Mushrooms grown in high-altitude soil exhibiting distinct alterations in growth, biochemical composition and antioxidant potential

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Abstract
Functional foods like mushrooms are high in secondary metabolites and bioactive chemicals with anti-inflammatory, antibacterial, antidiabetic, immune-stimulating and other health-promoting qualities. This study investigates four mushroom varieties including Cordyceps militaris, Pleurotus ostreatus, Pleurotus djamor and Agaricus bisporus, grown on Indian soil and natural environment for measurement of phenol and flavonoid content, along with their antioxidant properties and biochemical makeup. They had a large amount of phenolic and flavonoid content and high DPPH, H_2O_2 and NO scavenging inhibition percentages, demonstrating strong antioxidant potential. These mushroom extracts also contained a sizable amount of proline and nutritional content like carbohydrate, protein, lipids and fiber. Among all the mushrooms, the biochemical profile was significantly highest. Significant variations were also seen in the amounts of moisture and ash in each sample.

This research aimed to determine how mixing soil with compost substrate affected the yield and growth of mushrooms. The soil had a massive impact on forming more mycelial colonies which were necessary for the entire spawn run and spawning to pin head production and for the maturation of pins. It was discovered through this research that combining soil and compost substrate can aid in the growth of good-quality mushrooms and improve their productivity.

Keywords: Mushroom, antioxidant, phenol, nutrition, protein, flavonoids.

Introduction

Food production worldwide is in trouble due to climate change's adverse impact. India must consequently enhance agricultural production to nourish its expanding population while ensuring food safety and security. As a result, there are significant challenges to food security brought and the repetitive exploitation of agricultural land for an extended period, which has resulted in a remarkable decline in soil fertility[20,31]. It was estimated that 68% of India's population is dependent on agricultural outputs. A scientific, technological and economic strategy is necessary to ensure food security. One approach is to expand the production of mushrooms to enhance the nutrition and food security of the country.

Humans usually consume mushrooms, specifically Tramella, Auricularia, Agaricus, Lentinus, Pleurotus, Lentinula and Volvariella species[11,27]. Mushrooms grow more quickly than other field crops, requiring less space, water and agricultural waste[45]. Oyster mushrooms are farmed for commercial purposes worldwide because of their nutritional and other benefits[49]. Numerous civilizations use mushrooms as a delicacy including Ghana and China.

Mushrooms are a good source of essential amino acids, dietary fibre, non-starchy carbohydrates, proteins, vitamins and minerals[55,57]. They are a fantastic source of protein and are frequently substituted for meat in vegetarian diets. Mushrooms comprise of 26-31% protein, 2.51% fat, 18-43% sugar, 8-37% myco-cellulose and around 9-12% mineral content (potassium, phosphorus, calcium and sodium)[35]. Mushrooms have substantial dietary fibre and demonstrate immune-stimulating and anticancer effects[12].

Antioxidants stop the spread of free radicals and prevent their generation from destroying a series of events, leading to damage due to oxidative stress[44]. Antioxidant enzymes like catalase, glutathione peroxidase and superoxide dismutase are also neutralized free radicals which also keep cellular processes operating normally[4]. However, under specific circumstances, the biological antioxidant defence and healing systems become insufficient for the avoidance of oxidative damage[4,29] leading to illnesses like cancer, atherosclerosis, diabetes, neural diseases like Parkinson's and Alzheimer's and rheumatoid arthritis, along with the early aging process[44].

Several antioxidant chemicals have recently been found in the fungi kingdom[13,56]. Both fresh and processed mushrooms are commonly used as functional foods worldwide. Several species of mushrooms have been found to have antiviral, antibacterial, antidiabetic, cholesterol-lowering, hepatoprotective, immune-modulatory and anticancer properties because of their nutritional importance[8,10,51].

The nutritional or chemical makeup of mushrooms gives them their therapeutic properties. However, a variety of factors such as strain variations, the nature of the substrate, the cultivation method, the harvest stage, the analysis uses the portion of the fruiting bodies (FB) and techniques used for the measurement have an impact on nutritional composition and bioactive compounds[6]. Even though

numerous pieces of research have been performed to ascertain the nutritional composition of various growth of mushrooms under multiple conditions, further studies are required to understand the nutritional makeup of mushrooms better.

Due to lot of benefits of mushrooms, the present study was carried out using the four species of mushroom i.e. *Cordyceps militaris (C. militaris), Pleurotus ostreatus (P. ostreatus), Pleurotus djamor (P. djamor)* and *Agaricus bisporus* (*A. bisporus*). This study focuses on the production of mushrooms on a high-altitude soil sample mixed with compost substrate. It investigates mushrooms' quality and quantity, nutritional value and antioxidant potential.

Material and Methods

Mushroom sample collection and spawn preparation: Four edible mushroom kinds: *C. militaris, P. ostreatus, P. djamor* and *A. bisporus* (n=3) were procured from Thanvi Biotechnology, Bangalore, India. Cotton wool plugs were placed inside flasks containing 50 ml of potato dextrose broth before they were sterilized at 121.1°C for 15 minutes. Mycelial agar plugs (8 mm in diameter) collected from 7-day-old cultures of several mushroom fungi were individually added to the culture media after the medium was cooled at room temperature (RT). The flasks were cultured as static cultures for 28 days at RT (28 ± 2°C). These cultures were sub-cultured and kept alive on a PDA medium at 30 ± 2 °C in a B.O.D. incubator.

Preparation of Mushroom Spawn: The goat and horse dung were gathered in a 1:1:1 ratio with the chopped raw rice straw. The pile was retained for two weeks. The stack was then rotated once a week to let fresh air in and avoid overheating. Under this procedure, the composting proceeded for six weeks. Composted substrates were pasteurised in hot water at 80 °C for 2 h[30]. The pasteurised compost substrate was allowed to chill and remove excess water overnight (15–18 h). Manual packaging of the produced substrate into transparent bags was used. 5% of the substrate's moist mass was used to inoculate the spawn. For 3–4 weeks, the compost substrate with inoculation was kept for spawning at 25-30 °C in complete darkness.

Inoculation of spawn: Spawn run in the compost substrate was inoculated in the soil of land in the Vanzangi area of Eastern Ghats with an altitude of 4185 ft of Paderu division, Visakhapatnam district, Andhra Pradesh, India. The mushroom bed was prepared in the land by digging the 1ft square soil area and spreading the spawn with the compost substrate in 3 layers i.e. one layer of soil and another with compost substrate.

Physio-chemical analysis of soil and compost: A sample of 50 gm of soil was obtained from the study area. The soil samples were transferred to the lab on ice in zip-lock bags. In the laboratory, 50 g of compost and soil samples were kept in airtight conditions for chemical analysis. The physio-chemical analysis of soil and compost was performed at Regional Agriculture Research Station, Anakapalle, Andhra Pradesh, India.

Preparation of mushroom extract: To remove any leftover compost, the mushroom FB was wiped and rinsed with DH_20. They were blended into a coarse powder after being air-dried to a constant weight. Dried and powdered samples (5 g) were extracted using 25 mL ethanol. The extraction took place for 7 days with frequent shaking and mixing. The mixture was then filtered through filter paper. Using 10 mL of ethanol, the remnants were extracted once more. The extracts were kept at 4°C until further investigation.

Determination of Bioactive compound

Total phenol Content: The phenol level was evaluated by Folin- Ciocalteau assay[39] by using gallic acid as a standard. In a short, 1.5 mL of sodium carbonate solution was added, the sample extract (0.3 mL) was mixed and the Folin-Ciocalteau reagent solution (1.2 mL) was added. The mixture's absorbance was measured at 765nm after 1 h of incubation.

Total Flavonoid Content: The flavonoid content was quantified per aluminium-chloride colorimetric assay using quercetin as a standard[17]. Shortly, 1 M potassium acetate solution (0.1 mL), methanol (1.5 mL), 10% aluminium chloride (0.1 mL) and 2.8 mL DH_2O were added into the 0.5 mL of sample extract. The solutions' absorbance was measured at 415 nm after incubating for 30 minutes.

Total Proline Content: Proline was extracted and quantified spectrophotometrically using L-proline as a reference and ninhydrin and acetic acid as indicators[5]. Briefly, 0.5 gm of each mushroom sample powder was homogenized with 10mL of 3% of sulphosalicyclic acid and filtered through Whatmann filter paper. 2 mL of glacial acetic acid and 2 mL of filter extract were mixed with 2 mL of filter extract. The mixture was kept at 100 °C in a heated bath for 1h. The mixtures were chilled down and add 4 mL of toluene and extract the proline with toluene. The proline absorbance was measured at 520nm.

Proximate system of analysis: This analysis of each mushroom includes quantifying moisture, ash, lipid, fiber, carbohydrate and protein contents. 0.5 ml of mushroom extract from each sample was taken for quantification. Using BSA as a standard, proteins were measured following a previously described methodology[14]. Total sugars were measured following a previously described method by taking glucose as the reference standard. The results of protein and carbohydrate were expressed as mg of bovine serum albumin and glucose equals per gm mushroom respectively. Similarly, moisture content was quantified following the previous protocol[1] and ash and fiber content were measured following the previously published method[37].

Analysis of antioxidant activity: The antioxidant activity of four mushrooms was evaluated by following tests using ascorbic acid as standard.

DPPH Assay: This test evaluated the scavenging capacity of the sample extracts[9]. Briefly, 2.8 mL of the solution of DPPH and 0.2 mL of the sample extract were combined. The mixture was left to finish the reaction at RT for 30 minutes in a dark environment. The mixture's absorbance at 517 nm was then calculated and percentage inhibition was computed.

