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Study of Vitamin D_2 accumulation potential of two edible mushrooms from North-East India

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ABSTRACT

Vitamin D is a fat-soluble vitamin that plays an important role in calcium absorption from the intestine contributing to bone health and many other functions leading to health improvement. There are mainly two forms of Vitamin D, namely Vitamin D_3 (Cholecalciferol) and Vitamin D_2 (Ergocalciferol). Ergocalciferol (Vitamin D_2) is a form of Vitamin D found in food, especially mushrooms. Mushrooms contain ergosterol in their cell membrane; exposure to sunlight/ultraviolet (UV) light causes a photochemical reaction that isomerizes ergosterol to Vitamin D_2. Mushrooms are extensively grown in North-East India from time immemorial by the ethnic people. In this study, Vitamin D_2 from fruiting bodies of two Oyster Mushroom species, namely *Pleurotus florida* and *Pleurotus cornucopiae*, is quantified using a spectrophotometer at 264nm. This study aimed to determine the Vitamin D_2 accumulation potential of both the species and to select the best species with the highest Vitamin D_2 accumulation potential. Fresh cultures of *P. florida* and *P. cornucopiae* were maintained in CSIR-North East Institute of Science & Technology (CSIR-NEIST) Mushroom Germplasm Unit, Jorhat, Assam, India and cultivated in the cultivation unit. A total of 24 treatments were given to each sample by exposing it to UV rays ranging from UV-A (365nm and 395nm) and UV-B (280nm and 310nm) for different time periods. It was observed that Vitamin D_2 levels varied with different wavelengths used. In both the species, Vitamin D_2 levels increased in the UV-B range than in the UV-A range. Moreover, between the two species under study, *P. florida* showed the highest Vitamin D_2 accumulation potential at 280nm when exposed for 120 minutes. The level of Vitamin D_2 increased to an optimum level at 120 minutes of UV exposure and then declined upon the increase in the time period. The recommended daily allowance of Vitamin D is 400-800 IU/day according to WHO. In this study, post-UV treatment, the Vitamin D_2 levels drastically increased to a range of 1000-6000 IU approximately which is safe and can be used in the preparation of Vitamin D supplements in future.

Key words : *Pleurotus, Ergosterol, Ergocalciferol, Spectrophotometer.*

Introduction

There are approximately 202 known species of Oyster mushroom, which belong to the family Pleurotaceae (Basidiomycota, Agaricales) (Kirk, 2015). Oyster mushrooms are found naturally or cultivated worldwide due to their high nutritional value and low cost of cultivation. Few Oyster mushroom species are commonly grown by the ethnic people of North-East India (Barua *et al.*, 1998). The climate of Assam (September to April) is favourable for the cultivation of Oyster mushrooms. Button mushroom is not grown widely in Assam since it needs a very low temperature and high cost of cultivation. Mushrooms are commonly consumed by the ethnic people of North-East India from time im-

memorial which raises their demand as well as market value. The global Oyster Mushroom market rose to 12.74 million tons in 2018 and is expected to have a 6.41% CAGR (Compound annual growth rate) according to the latest market report (Fortune Business Insights and Regional Forecast 2019-2026).

Pleurotus fungi are cosmopolitan in distribution, i.e., found throughout the world (Miles, 2004). Earlier, taxonomists placed the oyster mushrooms under the broad genus *Agaricus* as *Agaricus ostreatus Jacq*. Paul Kummer separated the genus *Pleurotus* from Agaricus and established it as an independent genus in 1871.

Vitamin D is a fat-soluble vitamin that mainly regulates intestinal and renal calcium absorption (Norman, 2008). There are mainly two forms of Vitamin D, namely Vitamin D_3 (Cholecalciferol) and Vitamin D_2 (Ergocalciferol) (Bikle, 2014). Vitamin D_3 (Cholecalciferol) can be synthesized by the skin in the presence of sunlight and metabolized in the body. Vitamin D2 (Ergocalciferol) is a form of Vitamin D found in food, especially mushrooms.

