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Toxicity Test of Some Selected Wild Mushrooms of Nepal

Madhu Bilash Ghimire[1], Hari Prasad Aryal[1*], Pramesh Bahadur Lakhey[2], Rajeshwor Ranjitkar[3] & Yagya Raj Bhatta[4]
[1]Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[2]Department of Plant Resources, Thapathali, Kathmandu, Nepal
[3]Natural Products Research Laboratory, Thapathali, Kathmandu, Nepal
[4]Amrit Campus, Thamel, Kathmandu, Nepal
***Email:** hp.aryal@cdbtu.edu.np

Abstract

People have been using wild mushrooms in their diet and also as sources of income, but most of them do not have the proper knowledge to distinguish edible mushrooms from poisonous ones. The main objective of this research is to determine and document toxicity of some selected wild mushrooms of Nepal. Samples were collected based on their use practices by the local communities, representing different ecological belts. The tests were carried out following the protocol described in 'Test Guideline no. 425: Acute Oral Toxicity: Up-and-Down Procedure'. Mice (*Mus musculus*) were administered with aqueous solution of each sample in a single dose by gavage using a stomach tube on the basis of their body weight and were observed for the signs of toxicity. The tested samples of *Boletellus emodensis*, *Caloboletus calopus*, *Daedalea quercina*, *Lactifluus volemus*, *Lyophyllum decastes, Macrolepiota albuminosa, Phellodon niger, Phylloporus bellus, Russula delica, R. emetica*, *R. senecis*, *Strobilomyces strobilaceus*, *Termitomyces eurrhizus*, *T. le-testui*, *T. mammiformis*, *T. robustus*, *T. striatus* f. *brunneus*, *T. straitus* f. *pileatus*, *Trametes vernicipes* and *Trichaptum biforme* revealed LD50value > 2000 mg.kg^{-1} body weight indicating that they may be harmful if swallowed . However, in case of *Hapalopilus rutilans*, LD50value of 1212 mg.kg^{-1} body weight was observed confirming that it is harmful when swallowed.

Keywords: Indigenous knowledge, LD50, Mortality, Mushroom poisoning

Introduction

Various types of wild mushrooms grow in forests and meadows. Nepal is rich in mushroom flora because of its diverse ecological environment (Aryal et al., 2012). Mushrooms are one of the useful natural resources, especially for the communities residing in rural areas. They are important Non-Timber Forest Products (NTFPs) and are being used as food since time immemorial (Aryal & Budhathoki, 2014). Collection of wild mushrooms is very common and is important for livelihoods in rural areas (Adhikari 2000; Christensen & Larsen, 2005; Devkota, 2006). According to the updated data, 100 species of poisonous mushrooms have been reported in Nepal (Devkota & Aryal, 2020).

In Nepal, people have been using wild mushrooms not only in their diet but also as sources of income. However, they do not have adequate knowledge to distinguish edible mushrooms from poisonous ones (Aryal, 2009). Mushroom poisoning refers to deleterious effects from ingestion of toxic substances present in a mushroom (Patowary, 2010). The effects may vary from slight gastrointestinal discomfort to acute multiple organ failure (Avcý, et al., 2014). The toxic substances present in poisonous mushrooms are secondary metabolites produced in specific biochemical pathways in the fungal cell (Gopinath et al., 2011). Mushroom poisoning is most commonly seen in spring and autumn seasons with cool, damp evenings which favor the growth and development of mushrooms. Most of the times, nontoxic and poisonous mushrooms grow nearby and unfortunately, many mushrooms are difficult to identify even by a trained mycologist (Erguven et al., 2007). Mushroom poisoning is usually the result of ingestion of wild mushrooms due to misidentification of a toxic mushroom as an edible species. More than 95% of mushroom poisoning incidences around the world occur due to misidentification (Erden et al., 2013). Habit of eating naturally growing mushrooms is quite common in people living in rural areas. In Nepal, 20-30 people of age between 10-45 years die annually due to mushroom poisoning (Adhikari, 2014). Although mushroom toxicity has been extensively studied in Nepal on the basis of eco-morphological characters, Nepalese mushroom species have not yet been evaluated for acute oral toxicity.