H_2O_2 Scavenging Assay: The extracts' ability to scavenge H_2O_2 was assessed by performing the H_2O_2 Scavenging assay[38]. 0.6 mL of 40 mM of H_2O_2 and 3.4 mL of each sample extract were combined. The mixture was incubated at RT, their absorbance was calculated at 230 nm and the % of inhibition was also computed.

Nitric Oxide (NO) Scavenging Assay: The sample extracts' NO scavenging activity was assessed[10]. In short, phosphate buffer was used to form a mixture (3 mL) with 10 mM sodium nitroprusside and extract solutions. This combination was incubated at 25°C and 1 mL of the sulfanilic acid was added. The mixture was then given 1 mL of N-(1-Naphthyl) ethylenediamine and kept at RT for 30 minutes. The absorbance of mixtures was measured at 540 nm and % inhibition was computed.

FRAP Assay: In summary, based on previously published protocol[42], extracts (0.4 mL) were mixed with FRAP reaction mixture (3 mL) solution. The mixture was appropriately combined and incubated at 37°C for 30 minutes. At 562 nm, the solution's absorbance was measured and the % of inhibition of the formation of the fe^{2+} ferrozine complex was determined.

Reducing Power Assay: The sample extracts' reducing power test was examined following the stated methodology[22]. Phosphate buffer (2.5 mL) and each mushroom extract (0.2 mL) were combined. This mixture was kept at 50 °C heated bath and 10% trichloroacetic acid (2.5 mL) was added after chilling and centrifuged for ten minutes at 3000 rpm. After adding ferric chloride and distilled water to the upper layer of the solution at 700 nm, the absorbance was determined. The increase in absorbance indicates the increase in the reducing power.

Data collection: The duration between spawning and the end of the spawn run was noted. Days from the end of the run of spawn to pinhead development, the amount of time it took for pins to mature and the FB total number that was produced, were all noted. Quality was calculated by counting the number of abnormalities, sizes and colours. The fresh weight (gm) and dried weight (gm) of the mushrooms were used to calculate the yield. A substrate and a mushroom strain's efficacy were evaluated. The following equation was used to determine biological efficiency: Biological efficiency, BE (%)= harvested mushrooms' fresh weight / dry substrate's weight ×100.

Statistical Analysis: The studies were executed in triplicate. The analysed data were as per mean ± standard error (SE) using Microsoft Excel, 2018. ANOVA, a single factor analysis of variance, was used to determine the statistical significance of the studied data followed by post hoc Tukey's multiple comparison tests. At $p < 0.05$, differences were deemed significant. and at $p < 0.01$ they were considered highly significant.

Results

Physicochemical Characteristics of soil: The soil sample of the Vanzangi area of the Eastern Ghats regions of Visakhapatnam, AP, India, was selected to grow edible mushrooms; the soil sample was analysed for physico-chemical characteristic of soil. The result of the soil physio-chemical analysis are shown in table 1. The total moisture content was 7.62± 2.14 % and the total nitrogen and potassium were 215± 8.35 and 194 ± 12.36 kg/ha respectively. Further, the metals like Cu, Cd, Cr, Ni, Zn, Pb, Hg, As contents in this soil sample were 0.007 ± 0.001, 23.7 ± 2.41, 75.1 ± 6.74, 26.7 ± 4.52, 31.6 ± 3.85, 47.4 ± 2.63, 0.006 ± 0.001 and 0.009 ± 0.001 respectively. In addition, the pH of the soil is 7.45±1.73 which is slightly alkaline.

Physio-chemical characteristics of Compost: The natural compost rice straw, horse manure and goat manure were used as natural supplementation for the cultivation of mushrooms. The physio-chemical characteristics of the compost were analyzed. The compost differed in soil organic matter, electrical conductivity, chemical elements and pH. The pH value in horse manure compost (6.35±0.01) was significantly more ($p < 0.05$) than in other composts. Significantly higher ($p<0.05$) value in the content of soil organic material in goat manure compost (1.2±0.01%) was compared to other composts. Similarly, the electrical conductivity (0.15±0.002) and amount of P (82±3.4 kg/ha) and K (420±6.1 kg/ha) were significantly higher ($p<0.01$; $p<0.05$) in goat manure compared to rice straw and horse manure compost. Further, the nitrogen content was higher in rice straw (310±7.45 kg/ha) compost ($p<0.05$) compared to horse manure compost.

Comparative evaluation of growth and sporophore parameters of edible mushroom: The growth behaviour of the mushroom was evaluated by the following growth parameters: spawn spread period, pin head initiation and duration for the pin maturity. The sporophore characteristics were evaluated by calculating the parameters like FB's total number, fruiting bodies' weight, the yield of mushroom, FB's length, quality score and biological efficiency.

Spawn run period: The spawn run period of *C. militaris, P. ostreatus, P. djamor* and *A. bisporus* mushrooms was shown in table 3. After spawning, the time needed to finish the spawn run showed a significant difference ($p > 0.05$)

among *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus*. *P. ostreatus* took the shortest time (14±1.15 days) to finish the spawn run followed by *A. bisporus* and *C. militaris* with 15±1.15 and 16.33±0.33 days respectively. *P. djamor* took the most time (17.66±1.15 days) to finish the spawn run. It is clear that the spawn run period varied from a minimum of 14 days to a maximum of 17.66 days and the mean number of days was 15.75 days.

Pin Head Initiation: Table 3 displays the time from spawning to the first pinhead development. The data indicate a significant distinction ($p < 0.01$) between *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus.* They took time from spawning to the initial pinhead formation. *P. ostreatus* (24±1.15) followed by *C. militaris* (30±0.58) and *P. djamor* (31.33±0.88) observed the shortest number of days to pinhead formation. Moreover, *A. bisporus* (32.66±1.76) had the maximum duration between spawning and pinhead creation. It took between 24 and 32.66 days from spawning to pinhead development. The average number of days between spawning and pinhead development was 29.5.

Time taken for the pin maturity: To determine how long the mushroom took for pinheads to grow and mature, different edible mushrooms *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus* were investigated (Table 3). The *A. bisporus* pins matured in the fewest days (3.33±0.33), followed by those from *C. militaris* (4±0.15) and *P. ostreatus* (5±0.58). *P. djamor*'s pins required the longest (5.6±0.88) to mature after they had formed. 3.33 and 5.6 days passed between pin formation and pin maturation. The average time from pin development to maturity in this soil was 4.5 days.

Total number of FB: Total FB produced in the area of 30 cm^2 did not significantly ($p > 0.05$) vary among *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus* (Table 3). The minimum total FB was produced from *A. bisporus* (10±0.33) followed by *P. ostreatus* (21±0.88) and *P. djamor* (20±1.15). *C. militaris* produced the highest number of total FB, which is 25.33. The mean total number of FB was 19.08 produced in the soil of the study area.

Length of FB: The length of FB produced in the area of 30 cm^2 did not significantly ($p > 0.05$) vary among *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus* (Table 3). The minimum length of FB was produced from *C. militaris* (4.6±0.26 cm) followed by *A. bisporus* (6.34±0.09 cm) and *P. ostreatus* (8.26±0.23 cm). *P. djamor* produced the longest FB, which is 9.45±0.2 cm. The mean total number of FB was 7.16 cm produced in the soil of the study area.

Fresh and Dry weight of FB: The fresh and dry FB weights, which were cultivated in the soil, significantly differed among *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus* mushrooms (Figure 1). The data represented reveals that the average fresh weight of FB was recorded minimum in *C. militaris* (5.24±0.61 g) followed by *P. djamor* (8.53±0.58 g) and *P. ostreatus* (9.48±0.57 g). *A. bisporus* recorded the maximum fresh weight in the soil. The mean fresh weight of these edible mushrooms was 8.52±0.58 g. Moreover, the dry weight of the mushroom (Figure 1) was found to be minimum in *C. militaris* (3.32±0.57 g) followed by *P. djamor* (3.42±0.53 g) and *A. bisporus* (3.47 g). The maximum dry weight was recorded in *P. ostreatus* (4.17±0.99 g). The average fresh and dry weights of the FB cultivated in the soil were 8.52 and 3.6 g respectively.

Total yield of mushroom: The soil substrate influenced the total yield of mushrooms applied in this study did not statistically differ ($p > 0.05$) (Figure 2) with *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus* studied. The highest yield from the 30 cm^2 area of soil substrate was *C. militaris* (170.02±2.05 g) followed by *P. djamor* (158.95±1.58 g) and *P. ostreatus* (112.02±2.21 g). The lowest yield was in *A. bisporus* (96.17 g). The mean total yield was 135.12 g in the soil of the study area.

Table 1
Physio-chemical properties of Soil from the Vanzangi area of Eastern Ghats of Visakhapatnam, AP, India. Results are average ± SE (n=3) of triplicate measurements.

