Exploration, Sociobiology and Conservation of Mushrooms - My experience*

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I express a deep sense of gratitude from the core of my heart to the honorable members of Mycological Society of India for electing me as President of the Society for the year 2019 and also bestowing upon me the responsibility of Editor-in-Chief of KAVAKA being Transactions of Mycological Society of India. Before my election as President, the Society has honored me by giving me various positions of responsibility as member of Editorial Board of KAVAKA, Council Member and also as Vice President of the Society. To my esteemed teacher and mentor Late Dr. Satnam Singh Saini, former Professor and Head Department of Botany and Dean Life Sciences, Punjabi University, Patiala, I owe a great debt of gratitude. It was he who introduced me to the field of mycology in general and mushrooms in particular when I was a M.Sc. student in the Department from 1976-1978. He was instrumental in inducting me as Lecturer in Botany in the Department in 1987. Ever since I have been working along with my collaborators and students on various aspects including systematics, sociobiology, domestication, conservation, distribution, ecology, mycorrhiza and nutritional and nutraceutical evaluation of mushrooms of North West Himalayas and Punjab Plains.

MUSHROOM SYSTEMATICS

Work on mushroom systematics was initiated in 1977, when I was a M.Sc. student working for my dissertation on “Studies in the Taxonomy of Agaricales”, which I submitted in 1978. It was a preliminary work involving collection of mushroom samples from Patiala in Punjab. For M.Phil dissertation which I submitted in 1981 again I worked on the topic “Studies on the taxonomy of Agaricales” in which besides Punjab Plains, sample collection was also done from North West Himalayas. For my Doctorate thesis on “Studies on North West Himalayan Russulae”, which I submitted in 1985, I surveyed the whole of North West Himalayas from Kashmir to Nainital for the collection of research sample and for undertaking studies on the distribution and ecology of russulaceous mushrooms. During this period I published my first research paper on Russula foetens (Pers) Fr. in Current Science (Saini and Atri, 1981). Based upon the experience gained, a “Field Key to mushroom collector” was prepared, which was sent to Dr. Rolf Singer of Field Museum of Natural History Chicago, Illinois, U.S.A. for ratification in the beginning, and subsequently published (Atri and Saini, 2000; Atri et al., 2005a). During the preparation and giving final shape to the Field Key some of the authentic references including Smith (1949), Hesler and Smith (1979), Arora (1986), Singer (1986), Pegler (1977, 1983, 1986), Rayner (1968-70), etc. were frequently consulted. Recently an exhaustive document on the characterization of lamellate mushrooms was published in which again a modified version of the Field Key was made part of it (Atri et al., 2017). During all these years along with my students and collaborators, mushrooms belonging to families Agaricaceae, Amanitaceae, Bolbitiaceae, Tricholomataceae, Polyporaceae, Lyophyllaceae, Coprinaceae, Pluteaceae, Entolomataceae, Inocybaceae, Marasmiaceae, Mycenaceae, Pleurotaceae, Psathyrellaceae, Strophariaceae, Russulaceae, etc. were taxonomically investigated employing macroscopic, microscopic and need based molecular criteria. In all approximately 1700 collections belonging to 700 taxa were described. Out of these about 250 taxa were described for the first time from India and as many as 34 taxa, namely Macrocystidia indica Saini, Atri & Singer, Russula minutula var. robusta Saini, Atri & Singer (Saini et al., 1982); Russula natarajanii K. Das, J.R. Sharma & Atri (Das et al., 2006); Lactarius annulocystidiatus S. Sharma, M. Kaur & Atri (Sharma et al., 2012); Chlorolepiota indica B. Kumari, N.S. Atri & M. Kaur (Kumari et al., 2013a); Chlorolepiota brunneotincta N.S. Atri, B. Kumari & R.C. Upadhyay (Atri et al., 2014a); Leptiotopsis indica B. Kumari & N.S. Atri (Kumari et al., 2013b); Leptonia attenuispora B. Kumari & N.S. Atri (Kumari et al., 2013b); Russula sharmae K. Das, Atri & Buyck, Russula dubdiana K. Das, Atri & Buyck, Russula sikkimensis K. Das, Atri & Buyck (Das et al., 2013); Leucoagaricus albidus B. Kumari & N.S. Atri, Leucoagaricus barssii var. bulbobasilarus B. Kumari & N.S. Atri, Leucoagaricus tener var. brevisporus B. Kumari & N.S.

