

## Influence of supplementation on nutritional quality of Jew's Ear Mushroom (*Auricularia auricular-judae*)

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### ABSTRACT

**Addition of nutritional supplements is an effective way to enhance the yield and quality of mushrooms. This study investigated use of different supplements such as rice bran (10%), bengal gram powder (10%), urea (2%), ammonium sulphate (2%), poultry manure (10%), and spent mushroom compost (10%) in rubber sawdust substrate for the cultivation of Jew's ear mushroom (*Auricularia auricular-judae*) and their effect on the nutritional composition of the mushroom. Cultivation was done in polybags and harvested fruiting bodies were subjected to various chemical analyses using standard techniques. The results of the study revealed that there was a significant variation in the nutrient composition of *A. auricular-judae* cultivated on supplemented substrate. Rice bran supplementation enhanced carbohydrate and ash content, while urea treatment led to an increase in protein content, poultry manure supplementation enhanced mineral content of the mushrooms and spent mushroom compost treatment increased crude fibre content.**

**Key words:** Jew's ear mushroom, *Auricularia auricular-judae*, supplements, nutritional composition

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As “delicacies from the mountains,” wild edible mushroom fruiting bodies are a naturally available resources that are widely valued for their nutraceutical, pharmaceutical as well as cultural values (Moerman, 2008). The nutritional benefits and high delicacy of mushrooms have led to a rise in consumption throughout the world in recent years, facilitating their year-round growth and production (Rathore *et al.*, 2019).

*Auricularia auricula* commonly known by the name Jew's ear or Juda's ear mushroom is world's third most popular edible fungus (Zhao *et al.*, 2019) and valued for its flavour and medicinal properties in Asia. The Chinese traditional medicine uses this fungus as a topical treatment for sore throat, ophthalmia, staphylococcus, tonsillitis, and laryngocele by applying fresh mushroom to the sore body part. It is also

reported to be a source of antitumor compounds (Garibova *et al.* 1999). This mushroom is popular for their ability to add texture to soups (Chinese hot and sour soup), stir-fry and also use as salad (Verma and Verma, 2017).

The nutritional composition of mushrooms is affected by several factors including variation in strain, composition of substrate used, cultivation technique, stage of harvesting, and portion of the fruiting bodies used for analysis (Benjamin, 1995). Mushrooms can be used in bioremediation studies because of their capacity as a powerful bio-sorbents. Through their extensive mycelia network, mushrooms can absorb heavy metals from the substrate. In addition, they also hyper-accumulate some trace elements (Chatterjee *et al.*, 2017). Considering these, there is possibility of mushrooms to uptake nutrients

and other components from the environment or the growing substrate, which can enhance the level of nutrient and other bio-active components in mushrooms. Nutritional supplementation of substrate used in mushroom cultivation serves as an important practice to enhance the yield characters as well.

## MATERIALS AND METHODS

### Cultures used

Isolates of *A. auricula* were obtained by conducting surveys at three Agro Ecological Units (AEU 8, AEU 9 and AEU 12) of Southern Kerala. The collected mushrooms were subjected to tissue culture in Potato Dextrose Agar (PDA) medium at the laboratory of department of Plant Pathology, College of Agriculture, Vellayani. Fully grown mycelia of these cultured mushrooms were used for spawn preparation using paddy grains.

### Cultivation

Cultivation of the mushroom was done by compact polybag method as described by Baskaran *et al.* (1978). The basic substrate for cultivation comprised rubber sawdust and rubber wood chips in a 1:1 ratio with 2% calcium carbonate for all the treatments. Six supplements *viz.* rice bran (10%), bengal gram powder (10%), urea (2%), ammonium sulphate (2%), poultry manure (10%), and spent mushroom compost (10%) were added in separate treatments keeping non amended substrate as the control.