Physiochemical analysis of soil	
Total moisture (%)	7.62± 2.14
pH	7.45±1.73
Total Nitrogen (kg/ha)	215± 8.35
Potassium (kg/ha)	194 ± 12.36
Metals (in ppm)	
Cadmium (Cd)	0.007 ± 0.001
Copper (Cu)	23.7 ± 2.41
Chromium (Cr)	75.1 ± 6.74
Nickel (Ni)	26.7 ± 4.52
Lead (Pb)	31.6 ± 3.85
Zinc (Zn)	47.4 ± 2.63
Mercury (Hg)	0.006 ± 0.001
Arsenic (As)	0.009 ± 0.001

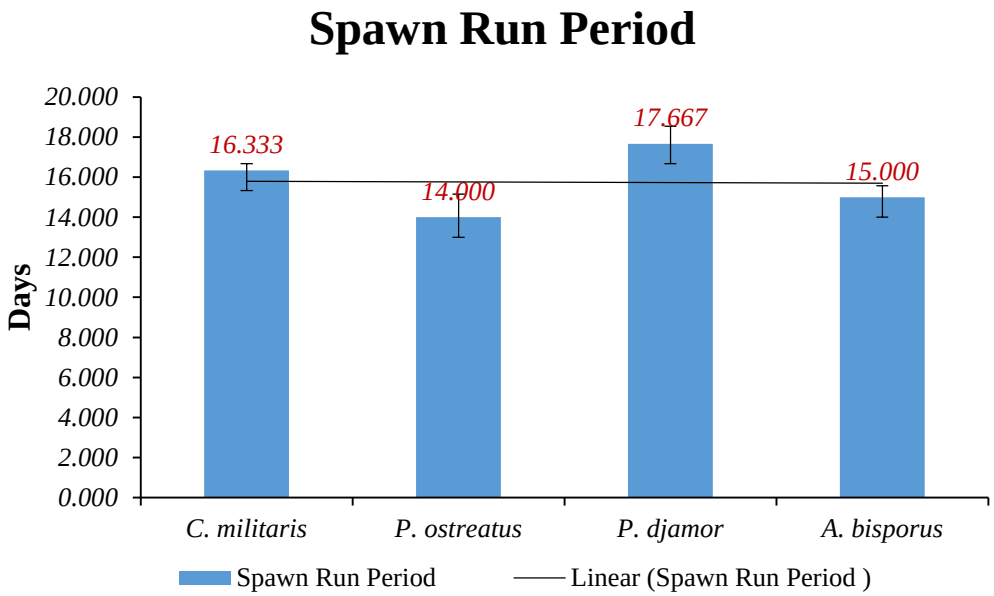
Table 2

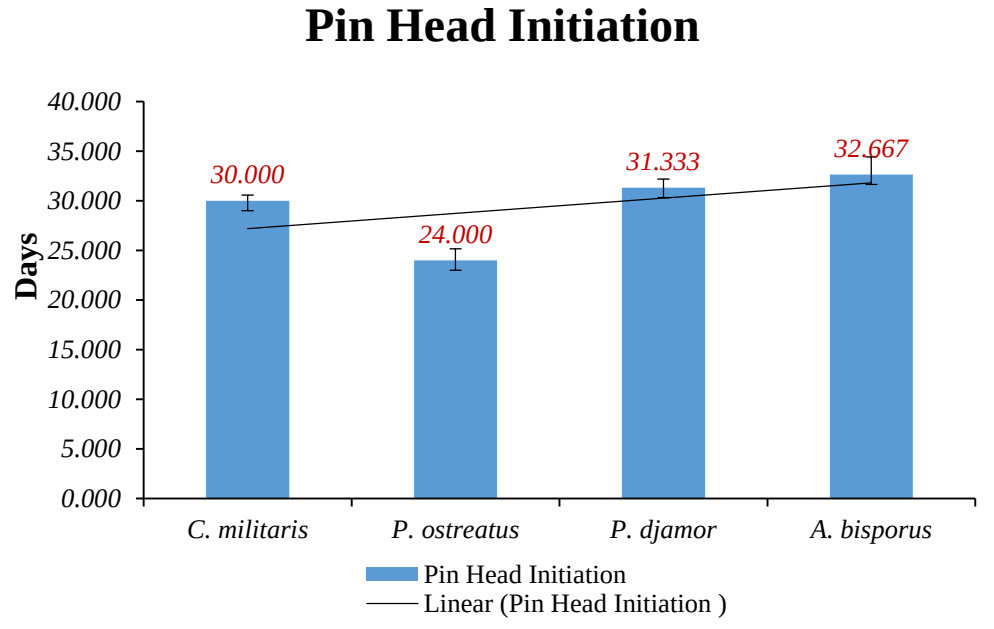
Physio-chemical properties of compost mixed with soil for cultivating the edible mushroom from the Vanzangi area of Eastern Ghats of Visakhapatnam, AP, India. Results are average ± SE (n=3) of triplicate measurements. The notation * and **, p < 0.05 and p < 0.01 stated that the average value differs significantly from others.

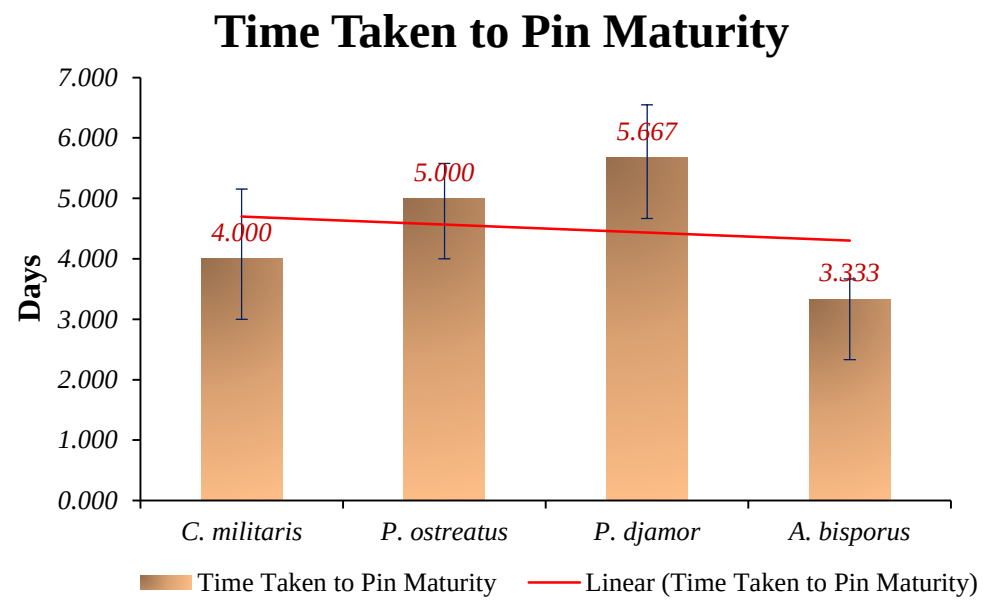
Physiochemical Analysis of Different Compost						
Types of Compost	**pH**	**Electrical Conductivity**	**Soil Organic Mater (OM-LOI %)**	**Nitrogen (kg/ha)**	**Phosphorus (kg/ha)**	**Potassium (kg/ha)**
Rice Bran	5.65±0.02	0.08±0.004	0.76±0.09	310±7.45[a]	74±2.1	175±3.2
Horse manure	6.35±0.01[a]	0.1±0.001	0.44±0.04	280±9.34	59±1.98	192±5.3
Goat manure	5.58±0.02	0.15±0.002[a]	1.2±0.01[a]	295±8.49	82±3.4[a]	420±6.1[aa]

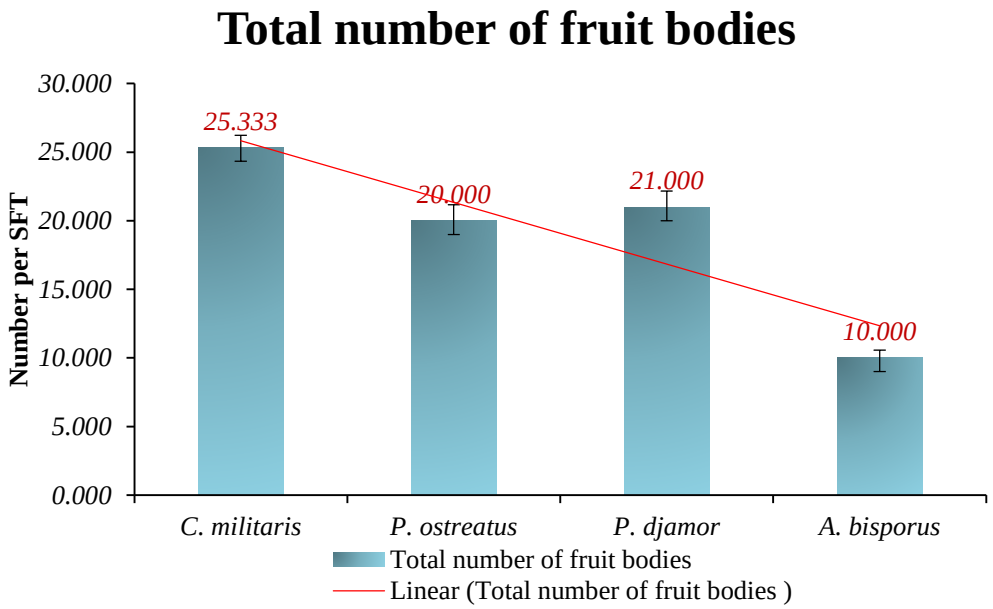
Table 3

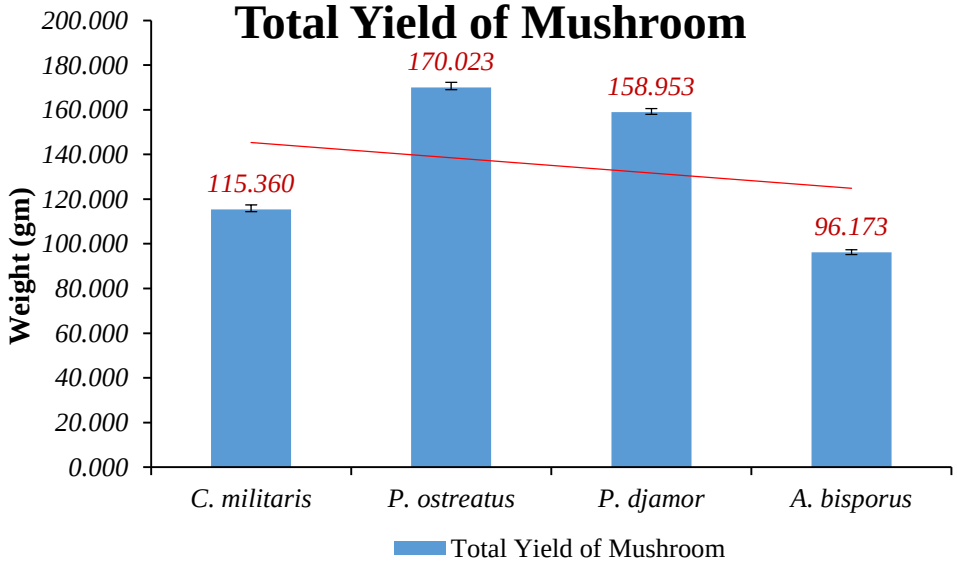
Evaluation of the growth and sporophore parameters of edible mushrooms cultivated in the soil of the Vanzangi area of Eastern Ghats. Three separate (n=3) measurements' means and standard errors are used to express values. The statistic, [a], p <0.01, shows that at least one component's mean value differs significantly from the others and within a row of identical letters differ significantly for each sample of mushrooms.