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Atri (Kumari and Atri, 2013); *Panaeolus africans* var. *diversistipus* Amandeep Kaur, N.S. Atri & Munruchi Kaur, *Panaeolus speciosus* var. *pilocystidiosus* Amandeep Kaur, N.S. Atri & Munruchi Kaur (Amandeep et al., 2013); *Coprinopsis radiata* var. *macrocarpus* Atri, A. Kaur & M. Kaur, *Coprinopsis cothurnata* var. *equisterca* Atri, A. Kaur & M. Kaur (Amandeep et al., 2014); *Conocybe microrhiza* var. *coprophila* Amand., Atri & M. Kaur (Amandeep et al., 2015a); *Agaricus lanipes* var. *macrosporus* Saini, Atri & A.K. Gupta (Saini et al., 1992); *Lactarius subvarious* var. *himalayensis* Atri, Saini, M.K. Saini & A.K. Gupta (Atri et al., 1993); *Termitomyces indicus* var. *patialensis* Atri, A. Kaur & Kour, *Termitomyces tyleriarius* var. *macrocarpus* Atri, A. Kaur & Kour (Atri et al., 2005b); *Agaricus stellatus-cuticus* Atri, M. Kaur & A. Kaur, *Agaricus flavistipus* Atri, M. Kaur & A. Kaur (Kaur et al., 2014a); *Lactarius deliciosus* var. *indicus* Atri, Saini & D.K. Mann (Atri et al., 1991); *Russula brunneoviolacea* var. *macrospora* M. Kaur, Atri, Sam. Sharma & Yadw. Singh (Kaur et al., 2011); *Russula rubra* var. *hymenocyistiata* Atri & Kour H., *Lactarius waltersii* var. *macrosporus* Atri & Kour H. (Atri and Kour, 2003); *Hebeloma sordidum* var. *microsporum* Saini & Atri (Saini and Atri, 1985); *Rhodocybe popinalis* var. *macrospora* Amandeep Kaur, N.S. Atri & Munruchi Kaur (Kaur et al., 2013a); *Psathyrella fimicola* N.S. Atri, Munruchi Kaur and Amandeep Kaur (Kaur et al., 2013 b); *Panaeolus cyanooannulatus* Atri, M. Kaur & A. Kaur and *Panaeolus lepistercoris* Atri, M. Kaur & A. Kaur (Kaur et al., 2014b) were new to science. For making comparison with the extralimital material, regular exchange of research material was done with the Herbarium of Field Museum of Natural History, Chicago (F), Herbarium of Royal Botanic Gardens, Kew (K) and few others.

The significance of exosporial ornamentation in the taxonomy of mushrooms was realized in 1924 when Melzer for the first time discussed the importance of this feature in *Russula* Pers. taxonomy. During our investigations while undertaking SEM study it was found that basidiospores of each species of russulaceous mushroom possesses unique ornamentation on the outer surface. Atri et al. (2016a) while attempting a review of the work done on various aspects of these mushrooms highlighted the importance of this character in systematics of russulaceous mushrooms. In most of the cases the ornamentation types are in conformity with the observation and the classification of ornamentation types given by Singer (1986) in his monumental monograph "Agaricales in Modern taxonomy". During the study of SEM details of basidiospores of 51 taxa of *Russula Pers.*, *Lactarius Pers.* and *Lactifluus* (Pers.) Roussel; eight varied ornamentation types were observed which have been designated as tuberculate type, catenulate type, winged type-A, winged type-B, incomplete reticulate type, complete reticulate type, ridged type and rugulose type (Atri et al., 2014b; 2016a). Based on the experience of working on these mushrooms spore ornamentation type has been found to be an important feature for segregating some of their closely allied taxa. This fact has been amply emphasized in the earlier works on the russulaceous mushrooms by Romagnesi (1967, 1996), Rayner (1968-1970), Hesler and Smith (1979), Singer (1986) and many others.