Polythene bags with a 150 gauge thickness and 60 x 30 cm dimensions were chosen for cultivation. Wood chips and rubber sawdust were soaked in water for twelve hours and sterilized by boiling method and then sun dried until a moisture level of around 60%. The supplements were sterilized by autoclaving for two hours at 121°C/15 lbs pressure. Substrate and supplements were mixed thoroughly and spawning was done in four circular layers. After spawning, bags were tied tightly at the top with 25 to 30 pin holes distributed all through the bags, labelled and kept for incubation in a dark room at 28°C temperature and 60 per cent relative humidity. Once the mycelium colonised the substrate, cut slits were made on the polybags and shifted to cropping room with 80 per cent relative humidity. Regular water spray was done on the bags. The fruiting bodies emerged were harvested and used for analysis.

### Nutritional Quality Analysis

Harvested mushrooms from each treatment were washed thoroughly, dried and made into powder form for various chemical analyses.

### Moisture content

Moisture content of the mushrooms was calculated on a fresh weight basis as per AOAC (2019). Hundred grams ( $w_1$ ) fresh sample of the mushroom was dried at 60 °C in a hot air oven till a constant weight ( $w_2$ ) was achieved. Weight of the dried sample was recorded and the per cent moisture content was estimated by the following formula.

Treatment no.	Treatment	Abbreviations used	Per cent supplementation
T1	Rice Bran	RB	10%
T2	Bengal gram Powder	BGP	10%
T3	Urea	Urea	2%
T4	Ammonium sulphate	AS	2%
T5	Poultry manure	PM	10%
T6	Spent mushroom compost	SMC	10%
T7	Not supplemented	Control	—

$$\% \text{ Moisture content} = \frac{w_1 - w_2}{w_1} \times 100$$

### **Carbohydrate content**

Estimation of total carbohydrate content was done using phenol sulfuric acid method (Masuko *et al.*, 2005). 100 mg of mushroom powder was added into a boiling tube, hydrolyzed it in boiling water bath for three hours with five ml of 2.5N hydrochloric acid and cooled to room temperature. It was then neutralized with sodium carbonate until the effervescence stopped. The volume was then made up to 100 ml and centrifuged for ten minutes at 5000 rpm. After centrifugation, the supernatant was collected and the aliquot was used for the analysis. A series of glucose stand solutions of volume 0.1, 0.2, 0.4, 0.6, 0.8 and 1ml was pipetted into a series of test tube for analysis and preparation of standard curve. A volume of 0.1 ml of the sample solution was also pipetted in a test tube and made up the volume to one ml with distilled water. A blank was set with 1 ml of distilled water alone. One ml phenol solution and five ml of 96 per cent sulfuric acid were added to each test tube and shaken well for 10 minutes and kept in a water bath for 20 minutes at 25-30°C. Absorbance of the solution was then read at 490 nm in a spectrophotometer. A standard curve of glucose was prepared keeping glucose concentration on X axis and absorption ( $A_{490}$ ) at Y axis. Total carbohydrate in the sample was then calculated by using standard graph.

#### Calculation

Absorbance corresponding to 0.1ml of the sample = 'x' mg of glucose

$$100 \text{ ml of the sample solution contains (mg glucose)} = \frac{'x'}{0.1} \times 100 = \% \text{ of total carbohydrate present.}$$

### **Crude protein content**

Crude protein content of the mushroom fruit bodies were assessed following Microkjeldhal method

as per the procedure given by Chang (2003). Ten ml of the digested mushroom sample in sulfuric acid was taken in the microkjeldhal flask and loaded on the distillation unit along with 10 ml of 40 per cent NaOH. The distillate was collected in 10 ml of four per cent boric acid with two to three drops of mixed indicator. The process of distillation was complete when colour of boric acid solution turned green from pink. The distillate collected in boric acid solution was back titrated against 0.02 N  $H_2SO_4$ . End point was indicated by the colour change from green to light pink. Titre value was noted and percentage of total nitrogen was calculated in the sample by the formula as given below. The N value thus obtained was converted to protein value by multiplying with the conversion factor, 6.25.

