NUTRITIONAL VALUE OF WILD MUSHROOMS FROM KHARAN DISTRICT OF BALOCHISTAN, PAKISTAN.

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خلاصہ

زیر تحقیق 18-2017 کے دوران بلوچستان کے ضلع خاران سے جمع شدہ مشر خاران سے جمع شدہ مشرومز کا پہلا مطالعہ ہے۔ وسیع پیمانے پر ضلع بھر میں سروے منعقد کئے گئے تا کہ مشرومز کو مختلف مقامات پر تلاش کیا جاسکے۔ مشروم کی پانچ مختلف اقسام موسم بہار سے موسم گرما کے دوران جمع کی گئیں جن کا تعلق دویژن Basidiomycota اور Agaricomycetes اور ڈر Agaricales اور Family Agaricaceae سے *Agaricus bisporus, Coprinus*, comatus, copruis sterquilinus Sprecies *Agaricus bitorquis*, اور *Coprinellus micaceus* کا تعلق فیملی *Psathyreaceae* سے ہے۔ ان کی غذائی اجزاء کا تجزیہ کیا گیا تھا، نمی کی مقدار *88.8%* سے *91.1%* تک تھی۔ سب سے زیادہ نمی *Agaricus bitorquis* میں ریکارڈ کی گئی۔ Ash content 9.9% سے 11.2% تک تھی۔ سب سے زیادہ 11.2٪ C, micacues میں ملی۔ سب مشرومز میں پروٹین کی مقدار فائدہ مند ہے، مشرومز میں لیڈز/ چربی کی مقدار ٪18 سے ٪28 پائی گئی۔ سب سے زیادہ لپڈز *Agaricus bitorquis* میں *28%* پایا گیا اس میں لپڈز کی مقدار فائدہ مند ہے۔ فیٹ coprius sterquilinus 2.0 میں ریکارڈ کی گئی۔ فیٹ، پروٹین اور لپڈز کی نسبت بہت کم پایا گیا۔ تمام مشروم میں وافر مقدار میں Mineral پایا گیا، سب سے زیادہ کیلشیم (7.4%)Ca پوٹاشیم Agaricus bisporus (15%)K اور میگنیشیم (7.2%)Mg *Agaricus bitroquis* میں غیر معمولی طور پر *MN* میکنیز، زنک *Zn*، سیلینیم Se بھی پایا گیا۔ تاہم یہ انسانی زندگی کیلیئے جائز حدود میں پایا گیا زیر نظر تحقیق سے ثابت ہوا کہ مصنوئی طور پر بنائی گئی انشورہائی پروٹین اور پروٹینکس پروٹین سپلیمنٹس کے مقابلے میں مشرومز کی غزائیت برابر پائی گئی

Abstract

This is the first study of wild mushrooms collected from Kharan district of Balochistan, Pakistan during 2017-18. Extensive survey was conducted throughout the district to find out wild mushrooms growing at different places. Five species of edible mushrooms were found growing at different sites during spring and summer seasons, belonging to Basidiomycota, class Agaricomycetes species are *Agaricus bitorquis*, *A. bisporus*, *Coprinus comatus*, *C. sterquilinus* Order Agaricales (Family Agaricaceae) while *Coprinellus micaceus* belongs to family Psathyrellaceae. These were analyzed for their proximate and mineral composition. The moisture content ranged between 88.8%-91.1%, highest moisture content was recorded in *A. bitorquis.* The ash content was recorded ranged between 9.9%-11.2% , highest ash content was found in *C. micaceus*. The high amount of Proteins ranged between 16%-32% was recorded from all 05 species. Maximum protein content (32 %) was found in *C. sterquilinus* and minimum was in *C. micaceus*. Lipids ranged between 18% - 28% in all species. *A. bitorquis* had maximum 28% amount of lipids. The highest fat content (2.0%) was recorded from *C. sterquilinus*.

Low fat content was found in all mushrooms as compare to proteins and Lipids. All mushrooms analyzed were found to be rich in proteins, lipids and minerals thus making these an excellent healthy food. The highest concentration of Ca (7.4%) and K (15.1%) was found in *A. bisporus*, while highest Mg (7.2%) was in *A. bitorquis.* Zn, Mn and Se were Present in negligible amounts however these were found in permissible limits for human consumption. Statistical analysis indicated that protein, fat and moisture contents were significantly high at $P \leq 0.05$ level, while the lipid was found to be non-significant. However Ca, K and Mg were also significantly high at the $P \leq 0.05$ level. The mushrooms analyzed, showed higher nutritional value which was comparable to the artificially made food supplement. 'Ensure high protein diet' and other proteins supplements.