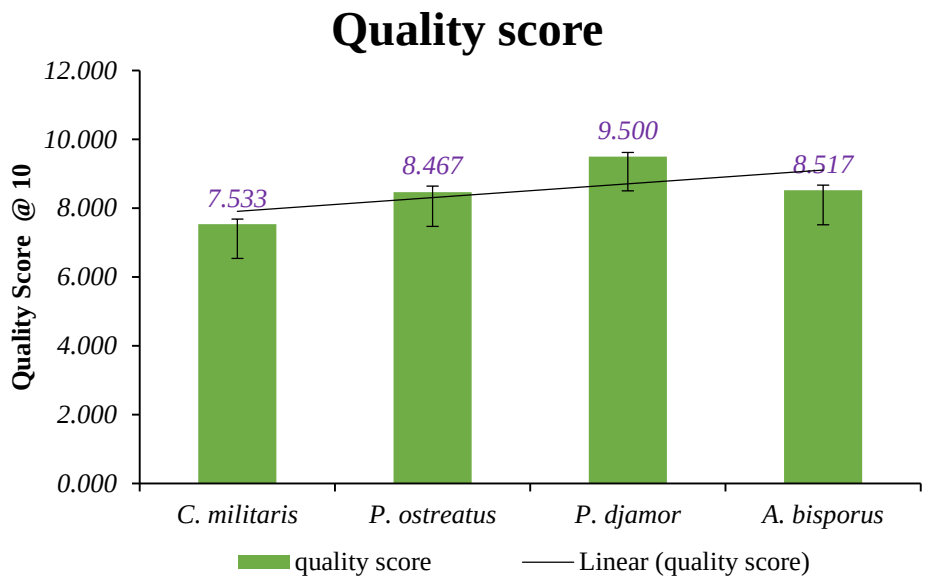





Time Taken to Pin Maturity
Days
7.000
6.000
5.000
4.000
3.000
2.000
1.000
0.000
4.000
5.000
5.667
3.333
C. militaris
P. ostreatus
P. djamor
A. bisporus
Time Taken to Pin Maturity
Linear (Time Taken to Pin Maturity)


Total number of fruit bodies
Number per SFT
30.000
25.000
20.000
15.000
10.000
5.000
0.000
25.333
20.000
21.000
10.000
C. militaris
P. ostreatus
P. djamor
A. bisporus
Total number of fruit bodies
Linear (Total number of fruit bodies)


Total Yield of Mushroom
Weight (gm)
200.000
180.000
160.000
140.000
120.000
100.000
80.000
60.000
40.000
20.000
0.000
115.360
170.023
158.953
96.173
C. militaris
P. ostreatus
P. djamor
A. bisporus
Total Yield of Mushroom


Quality score
12.000
10.000
8.000
6.000
4.000
2.000
0.000
Quality Score @ 10
7.533
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9.500
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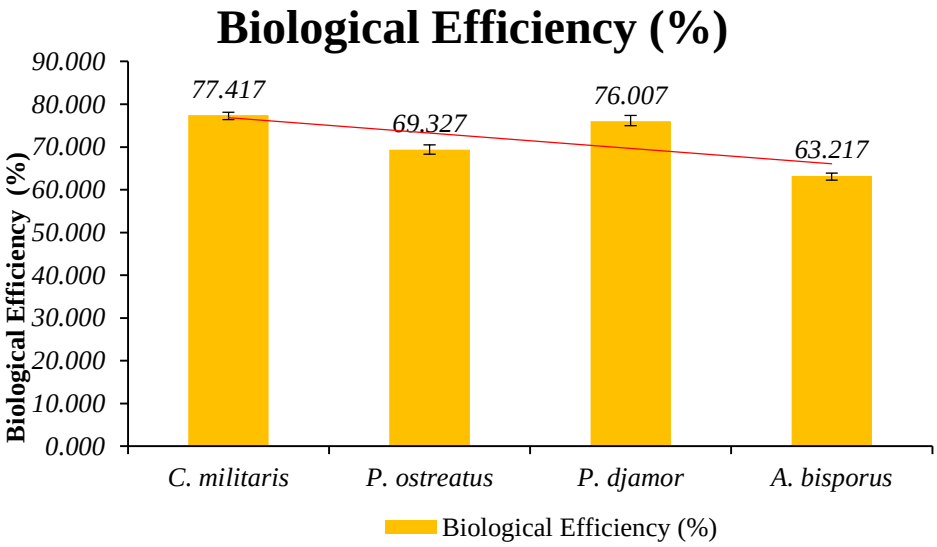
C. militaris
P. ostreatus
P. djamor
A. bisporus
quality score
Linear (quality score)


Biological Efficiency (%)
90.000
80.000
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0.000
Biological Efficiency (%)
77.417
69.327
76.007
63.217
C. militaris
P. ostreatus
P. djamor
A. bisporus
Biological Efficiency (%)

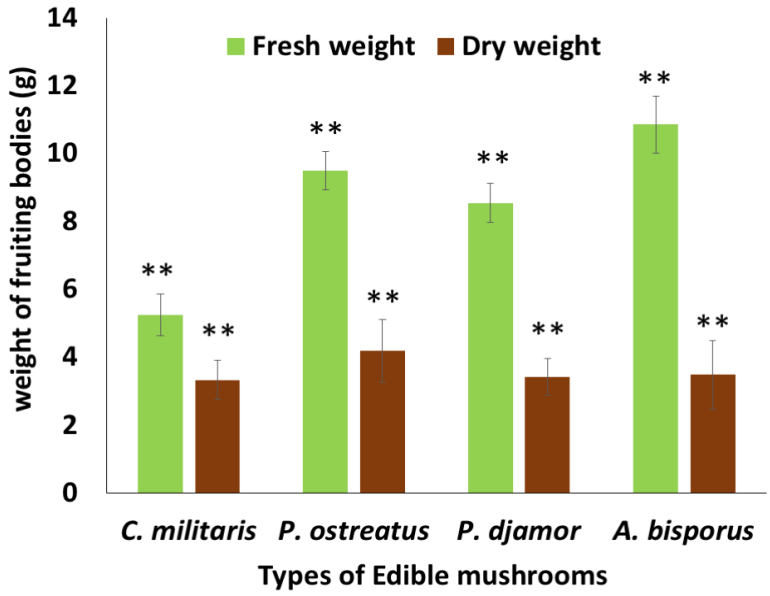
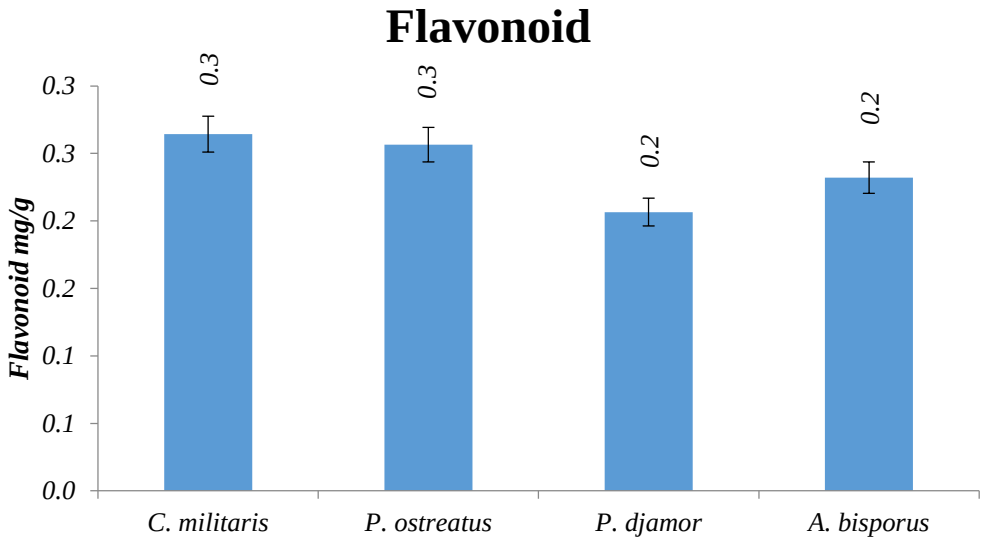
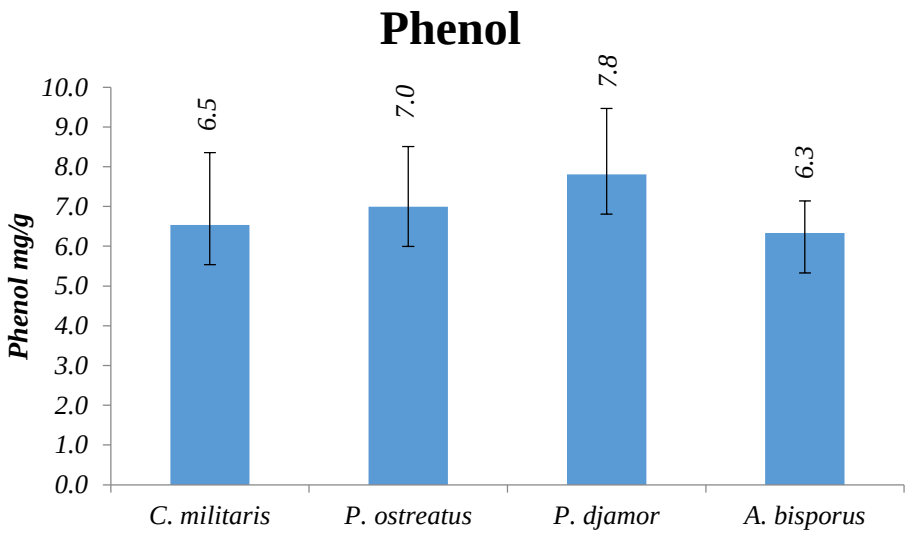