Materials and Methods

Study area and sample collections

Mushrooms samples (Table 3) were collected from different areas (Figure 1) of community-managed and natural forests of Kanchanpur (Laljhadi), Kailali (Godawari), Chitwan (Jugedi) and Kathmandu (Matatirtha and Chandragiri) districts of Nepal in the months of June-September, 2019.

Samples were collected taking proper scientific measures as described by Atri et al. (2005). They were sealed in sterile plastic bags, transported and were deposited at laboratory of Plant Pathology Unit (PPU) of Central Department of Botany (CDB), Tribhuvan University (TU), Nepal. The collected samples were identified on the basis of diagnostic morpho-taxonomic characteristics and microscopic examinations. For authentication, samples were confirmed using relevant literatures (Bakshi, 1971; Bels & Carlile et al., 2000; Dickinson & Lucas, 1979; Harkonen et al., 2003; Heim, 1977; Kumar et al., 1990; Pateregetvit, 1982; Singer, 1986;) expertise of PPU and web surfing on Index Fungorum and Mycobank.org.

Acute oral toxicity test

Tests were carried out at Pharmacology Laboratory of Natural Products Research Laboratory (NPRL), Kathmandu, Nepal on the basis of modified protocol of 'Test Guideline no. 425: Acute Oral Toxicity: Up-and-Down Procedure' recommended by Organization for Economic Cooperation and Development (2008). Tests were carried out in mice (*Mus musculus*). Each test was performed in five replicates. Twelve hours before starting the study, food was suspended while the body weight was monitored moments before