Vitamin D deficiency is emerging out to be a silent pandemic, affecting elderly people, women and children. Approximately 40% of Europeans are suffering from Vitamin D deficiency (Cashman, 2016). Vitamin D deficiency leads to rickets in children and osteoporosis in adults (Eriksen, 2002; Holick, 2007; Munns, 2016). Recent studies have shown a relationship between vitamin D deficiency and cancer, cardiovascular disease, diabetes, autoimmune diseases, and depression (Holick, 2005). Recently, Vitamin D deficiency has also gained significance due to its relation with respiratory infections (Martineau, 2017) and Covid-19 (Weir, 2020). Due to the modern lifestyle which resulted in less exposure to sunlight and lesser availability of natural sources of Vitamin D, people do not obtain the required amount of Vitamin D (Mattila, 1999).

The mushroom was considered to be included in the diet for the first time in the Report 'Dietetics and Nutrition' (Grand et al., 1972). The cell membrane of mushrooms contain a sterol named ergosterol which is considered equivalent to the cholesterol in the skin and it can convert to ergocalciferol (Vitamin D_2) following a photochemical reaction in the presence of sunlight or UV rays (Ko, 2008; Robert, 2008). Vitamin D_2 (Ergocalciferol) can be well metabolized in the human body and it contributes to bone health (Outila, 1999; Jasinghe, 2005; Koyyalamudi, 2009). There is a strong correlation between serum 25-hydroxyvitamin D concentrations and diet (Nakamura, 2000). Vitamin D deficiency can be prevented by consuming UV irradiated mushrooms (Ozzard, 2008). UV rays are proved to be much beneficial than sunlight in the respect that it does not alter the colour, appearance, folate composition, Vitamin B5, Vitamin B6, amino acids, fats, riboflavin, niacin etc. of the mushrooms whereas sun exposure leads to 26% loss of riboflavin, increases folate oxidation and ergosterol and also deteriorates the texture and colour (Simon, 2011).

Several studies have been done on mushrooms in the North-Eastern Region of India. Earlier studies mainly focused on mushroom poisoning (Seal, 1951; Banerjee,1962). Later on, edible mushrooms got popular due to their health benefits (Baruah, 1998; Chatterjee, 2017; Li, 2021). Other studies were also undertaken on glucans from mushrooms (Roy, 2009; Ojha, 2010; Sen, 2013), anticancer therapeutics (Panda, 2021), lignocellulosic degradation of the spent mushroom substrate (Devi, 2020).

The present study determines the Vitamin D_2 accumulation potential of two commonly cultivated Oyster mushroom species, *P. florida* and *P. cornucopiae.* The increase of Vitamin D2 with variable UV wavelengths was also studied. In this research, the species with higher Vitamin D accumulation potential between the two is determined so that farmers opt for the better species for cultivation which in turn contribute to nutraceutical industries.

Materials and Methods

Mushroom sample

Pure cultures of *P. florida* and *P. cornucopiae* were procured from the CSIR-NEIST Mushroom Germplasm Unit, Jorhat, Assam. These species are commonly grown in North-East India, especially in Assam. The culture tubes are stored at - 4 ^{0}C for further use. These pure cultures were then multiplied by subculturing in solid medium (PDA) followed by broth culture (PDB), spawn preparation and cultivation in the CSIR-NEIST Mushroom Unit (Fig. 1)

Chemicals

Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were purchased from Tulip Diagnostics (P) Ltd.; Calcium carbonate; Calciferol standard ($C_{28}H_{44}O$) from Tokyo Chemical Industry (TCI), Japan; Streptomycin Sulphate, Methanol and

Fig. 1(a). Mature fruiting bodies of *P. florida.*

Fig. 1(b). Mature fruiting bodies of *P. cornucopiae.*

Dichloromethane were purchased from Merck Life Sciences Pvt. Ltd.

Cultivation

The cultivation of edible mushrooms was first taken up by CSIR-NEIST, Jorhat in the year 1975 as a research project. It is worthwhile to mention that before the initiative of CSIR-NEIST there was no practice of mushroom cultivation in the North-Eastern region. In this study, the selected mushrooms were cultivated following the protocol developed by CSIR-NEIST, Jorhat. Oyster mushrooms are cultivated by using paddy straw as a substrate (Fig. 2a). Mushroom is harvested when the pileus reaches about 8-10cms in diameter (Fig. 2b).