14
12
10
8
6
4
2
0
weight of fruiting bodies (g)
Fresh weight
Dry weight
**
C. militaris
P. ostreatus
P. djamor
A. bisporus
Types of Edible mushrooms

Table 4

Evaluation of the edible mushrooms' bioactive compounds grown in the soil of the Vanzangi area of Eastern Ghats. Three separate (n=3) measurements' means and standard errors are used to express values. The statistic, [a], $p < 0.01$, shows that at least one component's mean value differs significantly from the others and within a row of identical letters differ significantly for each sample of mushrooms.





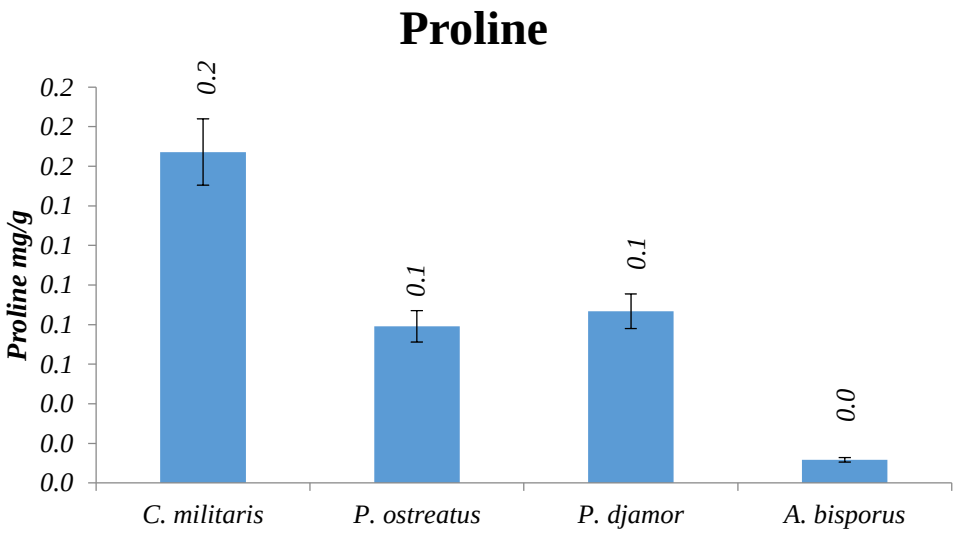
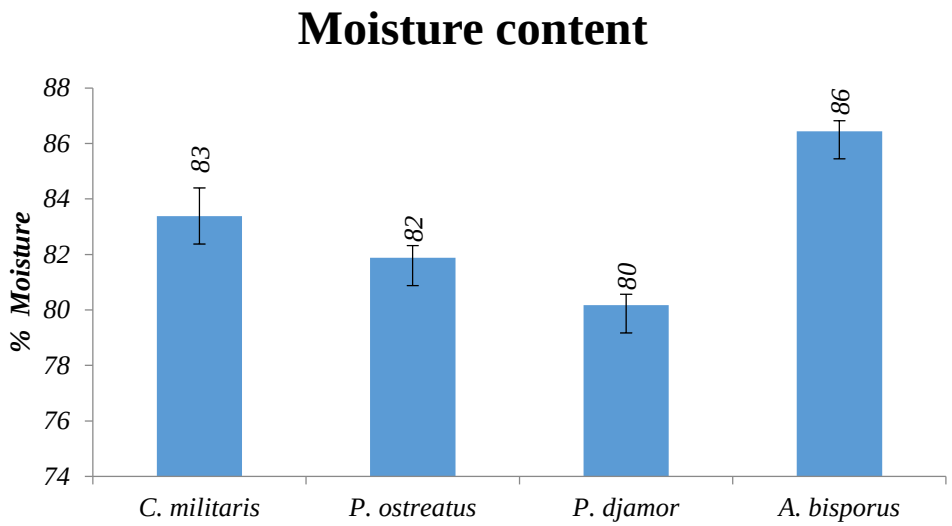
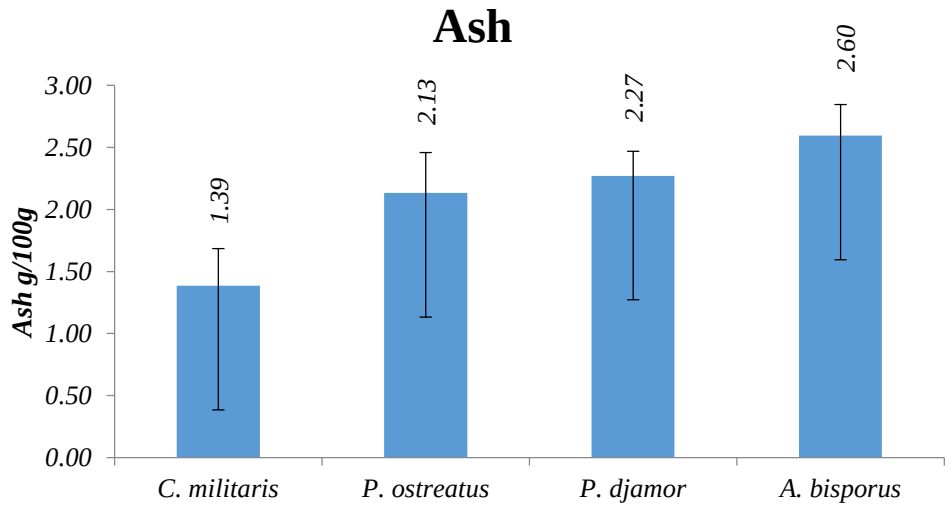
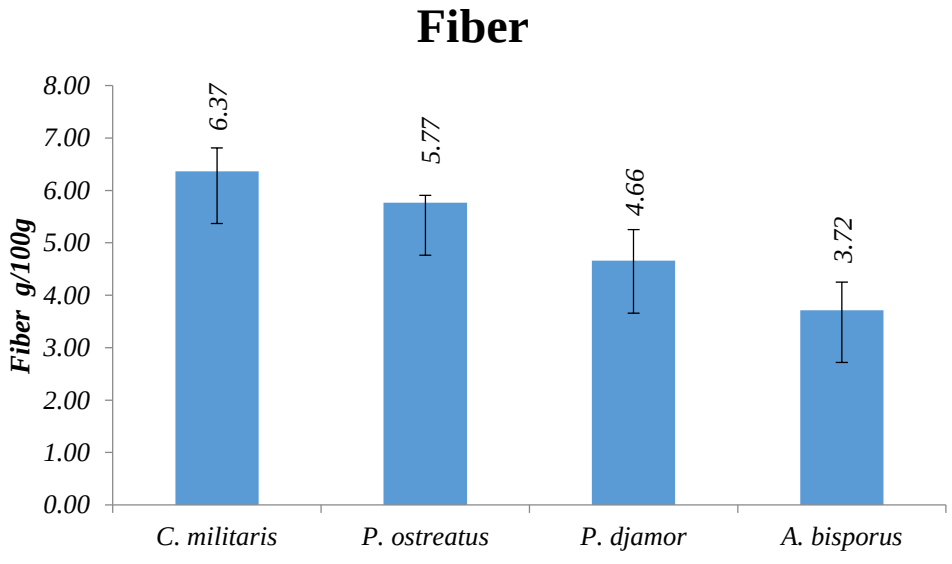


