

EFFECT OF VARIOUS ORGANIC SUPPLEMENTS ON NON-ENZYMATIC ANTIOXIDANT AND MINERALS EXPRESSION IN CALOCYBE INDICA

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ABSTRACT

Maintenance of equilibrium between free radical production and antioxidant defences is an essential condition for normal organism functioning. The intonation of the mushroom used now-a-days in the field of medicine, is the way where this is moderately achieved. In this regard the nutritional values of *Calocybe indica* which is popular among the cultivated mushrooms have been determined. The results showed that *Calocybe indica* were rich in non-enzymatic antioxidant and minerals. It is clear from the results that all the organic supplements added in the substrate at the time of spawning, showed higher concentrations of non-enzymatic antioxidant and minerals as compared to control. This study revealed the presence of non-enzymatic antioxidant in the samples and the striking feature of present work is to maximize the usage of mushroom in the powdered form when there is certain unstable condition for the usage of mushroom in the fresh form.

INTRODUCTION

Mushroom is a member of higher fungi seen with the naked eye and usually picked by hands, shaped fleshy fruiting body, widely used as food and food supplements for millennia. It is an important food item concerning human health, nutrition and disease prevention (Chang, 1996). In many countries mushrooms have long been valued as delicious and nutritional food. However, nutritional mushrooms provide a wide variety of medicinal properties and they are effective against certain critical diseases. The prominent species of cultivated mushrooms (*Agaricus bisporus*, *Pleurotus ostreatus*, *P. sajor-caju*, *P. florida* and *Calocybe indica*) are popular and widely accepted (Ruhul Amin *et al.*, 2007). In most countries, there is a well established consumer's acceptance of cultivated mushrooms, probably not only due to their unique flavor and texture but also for their physico-chemical properties and nutritional characteristics. Mushrooms are considered as valuable health foods since they are known for rich proteinaceous food, it consist of about 75% proteins and are low in calories, fat, fatty acids, vitamins and minerals. Dietary mushrooms provide a wide variety of medicinal properties and they are effective against certain life threatening diseases include anticancer, antibiotic, antiviral activities, immunity and blood lipid lowering effects. *Pleurotus florida* has antioxidant and antitumor activities (Nayana and Janardhanan, 2000; Manpreet *et al.*, 2004), *Pleurotus sajor-caju* has hypertensive effects through its active ingredients which affect the renin-angiotensin system (Chang, 1996), *P. ostreatus* possesses antitumor activity (Yoshioka *et al.*, 1985) and hypoglycaemic

effects in experimentally diabetic induced rats (Chorvathova *et al.*, 1993). Oyster and milky mushrooms are very effective in reducing the total plasma cholesterol and triglyceride level (Nuhu Alam *et al.*, 2007) and thus reduce the chance of atherosclerosis, cardiovascular and artery related disorders. These medicinal properties might be due to the presence of some important substance in dietary mushrooms, known for "medicines and foods have a common origin" (Kaul, 2001). Among the various edible mushroom *Calocybe* genus consists of about 20 species of mushroom, including *Calocybe indica*, which can be cultivated throughout the year in the entire of India even in hot humid climate (Kalha *et al.*, 2011). The fruit body of *Calocybe indica* is fleshy, milky white, umbrella like structure. *Calocybe indica* is an indigenous popular edible mushroom, possessing a variety of secondary metabolites such as phenolic compounds, terpenes and steroids possibly involved in their medicinal effects and the accumulation of these compounds depends on management, processing and maturity at the time of harvest. Antioxidants have been reported to prevent oxidative damage by free radical and reactive oxygen species (ROS) may prevent the occurrence of diseases. *Calocybe indica* have become an attractive functional food mainly because of their chemical composition, and this can be explained by the antioxidant capacity of mushrooms. Mushrooms are rich in protein, minerals and vitamins and they contain an abundance of essential amino acids (Sadler, 2003). Moreover, nutritional composition is affected by many factors; include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting,

portion of the fruiting bodies used for biochemical analysis (Benjamin, 1995). As far as milky mushroom is concern there is a lack of scientific investigations, the aim of present investigation was to improve the nutritional characteristics with a goal of increasing awareness and beneficial effects of edible *Calocybe indica* among the consumers. Therefore, a preliminary study was carried to find out the non-enzymatic antioxidants and minerals concentration of *Calocybe indica* by applying different organic supplements for health promoting benefits.