Fig. 2(a). Mushroom cultivation on paddy straw in the CSIR-NEIST Mushroom Unit.

Fig. 2(b). Harvested mature fruit bodies of mushroom in the CSIR-NEIST Mushroom Unit.

UV treatment

After harvest, a total of 100 g of basidiocarp of each mushroom species (*Pleurotus florida* and *Pleurotus cornucopiae*) were taken, cleaned and subjected to UV treatment using customized UV Chambers (*Green Spectrum*). A total of 24 treatments were given to fruiting bodies at 280nm, 310nm, 365nm, 395nm and the duration of the irradiation was varied from 60 minutes to 180 minutes (Table 1). Followed by the treatment, the fruit bodies were freeze-dried using a lyophilizer (*Scanvac Cool Safe*). The dried samples were powdered and stored for further experiments.

Table 1. Treatments used in the study

Sl.No.	Treatments	Conditions
1.	T-1-1-1	UV 280nm (ventral side),60 minutes
2.	T-1-1-2	UV 280nm (ventral side),120 minutes
3.	T-1-1-3	UV 280nm (ventral side),180 minutes
4.	T-1-2-1	UV 280nm (dorsal side),60 minutes
5.	T-1-2-2	UV 280nm (dorsal side),120 minutes
6.	T-1-2-3	UV 280nm (dorsal side),180 minutes
7.	T-2-1-1	UV 310nm(ventral side),60 minutes
8.	T-2-1-2	UV 310nm(ventral side),120 minutes
9.	T-2-1-3	UV 310nm(ventral side),180 minutes
10.	T-2-2-1	UV 310nm(dorsal side),60 minutes
11.	T-2-2-2	UV 310nm(dorsal side),120 minutes
12.	T-2-2-3	UV 310nm(dorsal side),180 minutes
13.	T-3-1-1	UV 365nm(ventral side),60 minutes
14.	T-3-1-2	UV 365nm(ventral side),120 minutes
15.	T-3-1-3	UV 365nm(ventral side),180 minutes
16.	T-3-2-1	UV 365nm(dorsal side), 60 minutes
17.	T-3-2-2	UV 365nm(dorsal side), 120 minutes
18.	T-3-2-3	UV 365nm(dorsal side), 180 minutes
19.	T-4-1-1	UV 395nm(ventral side), 60 minutes
20.	T-4-1-2	UV 395nm(ventral side), 120 minutes
21.	T-4-1-3	UV 395nm(ventral side), 180 minutes
22.	T-4-2-1	UV 395nm(dorsal side), 60 minutes
23.	T-4-2-2	UV 395nm(dorsal side), 120 minutes
24.	T-4-2-3	UV 395nm(dorsal side), 180 minutes

Extraction and quantification

Vitamin D extraction and estimation was done following a previously established procedure by Yuan *et al.* (2007) with some modifications.

***Standard preparation*:** 25 mg Vitamin D2 standard (TCI) was weighed accurately and mixed with a 25 ml solution mixture of methanol/dichloromethane (75:25, v/v). The solution was diluted to get different concentrations. The absorbance of each dilution was recorded at 264 nm against reagent blank and a standard graph was plotted taking concentration on the X-axis and absorbance on Y-axis (Table 2, Fig. 3).

Table 2. Different dilutions of Vitamin D2 Standard and their respective absorbance

Concentration	Absorbance at 264nm
0.5 mg/l	0.107
1 mg/l	0.169
2 mg/l	0.244
5 mg/l	0.554
50 mg/l	0.137
100 mg/l	1.726
Blank	0

***Extraction from samples and estimation*:** One gram lyophilized mushroom powder was extracted using methanol/dichloromethane (75:25, v/v) at room temperature in a 1:25 solid to liquid ratio using Soxhlet apparatus for three hours. The contents were centrifuged at 12000 rpm for 10 min and the supernatant was collected every time. The extraction procedure was repeated three times and the total supernatant collected was filtered and evaporated in a rotavapor at 50 ºC till it gets concentrated to 2-3 ml. The extract was dissolved in a methanol/dichloromethane (75:25, v/v) mixture. The mixture was filtered through a 0.45 µm syringe filter and analyzed for vitamin D using a spectrophotometer at 264 nm wavelength. The absorbance is converted to vitamin D2 using the formula y=0.015x+0.247.

