushroom yield and biological efficiency are important parameters that need to be understood and optimized by the mushroom cultivator. Different mushrooms and different strains of the same species will have a wide range of potential biological efficiencies. More delicate mushrooms, such as yellow oysters and slower growing mushrooms, such as reishi, tend to have lower biological efficiency values. In a way, increasing spawn:bulk substrate ratio is a way to increase nitrogen content, and is a form of supplementation. Again, too much spawn will eventually lead to diminishing returns. So, need to find out suitable amount. The objective of this study was to determine the quantity of substrate that would maximize reishi mushroom production.

MATERIALS AND METHODS

The experiment was conducted in the laboratory, workshop and the culture house of Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh, from March to August 2015. In this experiment sawdust was used as substrate and supplemented with rice bran for the cultivation of reishi mushroom. Different amount of substrate filled with different size of polypropylene bag was used as treatments. The treatments were T_1= 500g, T_2=750g, T_3=1000g and T_4=1200g. One strain of *Ganoderma lucidum*, namely GL-4 was used as test materials.

Preparation of pure culture: Pure culture of four strains were prepared on potato dextrose agar (PDA) medium containing 200g peeled and sliced potato, 20g dextrose and 20g agar per liter. The medium was poured into test tube at 10 ml/tube. The medium in test tube was sterilized in an autoclave for 20 minutes at 121^{0}C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the tubes were inoculated with the inoculants of above mentioned strain. After inoculation, the tubes were covered with cotton plug. All operations were done under sterile condition in a clean bench. The inoculated tubes were incubated in a growth chamber at 22 ± 2^{0}C. After completion of the whitish mycelium, this culture was used for inoculation of mother culture.

Preparation of mother culture: To prepare mother culture of test mushroom sawdust and rice bran mixed together at the ratio of 2:1 (v/v). Water was added to adjust moisture content at 65% and $CaCO_3$ was mixed at the rate of 0.2% of the mixture. Polypropylene bags of 18 cm × 25 cm size were filled with 300 g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the volume of the bag was made for space to put the inoculums. The neck was plugged with cotton and covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for one hour at 121^{0}C under 1.5 kg/cm^2 pressure. After sterilization the packets were cooled for 24 hours and transferred into a clean bench. Individually, a piece of stock PDA culture of test strain containing mycelium was placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned above. The inoculated packets were placed on a rack in the laboratory at 22 ± 2^{0}C temperatures for incubation. The substrate of the mother culture was colonized by the growth of whitish mycelium within 15-20 days after inoculation. The fully colonized packets were used for spawning.

Preparation of spawn packet: The substrate of spawn packets was prepared using sawdust and rice bran mixture at the ratio of 2:1. Water was added to make the moisture level about 65% and $CaCo_3$ was added at 0.2% (w/w) of the mixture. The substrate mixture was poured into 18 cm × 25 cm polypropylene bags at 500 g/bag. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the bag was made for space to introduce the inocula. The neck of each poly bags was plugged with cotton, covered with brown paper and tied with a rubber band. The packets were sterilized in an autoclave for 2 h at 121°C under 1.1 kg/cm^2 pressures. After sterilization, the packets were cooled and transferred to an inoculation chamber. The packets were inoculated separately with the mother culture of test strain at the rate of two tea spoonful per packet. The inoculated packets were incubated at 25°C.

Cultivation conditions for fruiting: The packets were kept in a dark room at 25°C for incubation. After mycelium maturation all the packets were transferred to the culture house with rubber bands, cotton plug and plastic neck. Then the packets were placed separately on the rack of culture house under natural condition. Water was applied per day as required and proper aeration was maintained in culture house for the release of excess CO_2 and supply of sufficient O_2 as required for the development of primordia and fruiting body.

Experiment design, data collection and analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications and each replication contain sixteen populations. Data on days to complete mycelium running, days required from opening to premature stage, days required from opening to antler initiation, days required from opening to conk formation, days to first harvest, number of fruiting bodies, length of stalk, diameter of stalk, diameter of pileus, thickness of pileus, biological yield and biological efficiency were recorded. Biological yield gm/packet was recorded by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion. The Biological Efficiency was determined by using the following formula:

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

Data were analyzed according to Gomez and Gomez method (1984) using MSTAT-c computer program and Excel software. Means separation were computed following Duncan's Multiple Range Test (DMRT) using the same computer program.

RESULTS AND DISCUSSION

Days required to complete mycelium running (DRCMR): Days to complete mycelium running showed significant variation. The variety GL-4 required maximum days (42.0) to complete mycelium running when the bags were filled with 1200g substrates which was statistically similar to 1000g substrate. Minimum days required (20.0) to complete mycelium running when the bags were filled with 500 g substrates (Table 1).

Days required from opening to premature stage (DROPS): Days required from opening to premature stage showed significant variation. The variety GL-4 required maximum days (12.50) to form premature stage when the bags were filled with 1000g substrates which

was statistically dissimilar to other treatments. Minimum days required (9.92) to complete mycelium running when the bags were filled with 500g substrates (Table 1).

Days required from opening to antler initiation (DROAI): Days required from opening to antler initiation stage showed significant variation. The variety GL-4 required maximum days (20.50) to form antler when the bags were filled with 1000g substrates which was followed by 1200g substrate. Minimum days required (14.33) to form antler when the bags were filled with 500 g substrates (Table 1).

Days required from opening to conk formation (DROKF): Days required from opening to conk formation showed significant variation (Table 1). The variety GL-4 required maximum days (32.50) to conk formation when the bags were filled with 1000g substrates which was statistically different to other treatments. Minimum days required (18.92) to conk formation when the bags were filled with 500g substrates.