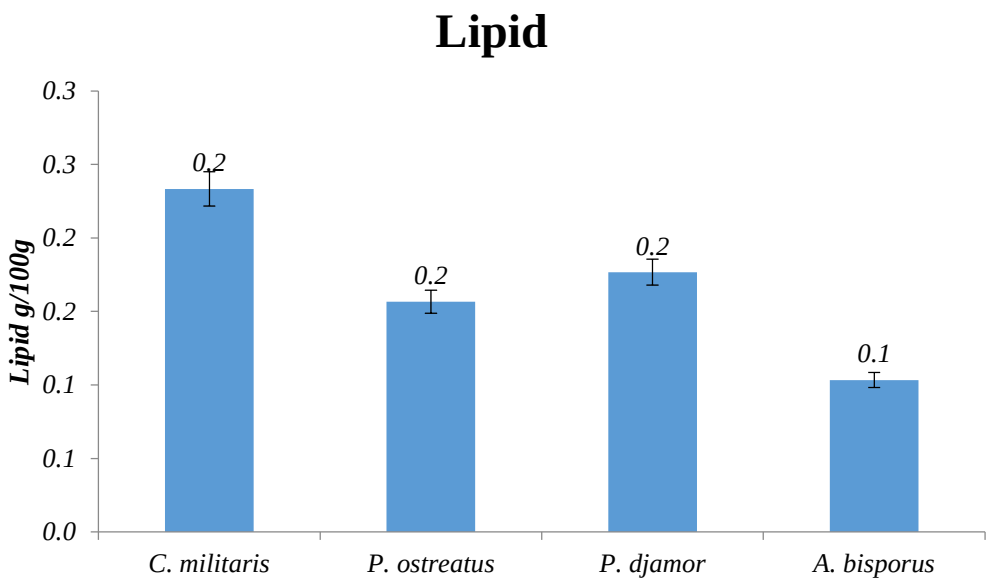
Table 5

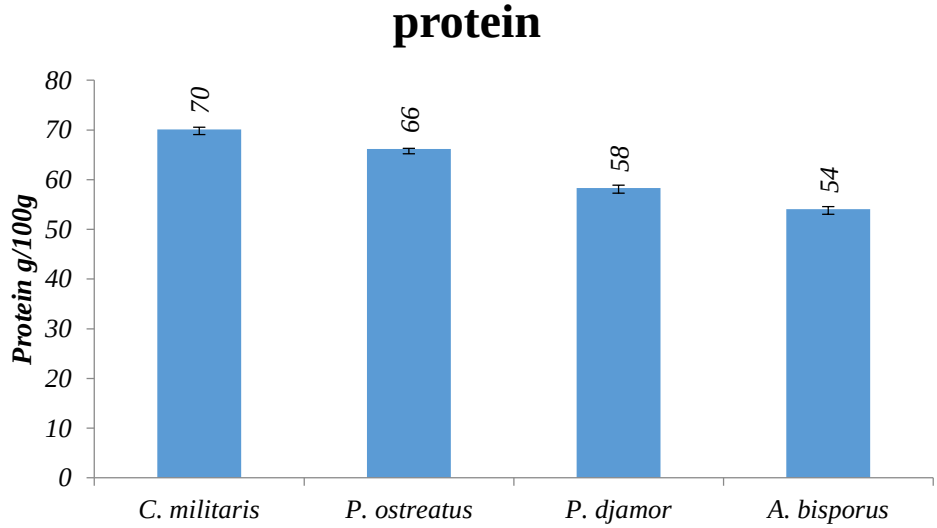
Evaluation of the nutritional value of edible mushrooms cultivated in the soil of the Vanzangi area of Eastern Ghats. Three separate (n=3) measurements' means and standard errors are used to express values. The statistic, [a], $p < 0.01$, shows that at least one component's mean value differs significantly from the others and within a row of identical letters differ significantly for each sample of mushrooms.

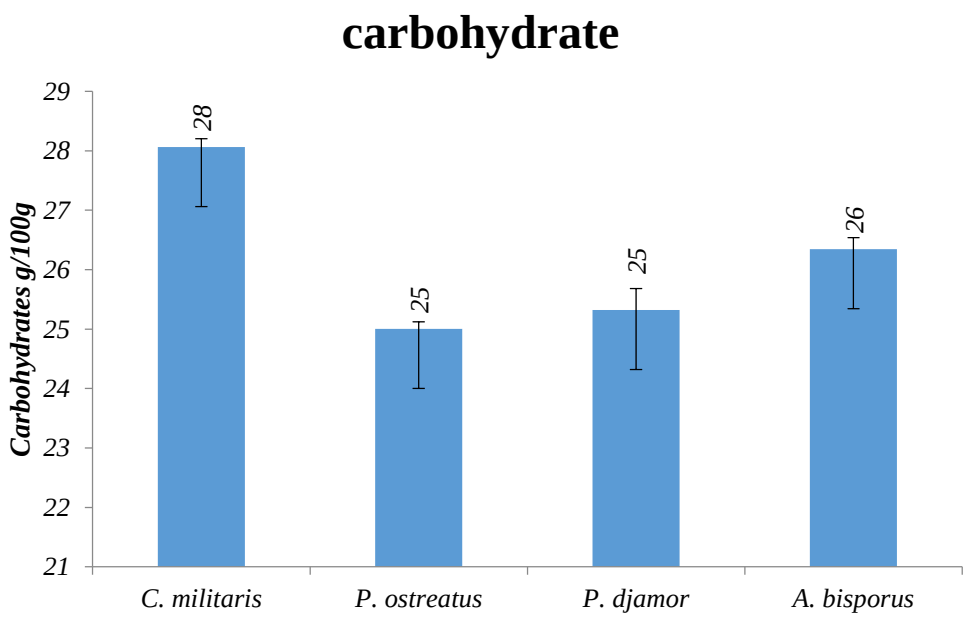







Lipid
Lipid g/100g
0.3
0.3
0.2
0.2
0.1
0.1
0.0
0.2
0.2
0.2
0.1
C. militaris
P. ostreatus
P. djamor
A. bisporus


protein
Protein g/100g
80
70
60
50
40
30
20
10
0
70
66
58
54
C. militaris
P. ostreatus
P. djamor
A. bisporus


carbohydrate
Carbohydrates g/100g
29
28
27
26
25
24
23
22
21
28
25
25
26
C. militaris
P. ostreatus
P. djamor
A. bisporus

Quality score (QS): The complete quality score of these mushrooms was investigated (Table 3) among *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus*. It is interesting to note that recorded QS was significantly higher ($p < 0.05$) in *P. djamor* (9.5±0.12) than other mushrooms. The results also show that the quality of *C. militaris* (7.53±0.15) was significantly ($p < 0.05$) very low compared to other mushrooms.

Biological efficiency: Biological efficiency (BE) reveals that *C. militaris* was significantly ($p < 0.05$) highest (77.41±0.74 %) from all the mushrooms studied followed by *P. djamor* (76±1.35%) (Figure 3). *P. ostreatus* and *A. bisporus* mushrooms recorded the BE percentage as 69.32±1.13 % and 63.21±0.68 % respectively.

Determination of bioactive compound: A UV-Visible spectrophotometer was used to measure the levels of bioactive components in FB, namely phenol, proline and flavonoid content in *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus*. The results are displayed in table 4. The ethanolic extract of these mushrooms contained statistically significant amounts of flavonoids overall ($p <0.01$). With a value of 0.26±0.003 mg/g of mushroom, *C. militaris* was found to have a greater concentration followed by *P. ostreatus* (0.25±0.004 mg/g) and *A. bisporus* (0.23±0.002 mg/g). According to research, *P. djamor* had the least quantity of flavonoid (0.20±0.005 mg/g).

All of the investigated mushroom species' phenol levels were found to be non-significant different ($p>0.05$). *P. djamor* had the highest concentration, measuring 7.8±1.66 mg/g of ethanolic extract followed by *P. ostreatus* and *C. militaris*, measuring 6.99±1.51 mg/g and 6.53±1.82 mg/g respectively. The mushrooms samples showed satisfactory results for proline content and there were differences between *C. militaris* (0.16±0.004 mg/g), *P. ostreatus* (0.07±0.002 mg/g), *P. djamor* (0.08±0.005 mg/g) and *A. bisporus* (0.01±0.0002 mg/g) which were statistically significant ($p <0.01$). It was found that *C. militaris* has more proline than the other studied mushrooms.

Estimation of proximate composition: Different edible mushrooms were taken to estimate moisture content, total ash content and nutritional compounds like fiber, carbohydrate, protein and lipid content to check the nutritive value of the mushroom cultivated in the soil. The findings of this study are represented in table 5. The moisture percentage and ash content recorded among all the species of mushrooms were not significant ($p > 0.05$). The highest percentage of moisture was shown in *A. bisporus* (86.77±6.62 %) followed by *C. militaris* (84.71±7.96 %) and *P. ostreatus* (81.54±8.62%). *P. djamor* (80.16±5.22 %) showed less percentage compared to others species. Further, total ash content was higher in *A. bisporus* (2.59±0.25 mg/g), followed by *P. djamor* (2.27±0.20 mg/g) and *P. ostreatus* (2.13±0.33 mg/g). *C. militaris* (1.38±0.30mg/g) showed a significantly lower percentage than other species.

Regarding the fiber content, carbohydrate content and protein content were not found to have significant ($p>0.05$) differences among *C. militaris*, *P. ostreatus*, *P. djamor* and *A. bisporus*. The fiber content in *C. militaris* was 6.36±0.98 mg/g followed by *P. ostreatus* (5.76±0.83 mg/g) and *P. djamor* (4.66±0.96 mg/g).

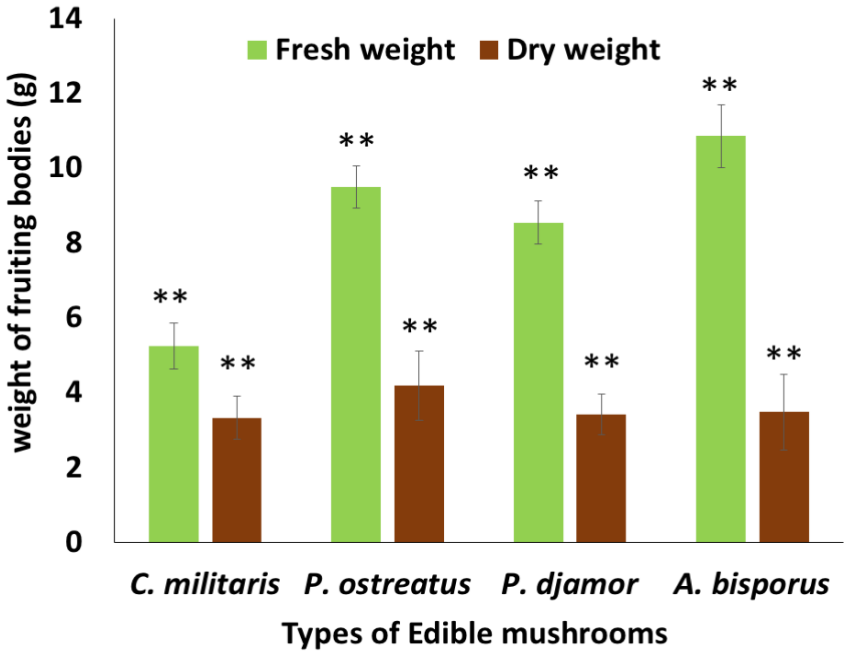


Figure 1: The approximate fresh and dry weight of mushrooms grown in the Eastern Ghats' Vanzangi region. The results are the average and standard errors of three independent (n=3) measurements. ** p <0.01 denotes a significant variance between the means of at least one component from the others.