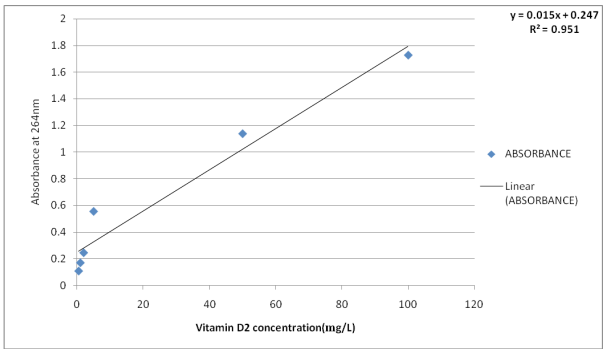

Fig. 3. Standard curve of Vitamin D_2 using Spectrophotometer.

Statistical analysis

Data were reported as mean ± SD. There were three replicates for each treatment and significant differences between Control and UV-treated samples within the same species and between the two species were calculated using Students t-test and One-Way ANOVA ($P \leq 0.05$). All the statistical tests were done using SPSS software (SPSS version 16.0).

Results and Discussion

Spectrophotometry is the standard and simplest

Table 3. Vitamin D_2 content of *P.florida and P.cornucopiae* upon varying wavelength and time period

Sl. No.	Treatment	Vitamin D_2 Concentration in µg/g(dry weight)	
		P.florida	*P.cornucopiae*
1.	UV 280nm ,60 minutes	70.44444 ± 0.23	52.40556 ± 0.14
2.	UV 280nm ,120 minutes	159.0667 ± 0.17	143.4444 ± 18.62
3.	UV 280nm,180 minutes	38.54028 ± 0.18	69.8 ± 0.17
4.	UV 310nm,60 minutes	58.1555 ± 3.04	73.46667 ± 0.11
5.	UV 310nm,120 minutes	41.15556 ± 0.20	79.08889 ± 0.23
6.	UV 310nm,180 minutes	88.97778 ± 0.25	156 ± 0.29
7.	UV 365nm,60 minutes	72.28889 ± 38.75	18.43806 ± 0.21
8.	UV 365nm,120 minutes	71.08889 ± 0.27	150.4 ± 0.24
9.	UV 365nm,180 minutes	119.7333 ± 0.17	103.7556 ± 0.20
10.	UV 395nm, 60 minutes	22.02222 ± 13.37	39.29194 ± 0.20
11.	UV 395nm, 120 minutes	32.54722 ± 0.26	81.71111 ± 0.10
12.	UV 395nm, 180 minutes	9.892778 ± 0.27	106.9111 ± 0.03
13.	Control	46.51111 ± 0.21	36.51111 ± 0.26

technique to quantify the amount of chemicals present in a solution mixture. In this study, the samples, analyzed by spectrophotometry at 264nm showed the presence of Ergocalciferol (Vitamin D2). Moreover, the amount of Vitamin D2 is also calculated for each sample using the standard graph (y=0.015x+0.247). 24 samples were analyzed along with 2 samples as control (Table 3, 4 and 5), (Fig. 4 and Fig. 5).

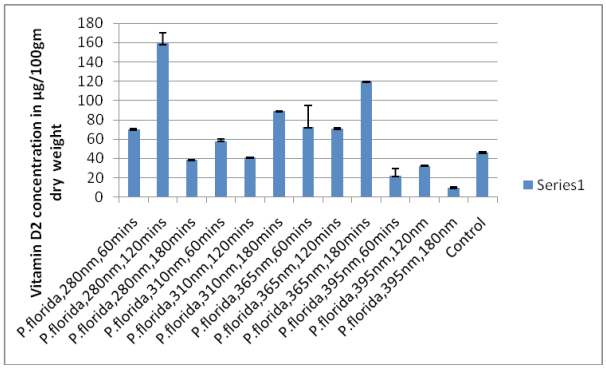

Fig. 4. Vitamin D_2 content of *P.florida* upon the varying wavelength of UV.