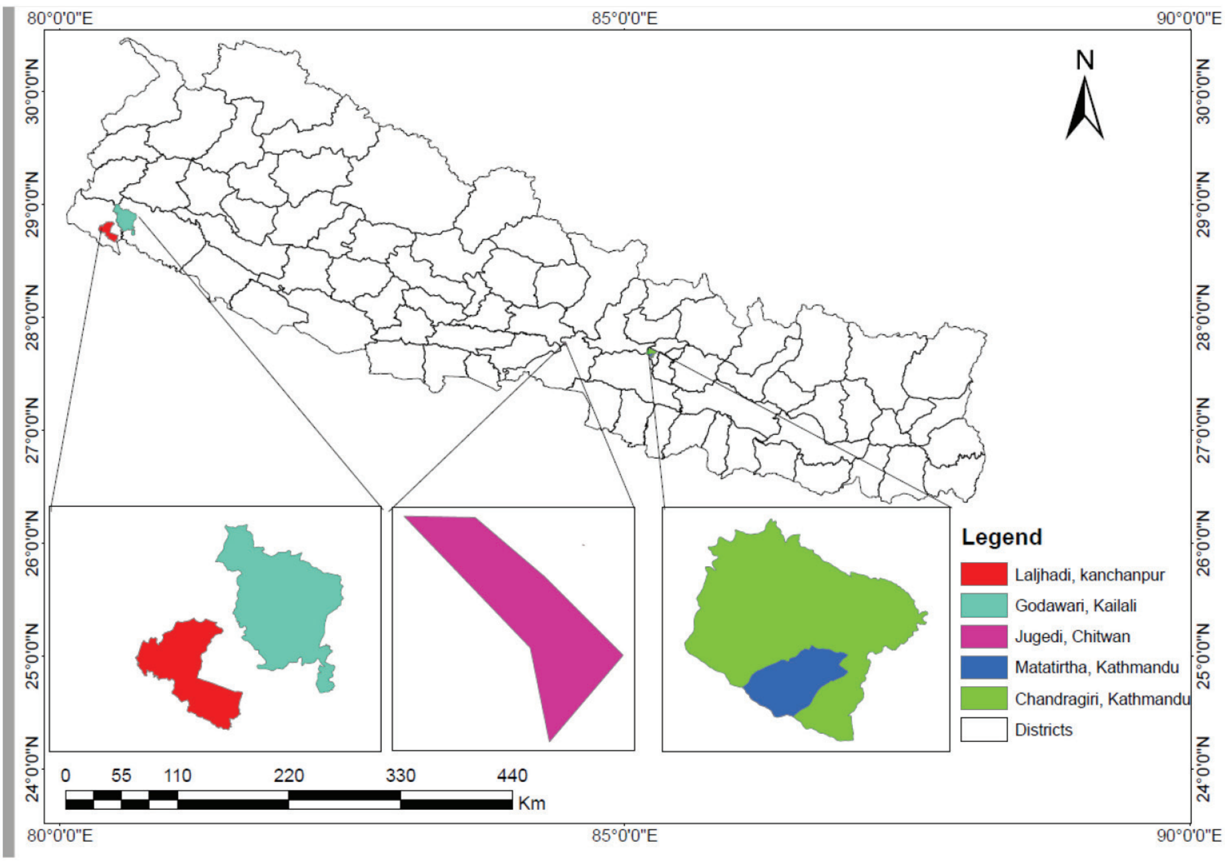

Figure 1: Mushroom collection sites.

the administration of the sample. Animals were randomly assigned in two groups: one, a control group treated with physiological saline while the other was experimental group treated with the sample at dose of 2000 mg.kg^{-1} of body weight using an orogastric tube. Clinical observations of animals were performed four times per day, paying attention to behavior, general physical condition, nasal mucosa, change in skin and fur, respiratory frequency, somatomotor activity and possible occurrence of signs such as tremors, convulsions, diarrhea, lethargy, drooling, low response to stimuli, sleep, photophobia, and coma. Based on 14 days of clinical observation in experimental group, level of toxicity was categorized according to classification criteria for acute toxicity recommended in Globally Harmonized System of Classification and Labeling of Chemicals (GHS, 4th edition) (United Nations [UN], 2011) as shown in Table 1.

Data analysis

The median lethal dose (LD50) value was calculated from a plotted graph of number of dead test animals against dose of the test material administered in Excel 2016 (Table 2).

Results and Discussion

During acute oral toxicity tests of dry mushroom (stipe and pileus) powders of *Boletellus emodensis* (Berk.) Singer, *Caloboletus calopus* (Pers.) Vizzini, *Daedalea quercina* (L.) Pers., *Lactifluus volemus* (Fr.) Kuntze, *Lyophyllum decastes* (Fr.) Singer, *Macrolepiota albuminosa* (Berk.) Pegler, *Phellodon niger* (Fr.) P. Karst., *Phylloporus bellus* (Massee) Corner, *Russula delica* Fr., *Russula emetica* (Schaeff.) Pers., *Russula senecis* S. Imai, *Strobilomyces strobilaceus* (Scop.) Berk., *Termitomyces eurrhizus* (Berk.) R. Heim, *Termitomyces le-testui* (Pat.) R. Heim, *Termitomyces mammiformis* R. Heim, *Termitomyces robustus* (Beeli) R. Heim, *Termitomyces striatus* f. *brunneus* Mossebo, *Termitomyces striatus* f. *pileatus* Mossebo, *Trametes vernicipes* (Berk.) Zmitr., Wasser & Ezhov and *Trichaptum biforme* (Fr.) Ryvarden, no mortalities were observed at oral dosage of 2000 mg. kg^{1}. Moreover, the body weights of the treated and control mice were almost identical and showed no significant differences during the experimental period. Further, no remarkable changes were observed in general behaviors between the control and treatment groups. These observations implied that these species had LD50 values greater than 2000 mg.kg^{1} indicating that they fall under Category 5 of GHS (4th edition) with hazard statement "may be harmful when swallowed" (Table 3). However, LD50 value for *Hapalopilus rutilans* (Pers.) Murrill was found to be 1212 mg.kg^{1} categorizing it in category 4 of GHS (4th edition) classified as "dangerous" with hazard statement "harmful if swallowed" (Figure 2, Table 2 and 3).

After survey and laboratory analysis, most of the collected mushroom species were found to be not significantly poisonous. On the other hand, *H. rutilans* showed higher LD50 value of 1212 mg.kg^{-1} and, hence, was classified as "Dangerous" according to GHS (4th edition). This is probably due to accumulation of hydrocarbon compounds from the riverine forest area (Igbiri et al., 2017). Similar results were also found by Fasidi and Kadiri (1995) for *Chlorophyllum molybditis, Cortinarius melliolens, Tricholoma lobayensis, Volvariella esculenta, Termitomyces robustus, Pleurotus tuber-regium* and *Lentinus subnudus*.