Key Words: Protein, Lipids, Fats, Calcium, Potassium, Magnesium, Maganese, Zinc, Selenium

Introduction

This is the first study of mushrooms from Kharan, Balochistan. Mushrooms belong to kingdom fungi, division Basidiomycota, class Agaricomycetes Order Agaricales. Mushrooms are delightful; grow at moist shady places on

waste and organic matter as decomposer or for agers. These eatable mushrooms are also utilized for therapeutic purposes and medicinal purposes by the local communities. Mushrooms are utilized as food because mushrooms are tasty, easy to digest and rich in nutritional value (Beluhan and Ranogajec 2011; Manzi *et al*., 2001).These mushrooms were analyzed for their dietary benefits for example; lipid, fat, protein and mineral substance including calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), zinc (Zn), selenium (Se). Now a day's people prefer organic food as compare to the processed diet (Ogazi, 2010; Ihediohanma *et al*., 2014). *Pleurotus sp* is used for its medicinal and nutritional value (Nayana and Janardhanan, 2000; Manpreet *et al*., 2004) while same used to treat cancer (Borchers *et al*., 2008). Nutrients in wild mushroom provide high calories due to sugar, minerals, fat, protein and vitamins (Manzi *et al*., 2001). Mushrooms also play a vital role in bio remediation (Smith and Ofosu, 1972; Stamets, 1993). Due to their high nutritional value these are cultivated, it is easy and reasonably-priced (Amin *et al*., 2007).

A substantial number of wild mushrooms are discovered from the province of Balochistan. They generally show up immediately after rain fall or in spring season and summer. The species grow overnight, spread and cover the whole region therefore the term mushroom growth is used. Mushrooms species are lived for a short time and vanishes in few days. The mushrooms are identified through their size, color, cap, stipe, gills and spores. The identification is also possible through chemical tests. A few mushrooms are harmful while some are non-poisonous and non-edible. In this manner, care ought to be taken to distinguish and report these species since eating toxic specie could be deadly for people and other living creatures. Some work on mushrooms of Pakistan has been done (Khan *et al*., 1980; Sultana and Qureshi, 2007). However very little work has been done on mushrooms of Balochistan, Pakistan. So far just 04 species have been reported in Balochistan (Sultana and Qureshi, 2007). (*Agaricus bisporus* was reported before anyway two new species are reported here for first time). Therefore, there was a need to identify and work on mushrooms of Balochistan. Considering the poisonous qualities of edible mushroom, there is a need to spread social awareness regarding this particular species.

Description of Area: Balochistan Province is divided into 34 districts and six divisions but Rakhshan is a new division it has four districts "Nushki, Chagai, Kharan & Washuk" Kharan is the headquarter of Rakhshan division (Report Revenue department, Government of Balochistan, 2018).Kharan is the capital of the district Kharan, it is a small town, and it lies at 28°35' latitude and 65°25' longitude. Its elevation is 692 meters (2270 feet) above sea level.

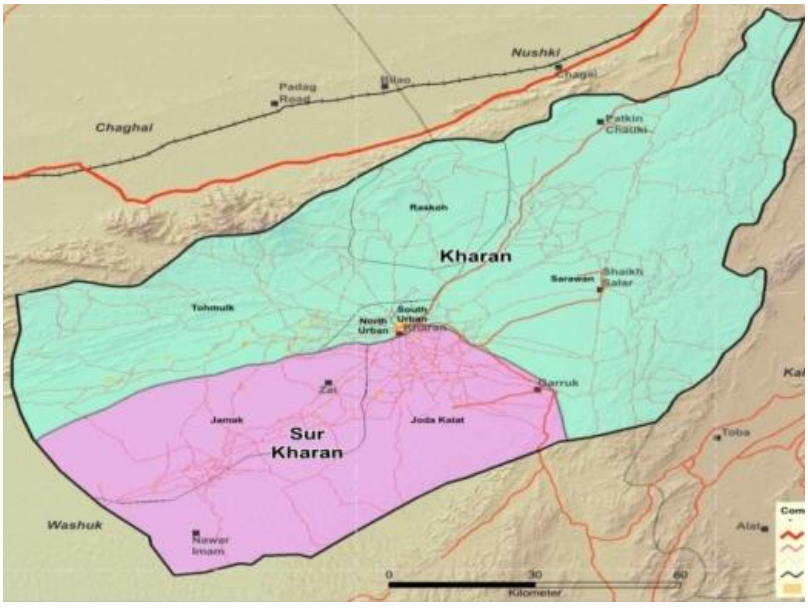

Fig 1. Map of Kharan, Source: https://www.google.com

The distance of Kharan from Quetta is 236 Km or 141 miles. The total area of the district Kharan is 8,958km.Kharan is a deserted area, summers are very hot and dry in this region with average 22mm rain fall**.** Summers are very hot during day time while nights are pleasant. Dust storms exist throughout the year especially

during the months of June to September and as a result travelling becomes impossible during these months. These destructive storms and scorching.