The published information on mushroom systematics by different Indian investigators scattered in literature from time to time was also compiled in the form of Check lists. These include "Check list of Indian Russulaceae (Atri et al., 1994), Check list of genus *Lentinus* (Sharma and Atri, 2015), Check list of Coprophilous agarics (Amandeep et al., 2015b), Catalogue of Russulaceous mushrooms of India (Sharma et al., 2018) and Check list of Russulaceous mushrooms from Jammu and Kashmir (Verma et al., 2019)". Besides these a "Documentary of Agaricomycetous Mushrooms of India" was also published (Upadhyay et al., 2017). The idea behind was to document all the validly published mushrooms in the book form which can serve as a ready reference for the future investigators interested in the taxonomy of mushrooms in India.

**ECOLOGICAL ASPECTS, CONSERVATION, SCREENING AND MYCORRIZA**

Besides taxonomic and diversity studies, simultaneously work was also carried out so as to understand the seasonal occurrence, distribution, habit, habitat, association and possibility for conservation of mushrooms in different parts of Punjab (Atri et al., 2009; 2010a, b) and North West Himalayan region (Saini and Atri, 1984; Atri and Saini, 1986; Atri, et al., 1997; Atri and Lakhanpal, 2002, Kaur et al., 2019). Further based on the field observations and the information gathered from literature the investigated mushrooms were screened into putatively mycorrhizal, edible and inedible types (Saini and Atri, 1984; Atri and Saini, 1986; Atri, et al., 1997; Atri and Lakhanpal, 2002 , Kaur et al., 2019). Further based on the field observations and the information gathered from literature the investigated mushrooms were screened into putatively mycorrhizal, edible and inedible types (Saini and Atri, 1984; Atri and Saini, 1986; Atri, et al., 1997, 2009; Kaur et al., 2019). For the purpose of conservation pure cultures of some of the edible mushrooms collected from the wild including *Lentinus connatus* Berk., *L. cladopus* Lév., *L. torulosus* (Pers.) Lloyd, *L. sajor-caju* (Fr.) Fr., *L. squarrosulus* Mont., *Pleurotus sapidus* Quél., *P. cystidosus* O. K. Mill and *Calocybe gambosa* (Fr.) Donk. were raised through tissue culture and subsequently submitted at MTCC Mohali and DMR Solan. The relevance of ECM studies in understanding the role of mutualistic association in plant growth and development was emphasized by Atri et al. (2016a). As a part of the investigations in this regard survey was undertaken during monsoon season from ending June, 2013 to early October, 2016 for the identification and characterization of ECM associates of *Shorea robusta* Gaertn. from Shivaliks falling in the jurisdiction of Himachal Pradesh, Haryana and Uttarakhand States in North Western part of India. During the survey species belonging to number of mushroom genera including *Russula Pers.*, *Agaricus L.*, *Amanita Pers.*, *Lactarius Pers.*, *Lactifluus* (Pers.) Roussel, *Xerula Maire, Termitomyces Heim, Pholiota* (Fr.) P. Kumm., *Inocybe* (Fr.) Fr. and *Asperoimocybe* Heim were documented. In all 50 mushroom species of these genera were documented from Sal forest, out of which as many as 22 taxa were found organically associated with the roots of Sal plants. Amongst these, *Russula Pers.* with as many as 12 species was found to be one of the dominant genera documented forming putative ectomycorrhizal association with the roots of Sal trees (Kumar, 2019). Some of the morpho-taxonomic details of mycorrhizal roots of Sal found in organic connection with
Lactifluus volemus var. volemus (Fr.: Fr.) Kuntze, Russula feugiana Sing., R. cremeoavallanea Sing., R. romagnesiana Shaffer, R. nigricans Fr., R. chlorinosoma Burl., R. azurea and R. cyanoxantha (Schaef.) Fr. and Lactarius sp. In all five experiment sets consisting of 40 polybags (20 x 21 cm²) in each including two controls were setup. Regular reading for the identified 19 parameters was taken after every 3 months for one year. The data generated was statistically analyzed using one way analysis of variance (ANOVA) and Tukey’s multiple comparison tests. Through artificial synthesis of mycorrhiza using three ECM associates, namely Russula kanadii A.K. Dutta & K. Acharya, R. cyanoxantha (Schaef.) Fr. and Lactarius sp. were confirmed as ectomycorrhizal associates of Shorea robusta Gaertn. The present study demonstrated that Sal seedlings exhibited positive growth response to inoculation with the ECM fungi when grown on natural sterilized soil. By the end of the 12 month experimental period, the length of shoots, length of roots, total length of seedlings, number of short roots, number of mycorrhizal roots, percentage of mycorrhizal roots, fresh weight of roots, fresh weight of shoots, total fresh weight of seedlings, dry weight of shoots, dry weight of roots, total dry weight of seedlings was found to increase significantly in case of artificially inoculated seedlings as compared to control seedlings. Out of the three ECM associates inoculated, maximum uptake of macronutrients was documented in the seedlings inoculated with Russula kanadii A.K. Dutta & K. Acharya. It was also observed that ECM infection inhibited the translocation of heavy metals from roots to shoots (Kumar, 2019). Mycorrhizal roots had significantly higher contents of heavy metals such as Al and Cr as compared to non mycorrhizal roots (Control).