Days required from opening to first harvest (DROFH): Days to first harvest showed significant variation (Table 1). The variety Gl-4 required maximum days (47.50) to first harvest when the bags were filled with 1000g substrate which was followed by 1000g substrate. Minimum days required (38.41) to first harvest when the bags were filled with 500g substrates which was statistically similar to 750g substrate. This result is partially supported by Hoq *et al.* (2016) who observed that the variety GL-4 required maximum days (29.78) to first harvest on sawdust based substrate filled with 500 g substrates.

Table 1. Effect of amount of substrates on growth of Reishi (Gl-4) mushroom

Treatments	Days required to complete mycelium running (DRCMR)	Days required from opening to premature stage (DROPS)	Days required from opening to antler initiation (DROAI)	Days required from opening to conk formation (DROKF)	Days required from opening to first harvest (DROFH)
T_1	20.00c	9.92d	14.33c	18.92c	38.41c
T_2	31.00b	10.75c	15.58b	20.17bc	38.42c
T_3	42.00a	12.50a	20.50a	32.50a	47.50a
T_4	42.00a	11.75b	15.91b	20.33b	41.00b
CV (%)	3.83	3.31	4.26	3.60	2.33

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. (T_1= 500g, T_2=750g, T_3=1000g and T_4=1200g).

Number of fruiting body: Number of fruiting bodies under different treatments differed significantly (Table 2). The highest number of fruiting bodies (3.25) was found in T_1 where bags were filled with 500g substrates and which was statistically different to other treatments. The lowest number (2.50) of fruiting bodies was found in T_3 which was statistically similar to T_4 and T_2.

Size of fruiting body: The length of stalk ranged from 2.13 to 3.42 cm with significant difference (Table 2). The highest length of stalk was found in T_4 (3.42cm) which was statistically dissimilar to other treatments. The lowest length of stalk was found in T_3 (2.13cm). The diameter of stalk was also significant and ranged from 0.78 to 1.11 cm (Table 2). The highest diameter of stalk was found in T_2 (1.11cm) while the lowest diameter of stalk (0.78cm) was found in T_4.

The diameter of pileus ranged from 4.46 cm to 5.25 cm with significant difference among the treatments (Table 2). The highest diameter of pileus (5.25cm) was found in T_4 followed by T_3 (5.00cm) and the lowest diameter of pileus (4.46 cm) was found in T_2. The thickness of pileus in different treatments differed significantly and ranged from 0.77cm to 0.93cm (Table 2).

Yield/Packet (g): Significant variation was observed in yield under different treatments (Table 2). The highest yield from 1st flush (30.42g) was found in T_1 followed by T_2 (22.25g) and T_3 (19.25g). The lowest yield was found in T_4 (13.58g). The highest total yield (68.50g) was found in T_1 followed by T_2 (45.55g) and T_3 (40.755g). The lowest total yield was found in T_4 (30.50g). Yield was counted in the harvest period 2nd flush. This result is partially supported by Hoq *et al.* (2012) who obtained the highest total yield of GL-4 mushroom 17.34g from 500 g sawdust based substrates.

Table 2. Effect of amount of substrates on yield and yield contributing characters of Reishi (Gl-4) mushroom

Treatments	Number of fruiting body (NFB)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Biological yield (g) (1st flush)	Total Biological yield (g) (1st & 2nd flush)
T_1	3.25a	3.00b	0.85b	4.83b	0.93a	30.42a	68.50a
T_2	2.50b	1.83d	1.11a	4.46c	0.76b	22.25b	45.55b
T_3	2.50b	2.13c	1.05a	5.00b	0.90a	19.25c	40.75c
T_4	2.67b	3.42a	0.78c	5.25a	0.77b	13.58d	30.50d
CV (%)	6.16	4.17	5.03	2.55	3.41	2.81	3.83

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. (T_1= 500g, T_2=750g, T_3=1000g and T_4=1200g)

Biological efficiency (Be%): The highest biological efficiency (39.12%) was found in T_1 followed by T_2 (17.35%) and the lowest biological efficiency was found in T_5 (7.26%) (Fig. 1) where Veena and Pandey (2010) reported a biological efficiency ranging from 4 to 13% when they cultivated *G. lucidum* on locally available sawdust in India. As substrate quantities increased, substrate utilization decreased.

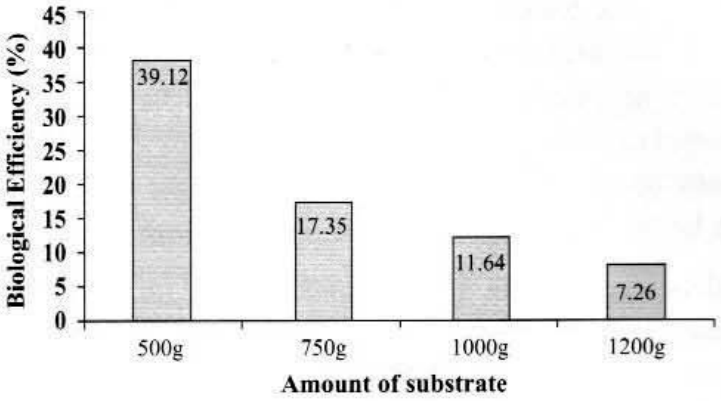

Fig. 1. Effect of different amount of substrates on biological efficiency of *Ganoderma lucidun* (GL-4) mushroom.

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