#### Calculation

$$\% \text{ of total N} = \frac{\text{Titre value} \times N \times 0.014 \times 100 \times 100}{W \times 10}$$

$$\% \text{ Protein content} = N \times 6.25$$

$$1 \text{ ml of } 1 \text{ N } H_2SO_4 = 0.014 \text{ g N,}$$

N- Normality of acid (0.02N)

W- Weight of the mushroom powder taken (0.5 g)

In fungi protein content is many times calculated as N x 4.38 (Kalac, 2013). In the present study we have used commonly used multiplier of 6.25.

### **Fat content**

Fat content of the mushroom samples was estimated by Soxhlet extraction method (James, 1995). Three grams of the mushroom was taken in a thimble and kept inside the soxhlet extractor. In order to properly distribute the solvent on the sample during extraction, a piece of cotton wool was placed at the top of the thimble. Ninety ml of petroleum ether was taken in a 150 ml round bottom flask which was then connected to the bottom end of the extractor and allowed to boil by setting it to a heating mantle at 70-90°. The extraction process continued for almost 6

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hours. The extract was vacuum evaporated in a rotary evaporator. Extract was then transferred into a pre-weighed Petri dish ( $w_1$ ), cooled in desiccators and weighed again ( $w_2$ ). Per cent fat in the mushroom sample was calculated by the following formula.

$$\% \text{ fat content} = \frac{w_1 - w_2}{w_1} \times 100$$

$w_1$  - Weight of petri dish + fat extract

$w_2$  - Weight of petri dish alone

$w$  - Weight of sample taken (3 g)

### **Crude fibre content**

Crude fibre content of the mushrooms was estimated by following the procedure of James (1995). Two grams of the dried mushroom powder was boiled with 200 ml concentrated sulfuric acid (1.25 %) for 30 min. The digested sample was then filtered through a muslin cloth and washed with boiling water. Again the sample was boiled with 200 ml of sodium hydroxide solution for 30 min and filtered through a muslin cloth and washed with 1.25 per cent sulfuric acid, 25 ml alcohol and three 50 ml portions of water. After this the residue of the sample was transferred to a pre-weighed ashing crucible ( $w_1$ ) and it was dried at  $130 \pm 2$  °C for two hours. Then the residue was cooled in desiccators and weight was recorded ( $w_2$ ). Further the residue was ignited at  $600 \pm 15$  °C in a muffle furnace for 30 min, cooled in a desiccators and noted the weight ( $w_f$ ).

$$\begin{aligned} \text{Per cent crude fibre} &= \frac{\text{Loss in weight}}{\text{Weight of the sample}} \times 100 \\ \text{in the sample} &= \frac{(w_1 - w_2) - (w_f - w)}{w} \times 100 \end{aligned}$$

### **Ash content**

Ash content of the mushrooms was estimated by following the procedure of AOAC (2019) using muffle furnace. Five gram dried mushroom powder was transferred to a pre-weighed silica crucible ( $w_1$ ). It was heated over a Bunsen burner at low flame until

the sample got charred and then transferred to a muffle furnace. In muffle furnace the charred sample was again heated to a temperature of 500 °C for around two hours until a white ash was obtained. It was then cooled and the final weight ( $w_2$ ) was recorded. Ash content in per cent was calculated using the following formula.

$$\% \text{ fat content} = \frac{w_1 - w_2}{\text{weight of sample}} \times 100$$

### **Calcium content**

Calcium content of the mushroom samples was calculated following EDTA titration method (AOAC, 2019). Five ml of the digested mushroom sample was pipetted out into a conical flask and added equal amount of distilled water. To this 10 drops each of hydroxyl amine hydrochloride and tri ethanol amine were added. A volume of 2.5 ml NaOH and 1 ml calcon solution were added to this solution. The resulting solution was then titrated with 0.01 N EDTA until an end point indicated by blue colour was obtained.