The results of the present study clearly demonstrated that the survival rates of ECM inoculated seedlings of Sal was significantly higher than those of control seedlings, suggesting that mycorrhizal seedlings exhibit more tolerance to heavy metals in comparison to uninoculated seedlings. Previous studies have shown that ECM enhancement of host heavy metal tolerance is largely realized through improved nutrient uptake, physical protection of hyphae and mantle, and inhibition of absorption of heavy metals from roots (Kumar and Atri, 2017).

For optimizing the production of Shorea robusta Gaertn. seedlings in the nursery, inoculation with Russula kanadii A.K. Dutta & K. Acharya as ECM partner could be a profitable proposition (Kumar, 2019). It can result in a decreased time to planting, less fertilizer input and more successful survival on out-planting, resulting in better quality nursery stocks with reduced production cost. Kumar and Atri (2017) discussed the progress made in ectomycorrhizal biology over a period of time in understanding the role of ECM symbiosis in providing tolerance to plants against various biotic and abiotic stresses and also in the maintenance of plant diversity for proper ecosystem sustenance.

**NUTRITIONAL, NUTRATEUTICAL AND SOCIOBIOLOGICAL ASPECTS**

Mushrooms represent subterranean sporophores of ascomyceteous and agaricomyceteous fungi having varied morphological forms. Some of the edible ones are represented by morels, tubers, coralloid forms, puffballs, earthstars, pleurotoid, lentinoid, agaricoid, coprinoid, volvarioid, amanitoid, termitophyllosus and russuloid mushrooms having coriaceous to fleshy consistency. Many of their species including Morchella esculenta (L.) Pers., Termitomyces heimiti Natarajan, T. mammiformis R. Heim, Russula cyanoxantha (Schaef.) Fr., Russula virescens (Schaef.) Fr., Lactarius deliciosus (L.) Gray, Lentinus squarrosulus Mont., Amanita vaginata (Bull.) Lam., Macrolepiota prorea (Scop.) Singer, Coprinus comatus (O.F. Müll.) Pers., Podaxis pistillaris (L.) Fr., Lycoperdon perlatum Pers., Volvariella volvacea (Bull.) Singer and species of Pleurotus (Fr.) P. Kumm. are being collected from the forested areas by the local inhabitants for their personal consumption including trading. Sharma et al. (2017) discussed the nutritional and nutrateutical potential of some russulaceous mushrooms from India. Atri et al. (2016a) while discussing researches on russulaceous mushrooms documented the work done on the nutritional and nutrateutical aspects of some edible species of Russula Pers., Lactarius Pers. and Lactiflus (Pers.) Roussel from all over the world. Saini and Atri (1999) while reviewing the work on pharmaceutical utility of mushrooms emphasized that besides their edibility, mushrooms have long been considered to have medicinal properties because of the presence of bioactive substances in them which are quite effective against fungi, bacteria and viruses.