Table 1: Classification of substances according to globally harmonized system of classification and labeling of chemicals (4th edition) (UN, 2011)

S.N.	Dose Ranges (mg.kg^{-1})	Category	Classification	Hazard Statement
1	>2000 mg.kg^{-1}	Category 5	Not classified	May be harmful if swallowed
2	> 300 ≤ 2000 mg.kg^{-1}	Category 4	Dangerous	Harmful if swallowed
3	> 50 ≤ 300 mg.kg^{-1}	Category 3	Toxic	Toxic if swallowed
4	> 5 ≤ 50 mg.kg^{-1}	Category 2	Very toxic	Fatal if swallowed
5	< 5 mg.kg^{-1}	Category 1	Highly toxic	Fatal if swallowed

Table 2: Effect of *Hapalopilus rutilans* at different concentration on mice

Doses ($mg.kg^{-1}$)	No. of dead mice
2000	5
1500	5
1250	2
1000	1

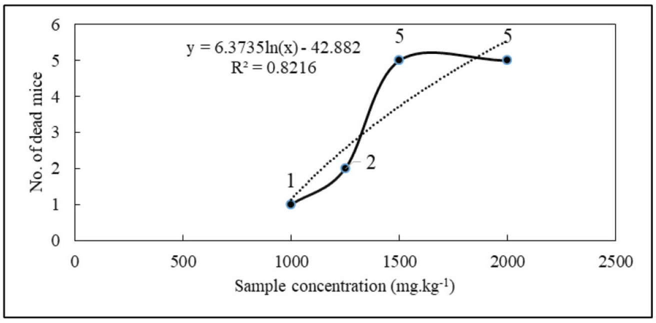

Figure 2: Calculation of median lethal dose (LD50) value from a plotted graph of number of dead test animal against concentration of the test material (*Hapalopilus rutilans*)

Table 3: LD50 values and classification of the tested wild mushroom samples collected from different parts of the country

S.N.	Scientific name	Family	Local name	Place of collection	LD50 ($mg.kg^{-1}$ BW)	Observation, and category, classification and hazard statement as per GHS (4th edition)
1	*Boletellus emodensis* (Berk.) Singer	Boletaceae	Katle chyau	Kailali	>2000	Observation: No death at 2000 $mg.kg^{-1}$ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
2	*Caloboletus calopus* (Pers.) Vizzini	Boletaceae	Kapase chyau	Chandragiri	>2000	Observation: No death at 2000 $mg.kg^{-1}$ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
3	*Daedalea quercina* (L.) Pers.	Fomitopsidaceae	Leech chyau	Kanchanpur	>2000	Observation: No death at 2000 $mg.kg^{-1}$ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
4	*Hapalopilus rutilans* (Pers.) Murrill	Polyporaceae	Gande chyau	Kanchanpur	1212	Observation: mentioned in Table 2 Category 4 Classification: Dangerous Hazard Statement: Harmful if swallowed
5	*Lactifluus volemus* (Fr.) Kuntze	Russulaceae	Dhudhe chyau	Chandragiri	>2000	Observation: No death at 2000 $mg.kg^{-1}$ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
6	*Lyophyllum decastes* (Fr.) Singer	Tricholomataceae	Jhuppe-Bagale chyau	Chandragiri	>2000	Observation: No death at 2000 $mg.kg^{-1}$ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed