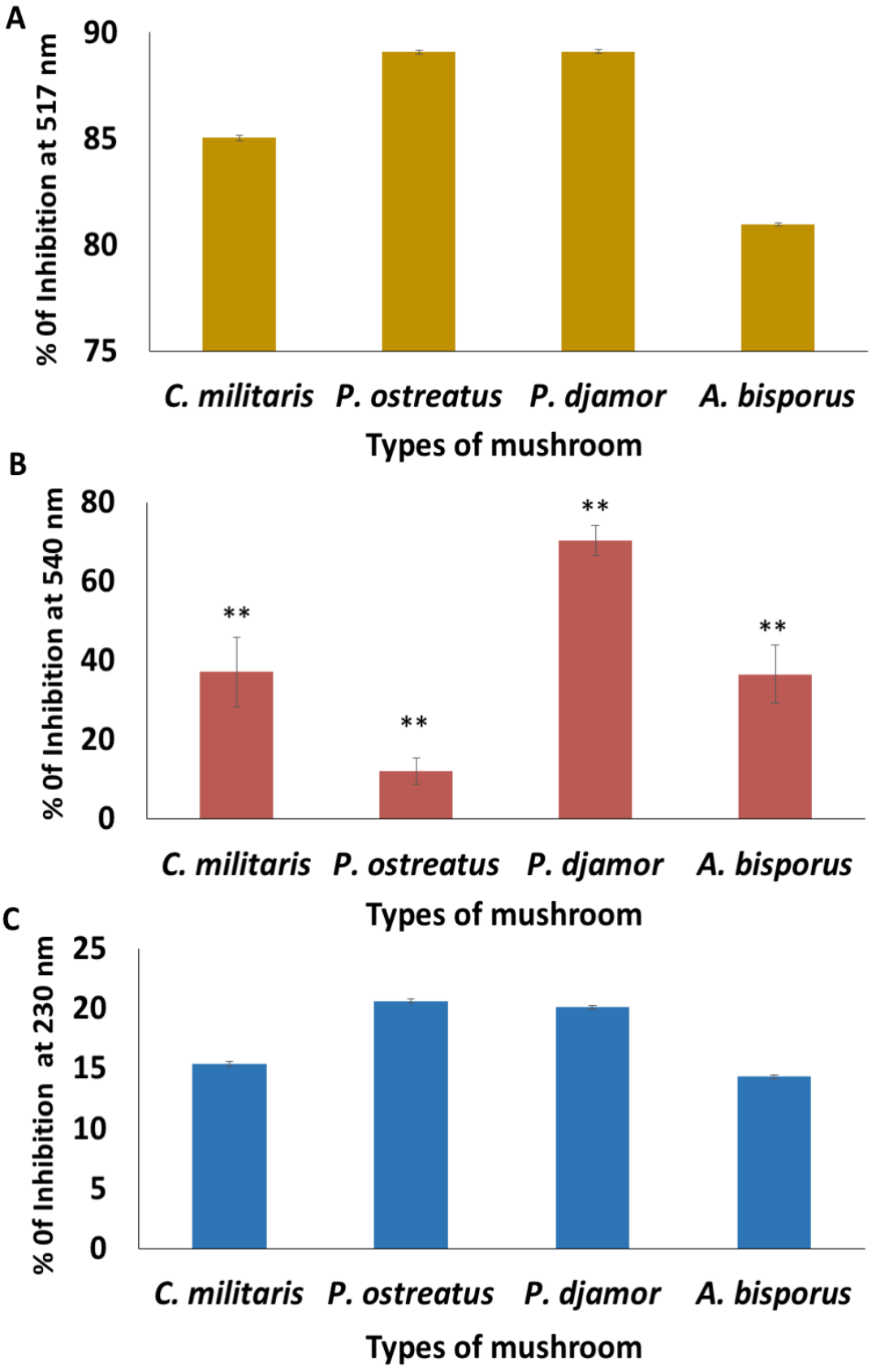


Figure 2: Evaluating the antioxidant ability of mushrooms grown in the Vanzangi region of the Eastern Ghats to scavenge free radicals ethanolic extract of mushrooms was tested using the following methods: A. DPPH free radical Scavenging Assay, B. Nitric Oxide Scavenging Assay and C. H_2O_2 Scavenging Assay. Three independent (n=3) measurements' means and standard errors are used to express values. The notation ** $p < 0.01$ means that at least one component's average value differs significantly from others.

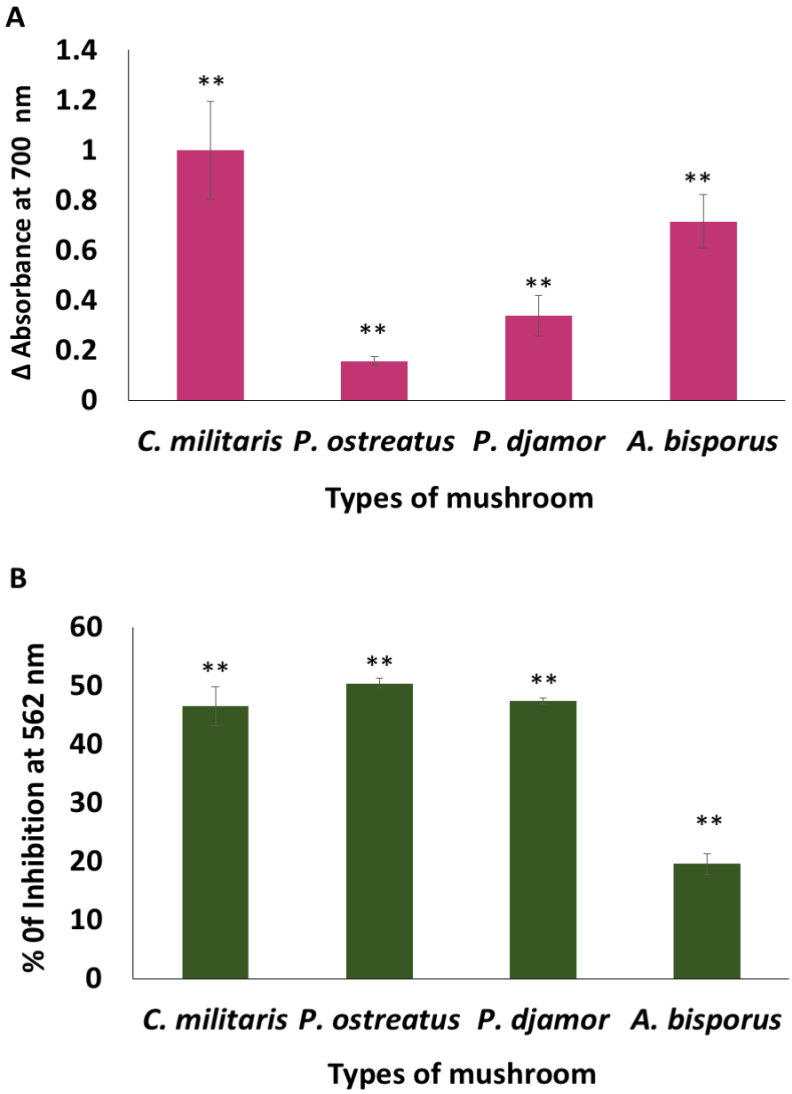


Figure 3: Evaluating the antioxidant potential of mushrooms grown in the Vanzangi region of the Eastern Ghats to scavenge free radicals ethanolic extract of mushrooms was tested using the following methods: A. FRAP Assay, B. Reducing power assay. Three separate (n=3) measurements' average and SE are used to express values. The notation ** p < 0.01, means that at least one component's average value differs significantly from others.

The *A. bisporus* (3.71±0.70 mg/g) showed significantly ($p<0.01$) less fiber content compared to other species. Moreover, *C. militaris* (7.72±0.48 mg/g) showed high carbohydrate content followed by *A. bisporus* (6.34±1.50 mg/g). The *P. ostreatus* (4.65±1.50 mg/g) and *P. djamor* (4.33±0.45mg/g) showed similar content of carbohydrates. Again, the protein content in *C. militaris* (20.08±0.94 mg/g) showed higher value compared to other species followed by *P. djamor* (8.64±0.36 mg/g and *P. ostreatus* (6.18±0.51 mg/g).

The least protein content was found in *A. bisporus* (4.73±0.45 mg/g). The lipid content among *C. militaris*, *P. ostreatus*, *P. djamor and A. bisporus* showed significant ($p<0.05$) differences. The high lipid content was observed in *C. militaris* (0.23±0.003 mg/g) followed by *P. djamor* (0.17±0.001 mg/g) and *P. ostreatus* (0.15±0.002 mg/g) and least found in *A. bisporus* (0.10±0.002 mg/g).