Their culinary and commercial value is primarily due to their organoleptic properties which include their texture, aroma, taste, acceptable flavor and digestibility (Crisan and Sands, 1978; Chang and Miles, 2004; Atri et al., 2016b, 2019). The world over lot of scientific data has been generated supporting their nutritional and nutrateutical credentials. ((Crisan and Sands, 1978; Chang and Miles, 2004; Agrahar-Murugkar and Subbulakshmi, 2005; Barros et al., 2007a, b; Pushpa and Purushothama, 2010; Gulati et al., 2011; Atri et al., 2013a; Sharma and Atri, 2014; Sharma et al., 2013, 2014; Kumari et al., 2012; Kumari and Atri, 2012, 2014; Atri et al., 2016b, Mridu and Atri, 2015, 2017; Atri et al., 2019). While investigating the North Indian mushrooms, number of these including Russula brevipes Peck, R. cyanoxantha (Schaeff.) Fr., R. heterophylla (Fr.) Fr., R. virescens (Schaef.) Fr., Lactarius sanguifluous (Pautel) Fr., L. deliciosus (L.) Gray, Lactiflus piperatus (L.) Kuntze, Isaria sinclairii (Berk.) Lloyd, I. tenuipes Peck, I. japonica Yasuda, I. farinosa (Holmsk.) Fr. and Cordyceps tuberculata (Lebert) Maire, Termitomyces badius Otienie, T. heimiti Natarajan, T. mammiformis R. Heim, T. mediis R. Heim & Grassé, T. microcarpus (Berk. & Broome) R. Heim, T. radicus Natarajan, T. striatus (Beeli) R. Heim, T. reticulatus Van der westh. & Eicker, Macrolepiota dolicihaula (Berk. & Broome) Pegler & R.W. Rayner, M. procera (Scop.) Singer, M.
hrocodes (Vittad.) Singer, *Pleurotus sapidus* Quél., *P. cystidiosus* O. K. Mill., *P. pulmonarius* (Fr.) Quél., *P. floridus* Singer, *Lentinus squarrosulus* Mont., *L. sajor-caju* (Fr.) Fr., *L. connatus* Berk., *L. torulosus* (Pers.) Lloyd, *L. cladopus* Lév., *Podaxis pistillaris* (L.) Fr. and *Calocybe gambosa* (Fr.) Donk were analyzed for their different constituents including proximate composition, essential amino acid and fatty acid profile, vitamins, and number of secondary metabolites including phenolic compounds, alkaloids, β carotenes, lycopene, lectins and antioxidant properties, etc. (Atri et al. 2014c; Kumari and Atri, 2012; Atri and Guleria, 2013; Sharma et al., 2015, 2016; Lata and Atri, 2017; Sharma et al., 2017; Mridu and Atri, 2015, 2017). Mushrooms when evaluated found to contain a rich amount of high quality digestible proteins and carbohydrates, low fat content and a moderate level of ash and crude fibers (Atri et al., 2013a; Atri et al., 2016b; Mridu and Atri, 2017). Just like higher plants, both macro and microelements, namely potassium, phosphorus, sodium, calcium, magnesium, copper, zinc, iron, molybdenum, selenium and cadmium having immense metabolic and nutraceutical utility are also present in sufficient quantities in all the evaluated wild edible species of mushrooms from North west India (Atri and Guleria, 2013; Atri et al., 2016b).

Many of the mushrooms including species of genera *Isaria* Pers., *Cordyceps* Fr., etc. are reported to contain biologically active polysaccharides which provide immunostimulatory and antioxidative and even antimicrobial properties (Chang and Buswell, 1996; Barros et al., 2007c; Ikekawa, 2001; Atri et al., 2014c; Sharma et al., 2015, 2016). This is also true in case of wild edible mushrooms as well. In view of this wild edible mushroom also offer large number of such related health benefits to the mycophagist society (Atri et al., 2012a, b; Kumari et al., 2013c; Kumari and Atri, 2014).

The indigenous knowledge about utility of wild mushrooms is an important area which needs to be explored scientifically for its wider use in human welfare. Information about local utility and sociobiology of wild edible mushrooms is an important component of ethnomycology. We have attempted to document the indigenous knowledge about wild edible mushrooms from local inhabitants of NW Himalaya. In different parts of Himachal Pradesh, Jammu and Kashmir and different parts of India was published by Atri and Mridu (2018).