S.N.	Scientific name	Family	Local name	Place of collection	LD50 (mg.kg^{-1} BW)	Observation, and category, classification and hazard statement as per GHS (4th edition)
7	*Macrolepiota albuminosa* (Berk.) Pegler	Tricholomataceae	Bagale chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
8	*Phellodon niger* (Fr.) P. Karst.	Thelephoraceae	Mayure chyay	Matatirtha	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
9	*Phylloporus bellus* (Massee) Corner	Boletaceae	Besare chyau	Matatirtha	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
10	*Russula delica* Fr.	Russulaceae	Seto chyau	Chandragiri	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
11	*Russula emetica* (Schaeff.)Pers.	Russulaceae	Rato chyau	Kailali	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
12	*Russula senecis* S. Imai	Russulaceae	Papree chyau	Matatirtha	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
13	*Strobilomyces strobilaceus* (Scop.) Berk.	Boletaceae	Bhut chyau	Matatirtha	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
14	*Termitomyces eurrhizus* (Berk.) R. Heim	Tricholomataceae	Bagale chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
15	*Termitomyces le-testui* (Pat.) R. Heim	Tricholomataceae	Dudhamunte chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed

S.N.	Scientific name	Family	Local name	Place of collection	LD50 (mg.kg^{-1} BW)	Observation, and category, classification and hazard statement as per GHS (4th edition)
16	*Termitomyces mammiformis* R. Heim	Tricholomataceae	Thuli Mugan chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
17	*Termitomyces robustus* (Beeli) R. Heim	Tricholomataceae	Bagale chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
18	*Termitomyces striatus* f. *brunneus* Mossebo	Tricholomataceae	Dhamire chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
19	*Termitomyces striatus* f. *pileatus* Mossebo	Tricholomataceae	Kalunge chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
20	*Trametes vernicipes*(Berk.) Zmitr., Wasser & Ezhov	Polyporaceae	Pankhey chyau	Kanchanpur	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
21	*Trichaptum biforme* (Fr.) Ryvarden	Polyporaceae	Chhale chyau	Kanchanpur	>2000	Observation: No death at 2000 mg.kg^{-1} Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed

Conclusion

In Nepal, since rural communities frequently consume wild mushrooms, consumption of poisonous mushrooms often results in several mortalities annually. In order to mitigate this problem, major emphasis has to be given on intensive research to determine the strength of toxicity of wild species which would further enable us to utilize mushroom species with food and medicinal value for the benefit of the people. Hence, this study focused on evaluating acute oral toxicity of wild mushrooms. Out of the twenty-one species tested for toxicity, only one species, viz. *Haplopilus rutilans*, was found to be conclusively toxic and was classified as "Dangerous" with the hazard statement "harmful if swallowed" as per GHS (4th edition). Remaining tested species were observed to have median lethal dose for acute oral toxicity greater than 2000 mg.mL^{-1} indicating that these species "may be toxic when swallowed". Further studies should be conducted to confirm non-toxicity (both acute and chronic) and to analyze nutrient and heavy metal contents of these potentially non-toxic mushrooms for the commercialization of those mushrooms which may promote economic growth of people and nation.

Author Contributions

The first author conducted field visit, sample collection and prepared manuscript. Second author helped in sample identification and supervised the overall work as well as he is guarantor of this research. Third and fourth authors helped in laboratory work. Fifth author helped in manuscript preparation.

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Figure 3: A. *Boletellus emodensis* (Berk.) Singer, **B.** *Caloboletus calopus* (Pers.) Vizzini, **C.** *Daedalea quercina* (L.) Pers., **D.** *Hapalopilus rutilans* (Pers.) Murrill, **E.** *Lactifluus volemus* (Fr.) Kuntze, **F.** *Lyophyllum decastes* (Fr.) Singer, **G.** *Macrolepiota albuminosa* (Berk.) Pegler, **H.** *Phellodon niger* (Fr.) P. Karst., **I.** *Phylloporus bellus* (Massee) Corner, **J.** *Russula delica* Fr., **K.** *Russula emetica* (Schaeff.) Pers., **L.** *Russula senecis* S. Imai