### Calculation

$$\% \text{ Ca in the sample} = \frac{\text{TV} \times \text{N} \times 0.02 \times 100 \times 100}{5 \times W}$$

TV=Volume of EDTA for sample titration (ml)

N= Normality of EDTA (0.01 N)

W= Weight of the dried and ground mushroom sample taken (1g)

### **Potassium content**

The samples were digested in tri-acid mixture [(HNO<sub>3</sub>: H<sub>2</sub>SO<sub>4</sub>and HClO<sub>4</sub>) in 10:1:4 ratio]. For digestion, 1 gm of powdered samples of dehydrated mushrooms were taken in 100 ml conical flask, 5 ml of concentrated HNO<sub>3</sub> was added to it and kept overnight. On next day, 10ml of tri-acid mixture was added and digested on hot plate as described by Piper (1966). After digestion, the material was filtered and

volume was made to 100 ml. Potassium content of the mushrooms was estimated by the technique of flame photometry (AOAC, 2019). Flame photometer was set up and aspirated working standards of 1, 2, 3, 4 and 5 ml of 100 ppm KCl solution. Five ml of mushroom extract was pipetted out to a 50 ml volumetric flask and made up the volume. Samples were then aspirated and readings (ppm) were noted.

#### Calculation

$$\% \text{ K in the sample} = \frac{\text{"X"} \text{ ppm} \times 50 \times 100}{5 \times 0.5 \times 10000}$$

X - Concentration in µg/ml of potassium in the standard solution

#### Sodium content

The samples were digested in tri-acid mixture [(HNO<sub>3</sub>: H<sub>2</sub>SO<sub>4</sub> and HClO<sub>4</sub>) in 10:1:4 ratio]. For digestion, 1 gm of powdered samples of dehydrated mushrooms were taken in 100 ml conical flask, 5 ml of concentrated HNO<sub>3</sub> was added to it and kept overnight. On next day, 10ml of tri-acid mixture was added and digested on hot plate as described by Piper (1966). Sodium content of the mushrooms was also

determined by flame photometry (AOAC, 2019). Flame photometer was set up and aspirated working standards of 1, 2, 3, 4 and 5ml of 100 ppm NaCl solution. Five ml of mushroom extract was pipetted out to a 50 ml volumetric flask and made up the volume. Samples were then aspirated and readings (ppm) were noted.

#### Calculation

$$\% \text{ Na in the sample} = \frac{\text{"X"} \text{ ppm} \times 50 \times 100}{5 \times 0.5 \times 10000}$$

X - Concentration in µg/ml of sodium in the standard solution

## RESULTS AND DISCUSSION

Nutritional composition of the Jew's ear mushroom upon supplementation with different amendments was found vary at a great extent (Table 1). A significant increase in the carbohydrate level was observed on supplementation with 10 per cent rice bran compared to that of control (61.21 ± 1.51 g/ 100 g and 54.12 ± 0.45 g/100 g respectively on dry weight basis). Rice bran is a good source of organic carbon and this organic carbon might have been