**Evaluation of Physical-Biochemical Parameters and substrates for Cultivation:** Study on the mycelial characters and growth pattern was undertaken in case of five species each of *Lentinus* Fr. (*L. squarrosulus* Mont., *L. sajor-caju* (Fr.) Fr., *Lentinus connatus* Berk., *L. cladopus* Lév and *L. torulosus* (Pers.) Lloyd) and *Pleurotus* (Fr.) P. Kumm. (*P. sapidus* Quél., *P. cystidiosus* O. K. Mill., *P. pulmonarius* (Fr.) Quél., *P. floridus* Singer and *P. sajor-caju* (Fr.) Singer) (Atri et al., 2013b; Atri and Sharma, 2012d). Along with this further investigations on the evaluation of incubation period, physical and biochemical parameters for the best mycelial growth was also undertaken in case of *L. squarrosulus* Mont., *L. sajor-caju* (Fr.) Fr., *L. connatus* Berk., *L. cladopus* Lév, *Pleurotus sapidus* Quél. and *P. cystidiosus* O. K. Mill. For this purpose number of solid and liquid media, variable temperature regimes, pH levels, different carbon sources, nitrogen sources, growth regulators, vitamins and trace elements were taken for evaluation so as to find out the best suited media and physical and biochemical parameters for the mycelial growth of these mushrooms (Tuitt, 1969; Madan and Thind, 1998). The results arrived at were statistically analyzed and published in series of publications from time to time (Atri et al. 2010c, 2011, 2013c; Atri and Guleria, 2013; Amita and Atri, 2017). It was found that each mushroom has its own specific requirements as for vegetative growth of mushroom mycelium is concerned.

Work on the evaluation of local lignocellulosic substrates for the cultivation of mushrooms was initiated somewhere in 2005 with the cultivation of *Calocybe indica* Purkayastha & A. Chandra (Milky mushrooms). For this the inspiration came from none other than Professor C. L. Jandaik, Indian mushroom mycologist from Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, who is well known for his pioneering work on the domestication of *Pleurotus sajor-caju* (Fr.) Sing. (Jandaik and Kapoor, 1976). With the culture and technology provided by him and inputs given by Dr. S.R. Sharma, Principal Scientist National Research Centre Mushrooms (ICAR), Chambaghat, Solan, milky mushroom was successfully cultivated at Patiala on rice straw, wheat straw, rice husk and 1:1:1 mixture of all the three substrates. On rice straw the mushroom gave 112.08% biological efficiency on fresh weight basis (Atri et al., 2012c). Continuing the work, domestication studies were undertaken on some wild edible mushrooms for which cultures were raised from the wild samples collected from different parts of North India. The mushrooms on which domestication studies were undertaken successfully using locally available substrates are *Lentinus squarrosulus* Mont, *L. sajor-caju* (Fr.) Fr., *L. cladopus* Lév., *L. torulosus* (Pers.) Lloyd, *Pleurotus sapidus* Quél. and *P. cystidiosus* O. K. Mill. These mushrooms were selected for domestication because these are being collected by the local people from the wild for their personal consumption, but none of them are under commercial cultivation. In fact these are specialty mushrooms, which are yet to be popularized among the masses. In their nutritional and nutraceutical profile, these are as good as any other commercially cultivated mushroom including *Agaricus bisporus* (J. E. Lange) Imbach. Presently for their cultivation different locally available substrates both for spawn production and cultivation were evaluated and biological efficiency of each mushroom on individual substrate was calculated on fresh weight basis (Atri et al., 2011; Atri and Lata, 2013; Atri et al., 2018). All of them can be cultivated easily on racks just like cultivation of dhingri mushrooms on unpasteurized lignocellulosic substrates without much improvisation in the cultivation room with excellent conversion rate.
CONCLUDING REMARKS

India is a rich repository of these mushrooms. Because of their importance in human nutrition and medicine they are considered as super food. They represent one of the important untapped resources of nutrition and quality food for future. They need to be inventorized, evaluated and conserved so as to mitigate the challenges being faced by mankind. It is high time that edible mushroom form a permanent part of the dietary recommendations. Due to their unique chemistry and high fiber content mushroom possess the ability to revitalize our immunity. Study for identifying the symbions of multipurpose trees in the forested areas and their use for raising semicultures for further use in afforestation and reforestation programmes needs impetus. Some of the mushrooms are already under cultivation and other nutritionally and nutraceutically important specialty mushrooms need to be identified conserved and domesticated. By doing this huge quantity of agro-wastes can be recycled into utility product. Mushrooms are one such bio-resource which serves the society and the environment because of three fold advantage they offer in the form of efficient bioconversion and utility as a rich source of nutritional and nutraceutical components with potential for utilization in human welfare.

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