Materials and Methods

Field trips were arranged for collection of mushrooms throughout the Kharan district during 2017-18.Mushrooms were found during spring and summer seasons at five different locations of district Kharan, Balochistan. These samples were identified up to species level the available literature (Largent and Stuntz, 1979; Hall *et al*., 2003; Desjardin *et al.*, (2015); Keirle *et al*,. (2004); Noordeloos *et al*., (2005).Nutritional value of dried mushrooms was determined by using following methods.

Nutritional Analysis:

Moisture analysis: Freshly collected clean mushroom samples were brought to laboratory and weighed. 20 gm was dried in oven at 60°C for 24 hours. The method of heating continued until a constant weight was achieved. Moisture content was calculated by using the following formula (Raghuramulu *et al*., 2003).

(Moisture (%) = (initial weight - final weight) × 100/weight of sample)

Determination of total Ash: One gm of dried sample was weighed in a crucible. Then these samples were placed in oven at 60°C for 24 hours and After 24 hours the samples were placed in a muffle furnace for approximately 4 to 6 hours at 600°C. Ash content was calculated by (Raghuramulu *et al.*, 2003) formula.

(Ash content = Dry weight-weight of ash/wt of sample×100)

Determination of total Protein: The protein contents were determined by Kjeldhal, **(1883)** method. 2gm of dried sample was taken in the Kjeldhal flask and equal amount of selenium mixture was added as Catalyst, It was mixed gently with 5ml of concentrated H_2SO_4 to digest the content and heated until contents became transparent. Total volume was raised to 100ml with distilled water. After few minutes 5ml sample was transferred in distillation chamber of Kjeldhal apparatus. Then 5ml NaOH was added to it and vapors were passed through the condenser into flask**.** Five ml of boric acid solution was mixed in it and one drop of methyl red was added. Ammonia vapors were treated with solution of boric acid, the color of mixture turned pink to green. Then ammonium borate solution was titrated against 0.014 N HCL. The volume of acid consumed for neutralization was recorded until the pink color appeared. The amount of N obtained was multiplied by Factor 6.25, to obtain protein; it was calculated by this formula.

(Protein = wt of atomic number 7 x 6.25)

Determination of Lipids: Total lipid content was determined by following the procedure of Folch *et al*., (1957). Dried ground mushroom sample 5g was dissolved in 50 ml of chloroform: methanol (2: 1 v/v) then mixed thoroughly and samples were allowed to stand for 3 days. The mixture was filtered and centrifuged at one thousand rpm. The supernatant methanol was taken through Pasteur pipette and chloroform was evaporated with the rise Temperature.

Determination of Fats: The fat content was evaluated through Soxhlet equipment. Dried 3-5 g of five samples was weighed in extraction Thimble then it was connected with the flask having petroleum ether filling 1/2 of the volume of flask. The extraction method was carried out by boiling the flask for six hours and then the ether was evaporated through rotary evaporator. The flask was cooled, dried and weighed. The fats content was calculated with the formula.

$$100\,[B - A]\,C$$

Wherever A = wt of flask (g), B = wt of flask containing fat (g), C = wt of sample (g)

Mineral evaluation: The mineral content was determined using standard method given by Ojeka and Ayodele, (1995) was followed. The dry mushroom samples were carried out after acid digestion with HNO_3 and Perchloric acid. The analysis of Calcium (Ca), Potassium (k), Magnesium (mg), Manganese (Mn) zinc (Zn) and Selenium (Se) was done through (AAS) Atomic Absorption Spectrophotometer.

Table 1. Moisture and ash content, protein, lipids and fats content in mushrooms.