**Table 1.** Effect of supplementation on proximate composition of *A. auricula*

Sl. No.	Treatments	Carbohydrate*	Crude protein*	Fat*	Crude fibre*	Ash*	Moisture content#
1	T1 (RB-10%)	61.21 ± 1.51 <sup>a</sup>	11.78 ± 0.34 <sup>c</sup>	1.59 ± 0.07 <sup>c</sup>	14.64 ± 0.12I <sup>''</sup>	5.33 ± 0.28 <sup>a</sup>	89.31 ± 0.58
2	T2 (BGP-10%)	56.27 ± 0.10 <sup>c</sup>	12.60 ± 0.35G <sup>''</sup>	1.78 ± 0.08G <sup>''</sup>	16.70 ± 0.06 <sup>bc</sup>	4.02 ± 0.09 <sup>cd</sup>	90.34 ± 0.62
3	T3 (Urea-2%)	56.47 ± 0.43 <sup>c</sup>	14.23 ± 0.20 <sup>a</sup>	1.63 ± 0.04 <sup>bc</sup>	15.50 ± 0.31H <sup>''</sup>	3.77 ± 0.33H <sup>''</sup>	89.42 ± 0.63
4	T4 (AS-2%)	52.31 ± 1.17I <sup>''</sup>	12.68 ± 0.34G <sup>''</sup>	1.65 ± 0.10 <sup>bc</sup>	16.43 ± 0.12 <sup>c</sup>	3.20 ± 0.29I <sup>''</sup>	89.00 ± 1.23
5	T5 (PM-10%)	58.27 ± 0.73G <sup>''</sup>	10.44 ± 0.27H <sup>''</sup>	1.68 ± 0.07 <sup>bc</sup>	16.98 ± 0.06G <sup>''</sup>	4.33 ± 0.11 <sup>bc</sup>	88.99 ± 1.94
6	T6 (SMC-10%)	55.47 ± 0.51 <sup>cd</sup>	9.74 ± 0.27I <sup>''</sup>	2.01 ± 0.16 <sup>a</sup>	18.37 ± 0.48 <sup>a</sup>	3.08 ± 0.23I <sup>''</sup>	87.95 ± 2.41
7	T7 (Control)	54.12 ± 0.45H <sup>''</sup>	9.39 ± 0.10I <sup>''</sup>	1.57 ± 0.12 <sup>c</sup>	15.32 ± 0.29H <sup>''</sup>	4.73 ± 0.13G <sup>''</sup>	87.90 ± 2.42
	<b>CD (0.05)</b>	<b>1.458</b>	<b>0.455</b>	<b>0.172</b>	<b>0.442</b>	<b>0.396</b>	<b>NS</b>
	<b>SE (m)</b>	<b>0.481</b>	<b>0.15</b>	<b>0.057</b>	<b>0.146</b>	<b>0.13</b>	<b>NS</b>

\*Dry weight basis, #fresh weight basis

Values are mean ± SD of three replications; Values followed by similar superscripts are not significantly different at 5% level. RB- Rice bran, BGP- Bengal gram powder, AS- Ammonium sulphate, PM- Poultry manure, SMC- Spent mushroom compost, NS- Non significant

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absorbed by the developing mushrooms which led to a rise in carbohydrate content. Amendment with ammonium sulphate (2%) yielded mushrooms with lowest carbohydrate content ( $52.31 \pm 1.17$  g/100 g), which was even lesser than that of the non-amended control. Amount of carbohydrate contained in mushrooms produced by supplementation with urea (2%) and bengal gram powder (10%) were found to be on par to each other ( $56.47 \pm 0.43$  g/100 g and  $56.27 \pm 0.10$  g/100 g respectively).

Supplementation with urea yielded mushrooms with highest content of crude protein ( $14.23 \pm 0.20$  g/100 g) compared to other treatments. Next higher level of crude protein content was observed in mushrooms cultivated by supplementation with ammonium sulphate ( $12.68 \pm 0.34$  g/100 g) and it was on par with that of treatment with bengal gram powder ( $12.60 \pm 0.35$  g/100 g). Among the six supplements used, spent compost yielded mushrooms with lowest crude protein ( $9.74 \pm 0.27$  g/100 g), which was on par with control ( $9.39 \pm 0.10$  g/100 g). Crude protein content in mushrooms is directly related to the amount of total nitrogen content in it. Urea is a rich source of nitrogen and nitrogen can be easily used by the developing mushrooms to convert it to proteins because the absorption of these molecules is more

efficient than synthesizing those (Nunes *et al.*, 2012). Fat content of the mushrooms was found to be the highest in treatment with spent mushroom compost ( $2.01 \pm 0.16$  g/100 g) whereas treatment with rice bran yielded low fat containing mushrooms ( $1.59 \pm 0.07$  g/100g), which was on par with control ( $1.57 \pm 0.12$  g/100 g).

A significantly higher content of crude fibre was observed in mushrooms produced by amending the substrate with spent mushroom compost ( $18.37 \pm 0.48$  g/100g). It can be attributed to the high level of cellulose, hemicellulose, lignin and pectin in the paddy straw based spent mushroom compost used as supplement (Stallcup, 1958). This was followed by treatment with poultry manure, where the crude fibre content was found to be  $16.98 \pm 0.06$  g/100 g. Crude fibre content in case of treatment with urea was on par with that of control ( $15.50 \pm 0.31$  g/100g and  $15.32 \pm 0.29$  g/100 g respectively). Lowest content of crude fibre was recorded in mushrooms produced by amending with rice bran ( $14.64 \pm 0.12$  g/100 g).