Antioxidant activity: The antioxidant activity of ethanolic extracts of these mushrooms *C. militaris, P. ostreatus, P. djamor* and *A. bisporus* were evaluated spectrophotometrically using different assays: DPPH, nitric oxide (NO) scavenging, FRAP and H_2O_2 scavenging. The antioxidant results are expressed in the percentage of inhibition and changes in absorbance of ascorbic acid as per mg of mushroom (Figures 2A-2E). *C. militaris, P. ostreatus, P. djamor* and *A. bisporus* showed a strong antioxidant capacity.

Figure 2A shows that the DPPH activity of the mushroom among the mushroom species was not significantly different. Still, both *P. ostreatus* (89.08±0.07 %) and *P. djamor* (89.10±0.09 %) showed higher activity followed by *C. militaris* (85.02±0.12%) and *A. bisporus* (80.95±0.08 %). Moreover, all these mushrooms efficiently scavenged nitric oxide radicals significantly ($p<0.01$), represented in figure

2B. The highest NO activity was shown by *P. djamor* (70.27±3.86%) extract followed by *C. militaris* and *A. bisporus* with activity percentages of 37.07±8.84 % and 36.56±7.26 % respectively. *P. ostreatus* scavenged 12.12±3.37 % NO radical, significantly lower than other tested species. Additionally, the H_2O_2 scavenging capacity in all the mushroom extracts was not significantly different and the result is represented in figure 3B. The scavenging capacity of hydrogen peroxide was high in both *P. ostreatus* (20.65±0.17%) and *P. djamor* (20.12±0.18%) followed by *C. militaris* (15.39±0.17 %) and the lowest scavenging capacity found in *A. bisporus* was 14.33±0.2 %. Further, the reducing power of the mushroom extracts showed a significant ($p<0.01$) difference among them (Figure 3A). The *C. militaris* showed the highest reducing power compared to other (1±0.19) tested species followed by *P. djamor* (0.71±0.1) and *A. bisporus* (0.33±0.08).

P. ostreatus (0.15±0.01) showed the lowest reducing power. The FRAP showed a significant ($p<0.01$) difference within the studied mushrooms (Figure 3B). The higher antioxidant capacity was found to be in *P. ostreatus* (50.42±0.81%), followed by *P. djamor* and *C. militaris* with activity percentages of 47.45±0.5% and 46.49±3.32% respectively and the lowest antioxidant capacity was found in *A. bisporus* (19.58±1.76%*)* in FRAP assay.

Discussion

Mushrooms are increasingly good meals in our worldly diets due to their medicinal characteristics and nutritional benefits. In this research, the performance of soil used as a substrate, along with compost made from rice straw and horse and goat manure on the growth and productivity of edible mushrooms *C. militaris, P. ostreatus, P. djamor* and *A. bisporus* was evaluated. The various physio-chemical compositions of the soil and compost led to considerable differences in the production of mushrooms, the morphological characteristics, biochemical composition and contents of bioactive compounds with the antioxidant potential of their FB.

The choice of substrate is crucial when growing mushrooms because they are productive for improved growth, maturation and yield. The growth of plants and other species in the soil is influenced by soil physicochemical qualities, particularly the soil's organic matter, pH value, moisture content and total nitrogen[48]. The soil's most prominent physical characteristic is pH which impacts the amount and uptake of solutes by the soil[3]. The total P, N, pH, EC and trace minerals are crucial for the mycelium's colonization and the FB formation. The soil pH of the Vanzangi area of Eastern Ghats was 7.45 which is neutral to moderately alkaline. The pH ranges from 7.2 -8.2, found to help in the better growth of mushrooms[25]; this indicates that the studied soil can help in the better growth of mushrooms.

The most crucial physical characteristic of soil is moisture. The soil's wetness affects how well nutrients are absorbed. The soil moisture between 7.86 to 8.85 is correlated positively with the distribution and fungi's growth[7]. Vanzangi area soil was closely similar to this range which is a good sign of the growth of good quality mushrooms. The soil and compost used for the growth of mushrooms showed a good amount of moisture, nitrogen and potassium content. This finding will help for the further detailed analysis of the soil which helps cultivate mushrooms more broadly.

By improving the porosity of the soil, soil organic matter may boost its ability to hold water[54]. Soil parameters affect soil characteristics, organic matter level, the capacity of cations exchange, salinity, permeability condition and subsurface features. EC is a rapid, easy and affordable way to assess the health of soils[46]. This is typical soil because the EC is far less than 1 (dS/cm)[15]. The compost used to cultivate mushrooms has an electrical conductivity of less than 1 which helps to produce higher-quality mushrooms. The numerous edible mushroom grows naturally on this soil of the current study with good antioxidant and antimicrobial activity[41]. In this case, we made the composted materials using rice straw combined with goat and horse manure. In addition to phosphate and potassium, nitrogen is a plentiful element in horse dung. The composition of the compost in this study contributed to a higher output of mushrooms.

According to this study's findings, compared to other cultivated mushrooms, *P. ostreatus* mycelia grew on the soil considerably faster (*C. militaris, P. djamor* and *A. bisporus*). The mycelia of the mushrooms ran on soil substrate for an average of 16 days. The findings are consistent with earlier research, which found that it takes 15 days for the spawn to mature while running on paddy straw waste. Nevertheless, other studies using identical substrates found that it takes between 13 and 16 days[23,36]. The difference in the time it takes a spawn to colonize a soil substrate fully depends on the fungus strain, the growth environment and the type of substrate[11,36]. Following the invasion of substrates by mycelia growth, pin-head production was noticed. The amount of time needed for pin-head production is comparable to other studies of a similar type conducted worldwide; for example, oyster mushrooms grown in various substrates form pin-heads between 23 and 27 days after spawning and last for 20–23 days[34].

However, it was discovered that pin-heads started to emerge after around 6 days[43]. When a particular mushroom type was grown on different kinds of substrates such as banana leaves, sawdust and bagasse, such changes in growth rate and colonization of mycelia were noticed[19,21,52,53]. In this case, the pin-head development took an average of 32 days to develop in the soil. More research is needed to determine the cause of the ghat area's slow growth of pin-head. In general, it was found from this research that the total cropping duration in this soil, or the amount of time between sowing spawn and harvesting, varied for each type of mushroom that was grown.

Each type of grown mushroom has a different average length and number of total FB produced. This suggests that *P. djamor* is the preferable mushroom for early harvest and length of the FB concerning the cropping period. For a greater yield of FB in this soil, *C. militaris* is preferable.

Substrates that produced better yields typically also produced higher BE values. This outcome demonstrates that *C. militaris* and *P. djamor* produced the maximum yield and BE when grown on the soil as a substrate and claim that their yield and BE make them ideal for mushroom production compared to other mushrooms. The extract of *C. militaris* in this investigation revealed higher amounts of flavonoid and proline than had previously been reported[26]. The result of proline shows that it can shield the structure of a protein from free radicals, neutralize them and make contact with enzymes to maintain the structure's integrity and proper functioning[50].

High flavonoid levels combined with other oxidative defences such as vitamins and enzymes, may give protection against oxidative stress, according to the literature[2]. *P. djamor* had a higher phenol content, indicating that it might be used as a natural antioxidant source. This study showed that the mushrooms grown in the Eastern Ghats area's soil had sufficient bioactive compounds. This could imply that these mushrooms have phenol, proline and flavonoid as bioactive components.

Carbohydrates, protein, ash, fiber and lipids were the most abundant macronutrients. This study reported that *A. bisporus* showed a higher percentage of moisture and ash content. *C. militaris* has a higher carbohydrate, protein, fiber and lipid content than other mushrooms. This indicated that *C. militaris* is the most nutritional value mushroom that can grow in the soil of the Eastern Ghats area. The protein amount is higher than other nutritive compounds in *C. militaris.* The amount of protein present in the mushrooms varies depending on the profile of genes of the type of mushroom. The physical and chemical properties of the medium used for growth were reported[40]. Thus, *C. militaris* is a good source of protein and has other nutritional compounds that can grow in the ghat soil.

There have been reports of higher secondary metabolite levels in the edible mushroom genera. These secondary metabolites are the mark of various biological effects such as the antioxidant's activity can act under various mechanisms in different mushroom species[24,33]. Hence, it is impossible to determine the antioxidant potential of these mushrooms using only one method. Therefore, this investigation examined *C. militaris, P. ostreatus, P. djamor* and *A. bisporus* for potential antioxidant activity using the DPPH, NO scavenging, FRAP, reducing power assay and H_2O_2 scavenging assays.

P. djamor shows higher DPPH, NO and H_2O_2 scavenging activity than the rest of the mushrooms which is related to the previous study[16,28,32]. *C. militaris* reported higher antioxidant activity in both FRAP and reducing power assay. The present study proved that the mushrooms grown in the soil are a source of antioxidants.

Conclusion

The findings of this experiment show that the soil in the Eastern Ghats region's Vanzangi area significantly affected the production of these mushrooms *C. militaris, P. ostreatus, P. djamor* and *A. bisporus*. The length of running of mycelium, the formation of a pinhead, the number of FB generated, the weight of FB and the mushroom's biological effectiveness were all influenced by the soil substrates. This could be crucial in helping the local growers achieve their desired mushroom harvests. According to the current study, edible mushrooms grown in the Vanzangi region have different nutritional profiles. Still, all species are healthy due to their more protein and fibre content and lower fat level.

Mushrooms are a healthy dietary choice because of their low fat and high fibre content, which makes them particularly protective against diabetes and heart disease. The soil in the Vanzangi area, which serves as the mushroom's growing medium, produces mushrooms with ample amounts of phenol and flavonoid contents. As a result, this study provided prospective reference material for additional research and offered a framework for future investigations from an economic and dietary perspective.

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