MATERIALS AND METHODS

Cultivation of milky mushroom on wheat straw in high density polythene bags (60 cm X 40 cm) with layer spawning was followed as per standard procedure (Panday and Tewari, 1993). Various organic supplements and biofertilizers were tried viz. rice bran, wheat bran, gram dal powder, Azotobacter and pseudomonas. The supplements were added at the time of spawning the substrate. One hundred grams of each material was used in each of the four layers in equal quantities. Substrates without any supplement served as control. Three replications were maintained for each treatment. Matured fruiting bodies were harvested from two flushes and observations regarding the number and weight of sporophores were recorded. Biological efficiency was calculated as a ratio between the fresh weight of harvested mushrooms and the dry weight of substrate per bag and was expressed as a per cent. Data pertaining to yield were analyzed statistically. The fresh mushroom sample was collected, shade dried and coarse powder of the sample was used for the study.

Estimation of Vitamin A

The fresh sample of mushroom was collected and shade dried, sample of mushroom was extracted with 10 ml proportions of petroleum ether (40-60°C). Wash the extracts with water for separating the layers using separating funnels. Added sodium sulphate to remove the moisture. Ether extract 1mL was taken and evaporated to dryness at 60°C. The dried residue was dissolved in 1mL chloroform and used for estimation of vitamin A by the method of (Bayfield and Cole, 1980).

Estimation of Ascorbic Acid

Grind about 10g of sample and homogenized at 2000 rpm for 10 minutes. The supernatants obtained were treated with a pinch of charcoal, shaken well and kept for 10 minutes and centrifuge to remove charcoal residues. The clear supernatants obtained were used for assay. The estimation of ascorbic acid in the mushroom sample was done by the method of (Roe and Keuther, 1953).

Estimation of α - Tocopherol

The sample were homogenized in a blender, weighed accurately 2.5g of homogenised tissues into a conical flask, add 50mL of 0.1 %N sulphuric acid and allowed to stand overnight. Shaken the flask and filtered, discarding the initials. Aliquots of the filtrate were used for estimation of α -tocopherol by a method described by (Rosenberg, 1992).

Estimation of Reduced Glutathione

Take 1g sample and homogenised in 5% TCA give 20% homogenate. The precipitated proteins centrifuged at 1000

rpm for 10 minutes, cooled on ice and 0.1 ml of the supernatant was taken for estimation. Reduced glutathione was carried out by the method (Moron *et al.*, 1979).

RESULTS AND DISCUSSION

The non enzymatic- antioxidants include Vitamin A, Ascorbic acid ,Tocopherol and Reduced Glutathione, which scavenge wide variety of free radicals. The Non- Enzymatic Antioxidants in *Calocybe indica* are presented in Table 1.