S. No	Mushroom species	Moisture Content % (g)	Ash Content % (g)	Protein (g)	Lipids (g)	Fats Content (g)
1	*Agaricus bitorquis*	91.1±1.09	9.9±0.06	25±0.85	28±0.86	1.32±0.21
2	*Agaricus bisporus*	89.8±1.36	10.2±0.56	30±0.81	23±0.92	1.25±0.19
3	*Coprinellus micaceus*	88.8±1.45	11.2±0.87	16±0.86	20±0.87	1.24±0.13
4	*Coprinus comatus*	90±1.09	10±0.21	31±0.89	18±0.89	1.99±0.18
5	*Coprinus sterquilinus*	89.7±1.25	10.3±0.52	32±0.88	19±0.86	2.0±0.18
	LSD (0.05)	0.17*	0.18*	2.4*	3.52*	0.5*

Each value is the mean of 5 replicates ± Standard Deviation, ANOVA $P \leq 0.05$

Table 2. Nutritional content in five species of wild edible mushrooms.

Mushroom Species	Macro minerals			Micro Minerals		
	Calcium Ca (PPM)	Potassium K (PPM)	Magnesium Mg (PPM)	Manganese Mn (PPM)	Zinc Zn (PPM)	Selinium Se (PPM)
Agaricus bitorquis	6.1a±0.62	14.5*a* ±0.93	7.2a ±0.62	0.5a±0.08	1.7b±0.59	0.09a±0.014
Agaricus bisporus	7.4a ±0.62	15.1*a* ±0.89	6.4a±0.62	0.3ab±0.08	1.6b±0.54	0.09a±0.014
Coprinellus micaceus	4.1b ±0.14	9.5*ab* ±2.69	4.1ab±0.17	0.3ab±0.08	1.7b±0.26	0.06b±0.1
Coprinus comatus	5.3ab ±0.2	5.2*b* ±0.2	3.4b±0.18	0.18b±0.2	2.5a±0.14	0.07ab±o.038
Coprinus sterquolinus	5.2ab ±0.08	5.4b ±0.14	3.1b±0.16	0.17b±0.14	2.3a±0.24	0.08a±1.03
LSD (0.05)	1.34*	2.75*	2.31*	0.12*	0.80*	0.02*

(Each value is the mean of 5 replicates ± Standard Deviation, ANOVA $P \leq 0.05$)

Results and Discussion

Five species were found growing at five localities of Kharan, Balochistan, these were collected and brought to the laboratory. These were identified by the help of available literature and by the help of Research Officers of Pakistan Natural History Museum Islamabad. The five species identified are *Agaricus bitorquis*, *Agaricus bisporus*, *Coprinus comatus* and *Coprinus sterquilinus.* These specimens are preserved and deposited in herbarium, Botanical garden, University of Balochistan. Mushrooms in Balochistan and uploaded in online herbarium. The mushrooms in Balochistan are threatened because of the over collection, urbanization, drought and deforestation. Some of the species previously seen by the locals are no more available. A large number of edible species about (54) of mushrooms have been reported from Pakistan by Sultana and Qureshi (2007) including 04 from Balochistan, 03 from Sindh, 05 from Punjab and maximum species 44 were recorded from NWFP and Azad Kashmir. Only 02 species were found from Kharan *Agaricus bitorquis*, *A. bisporus* have been reported by Sultan and Quereshi (2007). However 03 species *Coprinus comatus, Coprinus sterquilinus* and *Coprinellus micaceus* have been reported for the first time from Balochistan, Pakistan. Some species being commercially used in the world are *Agaricus bisporus*, *Coprinus comatus*, *Lentinus edodes*, *Pleurotus ostreatus, Stropharia rugosoannulata* and *Volvariella volvacea.*

The result in Table 1 showed the moisture content in *A. bitorquis, A. bisporus, C. micaceus, C. comatus, C. sterquilinus* (91.1%, 89.8%, 88.8, 90.0%, 89.7% respectively), highest moisture content was recorded from *A. bitorquis* (91.1%). The ash content (9.9%, 10.2% .11.2%, 10.0%, 10.3% respectively) were in recorded *A. bitorquis, A. bisporus*, *Coprinellus micaceus C. comatus. C. sterquilinus,*The highest ash content was recorded from *Coprinellus micaceus* (11.2%). The high concentration of protein was between (16-32%) while maximum protein recorded from *C. sterquilinus* it was 32%. Almost similar results were found by Mohiuddin *et al*., (2015). High amount of proteins were found in these wild mushrooms these are comparable with the amount of protein found in meat and egg (Aletor, 1990). The proteins, carbohydrates and vitamins in mushrooms are more than in wheat and milk (Aletor, 1990; Thatoi and Singdevsachan, 2014). Mushrooms are highly recommended as food for the people of developing countries like Pakistan with high rates of malnutrition where people cannot afford to purchase meat on regular basis. Therefore these mushrooms are recommended to be used as an excellent source of protein.