P. florida and *P. cornucopiae* are Oyster mushrooms that are widely grown all over the world including North-East India. White Oyster mushroom, *P. florida* is a commonly grown mushroom species due to its high nutritional value, ease and low cost of cultivation. Branched Oyster mushroom, *P. cornucopiae* is also similar to *P. florida* but it has some distinguishable morphological character, i.e., its gills extend downwards through the stipe (decurrent).

The ability of mushrooms to convert ergosterol into ergocalciferol (Vitamin D2) has gained enormous recognition since the whole world is suffering from Vitamin D deficiency without awareness. Vitamin D deficiency is no doubt the most common nutritional and medical problem in the world, affecting a large number of individuals of the world's population which can be considered as a silent epidemic (Holick, 2005).

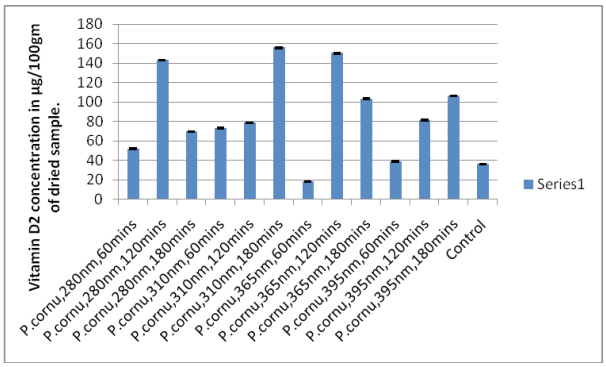

Fig. 5. Vitamin D_2 content of *P.cornucopiae* upon the varying wavelength of UV.

The results showed that the ergocalciferol concentration varied with different UV irradiation. It could be observed from the results that the species have shown a significant increase in Vitamin D concentration on UV irradiation irrespective of the wavelength used in comparison to the control. A maximum increase in vitamin D concentration in all the treatments was observed upon UV irradiation by 280 nm wavelength, which falls under the UV-B range when exposed for a period of 120 minutes.

Conclusion

During the present study, it was observed that both

Table 4. Vitamin D content of *P.florida* upon the varying wavelength of UV irradiation

Species	Time	Vitamin D2 concentration in IU/g(dry weight)				
P.florida	(mins)	280nm	310nm	365nm	395nm	Control
	60 min	2817.78	2326.22	2891.56	880.889	
	120 min	6362.67	1646.22	2843.56	1301.89	1860.44
	180 min	1541.61	3559.11	4789.33	395.711	

Table 5. Vitamin D content of *P.cornucopiae* upon the varying wavelength of UV irradiation

Species	Time	Vitamin D2 concentration in IU/g(dry weight)				
	(mins)	280nm	310nm	365nm	395nm	Control
P. cornucopiae	60 min	2096.22	2938.67	737.522	1571.68	
	120 min	5737.78	3163.56	6016	3268.44	1460.44
	180 min	2792	6240	4150.22	4276.44	

the test species of mushroom produced maximum vitamin D_2 when exposed to UV irradiation in the range of UV-280 to UV-310. Moreover, it was observed that Vitamin D_2 concentration tends to decrease on exposure to UV-A for a period of more than 120 minutes. The vitamin D_2 produced in mushrooms can very well take care of vitamin D deficiency in human beings as the physiological functions of vitamin D_2 and D_3 are more or less similar and vitamin D_2 can also be metabolized in the human body in a similar process as Vitamin D_3 (Jasinghe *et al.*, 2005). Moreover, 120 min UV irradiation was proved to produce the maximum amount of Vitamin D in mushrooms. The recommended daily allowance of Vitamin D is 400-800 IU/day according to WHO. In this study, post-UV treatment, the Vitamin D_2 levels drastically increased to a range of 1000-6000 IU approximately which is safe (Patrick *et al.*, 2019) and can be used in the preparation of Vitamin D supplements in future.

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Conflict of interest

The authors declared that they have no competing interests.

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