The data presented in Table 1 shows that, Vitamin A in organic supplemented sample of *Calocybe indica* was recorded highest in Wheat Straw + Rice Bran (0.450 mg/g) followed by Wheat Straw + Gram Dal Powder (0.358mg/g), Wheat Straw + Wheat Bran (0.315mg/g), as compared to control (0.210mg/g). Addition of organic supplements at the time of spawning increase the conc. of Vitamin A in the fruit of mushroom, which is fat soluble stored in the body and is necessary for clear vision in dim light which maintains integrity of epithelial tissue (Gopalan *et al.*, 2000). Vitamin A and retinoid, either topically or orally administered, were able to induce complete remission a high proportion of patients with basal cell and advanced squamous cell carcinoma (Lehninger *et al.*, 1996). Vitamin A also acts a neutralizing agent for the free radicals. The level of Vitamin C (ascorbic acid) in the organic supplemented samples of *Calocybe indica* was recorded highest in Wheat Straw + Rice Bran (0.370 mg/g) followed by Wheat Straw + Gram Dal Powder (0.355mg/g), and Wheat Straw + Wheat Bran (0.326mg/g), as compared to control (0.320mg/g). (Powdered samples). Ascorbic acid is regard as the first line natural antioxidant defence in plasma and a powerful inhibitor of LPO (Maxwell, 1995). Vitamin C is a water soluble antioxidant that acts as a free radical scavenger, scavenges peroxy radicals (Sies, 1993). Vitamin C protects non-smokers against the harmful effects of ROS from passive smoking (Jacob, 2000). Tocopherol is a fat soluble vitamin mainly formed intercalated within the membrane. It prevents the attack of reactive oxygen species of the membrane PUFA (Sies, 1993). Vitamin E (α -tocopherol) is probably the most important lipid-soluble antioxidant protecting membranes, lipids and lipoproteins (VanBakl *et al.*, 2000). The highest concentration of Vitamin E in the additive powdered samples of milky mushroom was recorded highest in Wheat Straw + Rice Bran (0.745 mg/g) followed by Wheat Straw + Gram Dal Powder (0.675 mg/g) and Wheat Straw + Wheat Bran (0.512 mg/g), as compared to control (0.425 mg/g). Vitamin E is one of the few nutrients for which supplementation with higher than recommended levels have been shown to enhance immune response and resistance to diseases (Bendich, 1997). Many studies have suggested that high intake of Vitamin E may down the development and progression of atherosclerosis. Some clinical trials also reported beneficial effects of Vitamin E supplementation in the secondary prevention of cardiovascular events (Meydani, 2000). The glutathione (GSH) is the most abundant non protein, thiol GSH / GSSG pair forms the major intracellular redox system. GSH in the supplemented samples of *Calocybe indica* was noted as Wheat Straw + Rice Bran (0.128 μ moles/g) followed by Wheat Straw + Gram dal Powder (0.112 μ moles/g), and Wheat Straw + Wheat Bran (0.102 μ moles/g), in comparison

Table 1: Levels of non enzymatic-anti oxidants in milky mushroom (Powdered sample)

Substrate with supplementation	Vitamin A(mg/g)	Vitamin C(mg/g)	Vitamin E(mg/g)	Reduced glutathione(n moles/g)
Wheat Straw + Rice Bran	0.450	0.370	0.745	0.128
Wheat Straw+Wheat Bran	0.315	0.326	0.512	0.105
Wheat Straw+Gram Dal Powder	0.358	0.355	0.675	0.112
Control	0.210	0.320	0.425	0.098

Table 2: Minerals contents of Calocybe indica in dried form (mg)

Substrate +Supplements	Calcium (mg/g)	Phosphorus (mg/g)	Iron (mg/g)	Zinc (mg/g)	Magnesium (mg/g)	Manganese (mg/g)	Arsenic (mg/g)
Wheat Straw + Rice Bran	19.70	440.0	56.75	12.45	12.65	1.56	0.52
Wheat Straw+Wheat Bran	18.45	445.0	55.12	12.34	12.24	1.15	0.47
Wheat straw+ Gram Dal Powder	18.68	438.0	55.22	11.92	12.55	1.51	0.49
Control	18.0	435.0	55.30	11.76	11.70	1.42	0.45

to control (0.098 μ moles/g). Functions of GSH in reductive processes are essential for the synthesis and also the degradation of the levels of proteins, formation of DNA, regulation of enzyme activities and protection of the cells against ROS and free radicals produced even in normal metabolism (Gul *et al.*, 2000). The present work also supported by (Hema, 2000) that dietary intake with addition of antioxidants may provide great relief to the problem caused by ROS.

The analysis of *Calocybe indica* for mineral/ nutrients composition are presented in Table (2). The maximum amount of minerals constituents present in 100 g mushroom was found in rice bran supplemented dried sample contained Calcium (19.70mg), iron (56.75mg), zinc (12.45mg), magnesium (12.65mg), manganese (1.56mg) and arsenic (0.52mg). followed by gram dal powder which contain calcium (18.68mg), iron (55.22mg), manganese (1.51mg) and magnesium (12.55mg) as compared to unsupplemented sample like Ca (18 mg), P(435.0), Fe (55.30 mg), Zn (11.76mg), Mg (11.70mg), Mn (1.42mg) and As (0.45mg). The present finding was supported by (Bano and Rajarathnam, 1982), who studied the nutritional status of *Pleurotus* mushroom. The maximum phosphorus (445mg) present in wheat bran followed by rice bran (440mg) as compared to control (435 mg). The findings of present investigation are comparable to previous studies reported by different authors like Shin *et al.* (2007) and Dundar *et al.* (2008).

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