High levels of total Lipids from five species, maximum lipids were recorded from *A. bitorquis*, (28%). Similar amount of lipids were reported by Saiqa *et al*., (2008). They also found that *A. bisporus* and *A. bitorquis* have high nutritional and commercial value; with good quantity of Lipids, Protein and carbohydrates. The amount of protein, carbohydrates and fiber recorded from wild mushrooms were almost similar to that report from cultivated mushroom *Plesurotus sps* (Sharma and Madan, 1993).

The amount of Fats was recorded from all mushrooms studied. The quantity of fats was quiet low as compare to protein and lipids. However lipids are the main source of energy when surplus are deposited in the mushroom of fat

in body. Low fats prove that mushrooms are a good source of food with low fats. Sultana and Qureshi (2007) also found similar results while working on mushrooms of Pakistan, they have reported that these are used as perfect diet for heart and cardiovascular patient. The fatty fractions of mushrooms contain unsaturated fatty acids such as linoleic acid Zhang, 2016). Atila *et al.,* (2017) reported that good quality carbohydrates and fatty acids are found in mushrooms. These have anticancer, antimicrobial and antihypertensive activities. Many mushrooms contain unsaturated fatty acids, polysaccharides, vitamins and minerals. Many of these compounds are biologically active. They showed activities against many diseases, high antimicrobial, and antioxidant, anti diabetic and hepatic protective activities.

The result in Table 2 showed the mineral composition of these five species. *A. bitorquis* (Ca 6.1%, K 14.5%, Mg 7.2%,Mn 0.5%, Zn 1.7%, Se 0.09%), *A. bisporus* (Ca 7.4%, K 15.1%, Mg 6.4%, Mn 0.3%, Zn 1.6% , Se0.09%),*C. micaceus* (Ca 4.1%, K 9.5%, Mg 4.1%,Mn 0.4%, Zn 1.7% , Se 0.06%), *C. comatus* (Ca 5.3 %, K 5.2%, Mg 3.4%,Mn 0.18 %, Zn 2.5% and Se 0.07%), *C. sterquilinus* (Ca 5.2%, K 5.4%, Mg 3.1%, Mn 0.17%,Zn 2.3% and Se o.o8%). The mushrooms studied, had good amount of minerals including trace minerals. Maximum Ca and K were recorded from *A. bisporus*. Highest Mg and Mn were found in *A. bitorquis* while highest Zn was found in C. *sterquilinus.* These values are also almost equal to that reported from cultivated mushrooms *Pleurotus spp* that is used as healthy food because of its high nutritional value (Leghari *et al.,* 2017). Low levels of selenium were found in all species tested these were found in all species which was in permissible limits. Therefore, it is recommended that all mushrooms can be used as food however, *A. bisporus, A. bitorquis* have high minerals also used as food as a complete diet or be added to other foods for enriching the product. Similar results were found by Monteiro (2008) he added the *A. brasiliensis* mushroom to tomato sauce. These species are not poisonous. Chang (1982) found high levels of 4.4/100g of moisture, 39.4 /100g of protein, 45.6/100g of carbohydrate, 3.0/100 g of lipid, and 7.6/100 g of minerals as compare to the amount found in this study. The difference is probably due to species and climatic conditions.

The mushrooms contain high macro and micro mineral elements (Bano and Rajarathanam, 1982; Mudassir and Mansoor, 1999). Mushrooms have been identified as alternative source for healthy diet rich in protein and minerals. Mushrooms are also recommended as food for the people of developing countries like Pakistan with high rates of malnutrition where people cannot afford to purchase meat on regular basis. C. *comatus, C. micaceus* and *C. sterquilinus* are edible when young; they become non-edible when mature as these mushrooms release black color liquid (spore) which makes it unpleasant for consumption.

Conclusion

Extensive survey was conducted to report wild mushrooms and their nutritional value from Kharan district of Balochistan, Pakistan during 2017-18. Five species of edible mushrooms belonging to Basidiomycota, class Agaricomycetes were recorded namely*Agaricusbitorquis, A.bisporus*,*Coprinuscomatus, C. sterquilinus*and *Coprinellusmicaceus.* These were analyzed for their proximate and mineral composition. All wildmushrooms had significantly high moisture, protein, lipid with low fats.Significantly high concentration of Ca, K and Mg was also recorded. However Zn, Mn and Se were present in negligible amounts. It is therefore concluded that these mushrooms have high nutritional value comparable to food and protein supplements.

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