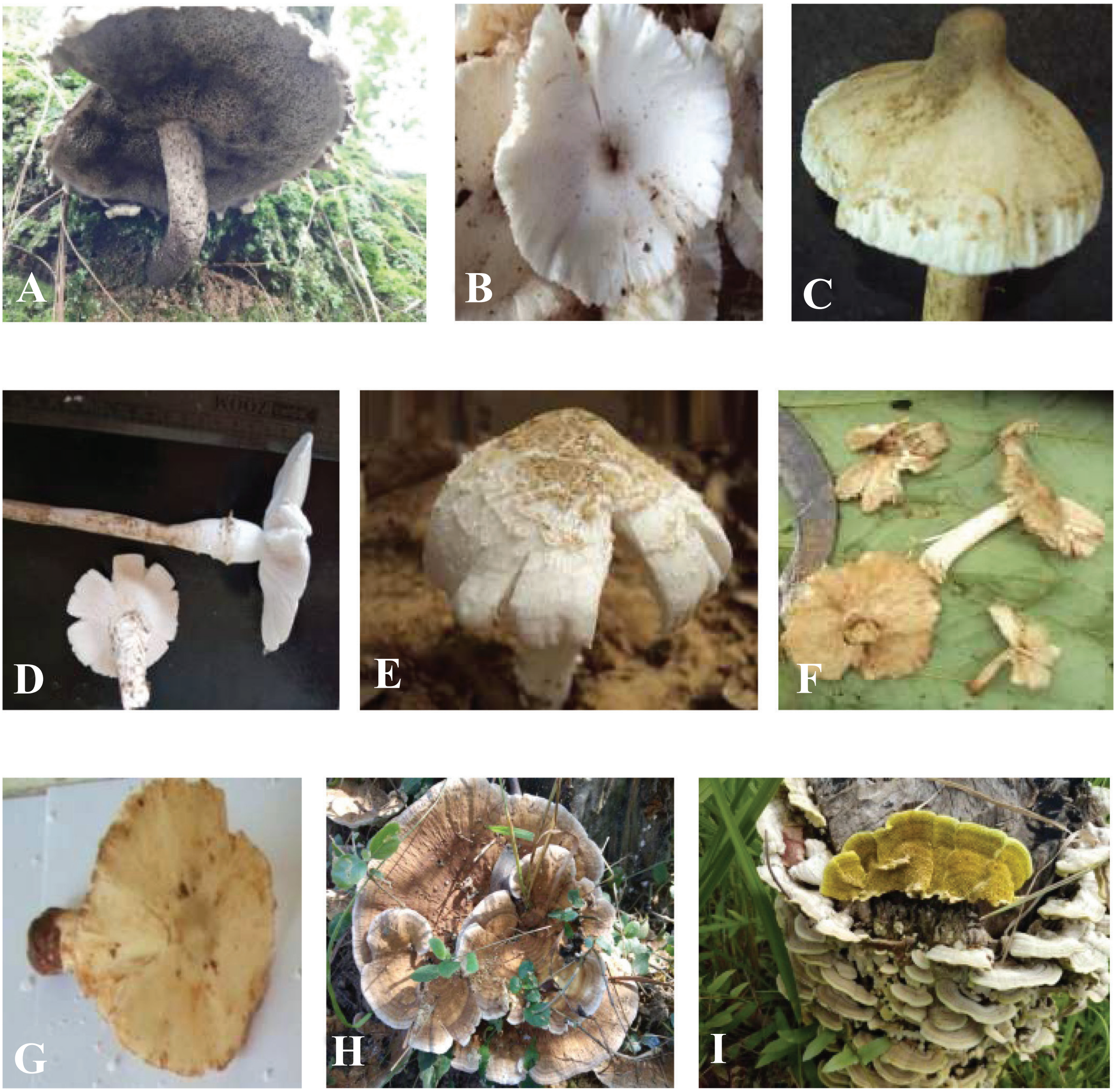

Figure 4: A. *Strobilomyces strobilaceus* (Scop.) Berk., **B.** *Termitomyces eurrhizus* (Berk.) R. Heim, **C.** *Termitomyces le-testui* (Pat.) R. Heim, **D.** *Termitomyces mammiformis* R. Heim, **E.** *Termitomyces robustus* (Beeli) R. Heim, **F.** *Termitomyces striatus* f. *brunneus* Mossebo, **G.** *Termitomyces striatus* f. *pileatus* Mossebo, **H.** *Trametes vernicipes* (Berk.) Zmitr., Wasser & Ezhov, **I.** *Trichaptum biforme* (Fr.) Ryvarden