Rice bran supplementation yielded Jew's ear mushrooms with highest amount of ash content, which was around  $5.33 \pm 0.28$  g/100 g on dry weight basis. Major component of ash is carbon (Vassilev *et al.*,

**Table 2.** Effect of supplementation on mineral content of *A. auricula*

Sl. No.	Treatments	Calcium (g/100g)	Sodium (g/100g)	Potassium (g/100g)
1	T1 (RB)	$1.61 \pm 0.06^c$	$0.94 \pm 0.02G''$	$1.83 \pm 0.03G''$
2	T2 (BGP)	$1.73 \pm 0.02G''$	$0.71 \pm 0.04H''$	$1.71 \pm 0.09^c$
3	T3 (Urea)	$1.43 \pm 0.06H''$	$0.79 \pm 0.02^c$	$0.57 \pm 0.03M''$
4	T4 (AS)	$1.28 \pm 0.04I''$	$0.79 \pm 0.06^c$	$1.08 \pm 0.04I''$
5	T5 (PM)	$1.92 \pm 0.04^a$	$1.21 \pm 0.03^a$	$1.94 \pm 0.03^a$
6	T6 (SMC)	$1.80 \pm 0.04G''$	$0.89 \pm 0.02G''$	$1.54 \pm 0.04H''$
7	T7 (Control)	$1.39 \pm 0.06H''$	$0.68 \pm 0.03H''$	$0.66 \pm 0.03''$
	<b>CD</b>	<b>0.085</b>	<b>0.062</b>	<b>0.081</b>
	<b>SE(m)</b>	<b>0.028</b>	<b>0.02</b>	<b>0.027</b>

Values are mean  $\pm$  SD of three replications; Values followed by similar superscripts are not significantly different at 5% level. RB- Rice bran, BGP- Bengal gram powder, AS- Ammonium sulphate, PM- Poultry manure, SMC- Spent mushroom compost

2010) and rice bran is a rich source of organic carbon. High levels of ash content mushrooms produced by supplementation with rice bran may be attributed to high carbon content in rice bran. Ash content was found to be lowest in treatment with spent mushroom compost ( $3.08 \pm 0.23$  g/100 g). There was no significant variation in moisture content with respect to all the treatments which ranged from 87 to 91 per cent in various treatments (Table 1).

Minerals viz., calcium, sodium as well as potassium were found to be the highest in mushrooms cultivated by amending the substrate with poultry manure ( $1.92 \pm 0.04$  g/100 g,  $1.21 \pm 0.03$  g/100 g and  $1.94 \pm 0.03$  g/100 g respectively). This can be attributed to the fact that poultry manure is a rich source of these minerals (Foreman and Long, 2013). Lowest content of calcium was observed in mushrooms produced by supplementation with ammonium sulphate ( $1.28 \pm 0.04$  g/100 g). Comparatively lowest amount of sodium was found in untreated control ( $0.68 \pm 0.03$  g/100 g), which was on par with supplementation by bengal gram powder ( $0.71 \pm 0.04$  g/100 g). Compared to other treatments, urea amendment yielded mushrooms with lowest potassium content ( $0.57 \pm 0.03$  g/100 g). Nunes *et al.* (2012) reported that addition of urea inhibited the assimilation of minerals like potassium and phosphorous in the substrates and urea may act as a chelator by reducing the availability of such minerals.

## CONCLUSION

The major results of the present study revealed that there was a significant variation in the nutrient contents of fruiting bodies of Jew's ear mushroom upon supplementation with different amendments. Different supplements attributed enhancement in different constituents. Hence it can be concluded that a combination of several amendments in right proportion can yield nutrient rich fruiting bodies of *A. auricula*.

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