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Ethnomycology Study of an Ectomycorhizian Mushroom Used in Cynegetic Art in Tshopo Province (Democratic Republic of the Congo)

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Abstract: A survey was done during 66 months (January 2010 to July 2015) in Tshopo province, Democratic Republic of the Congo, on the use of a vegetal specie called "Zila bokilo" in local language. This specie was identified as an ectomycorhizian mushroom, *Tuber sp*, used in cynegetic art as hunting bait in some villages in Tshopo province. The animals attracted by this mushroom include not only rodents (36.4%) among which Gambian pouched rat (*Cricetomys eminii*) but also animal of the Artiodactyla order (27.3%) such as the bush pig (*Potamochoerus porcus*), bay duiker (*Cephalophus dorsalis*), blue duiker (*Cephalophus monticola*); mycophagous animals such as monkey (*Cercopithecus sp*), turtle (*Kinixys erosa*), snail (*Achantina sp.*), Thomas's rope squirrel (*Funisciurus anerythrus)* and curiously carnivore such as the jackal (*Canis adustus*). *Tuber sp* develops better on the roots of *Gilbertiodendron dewevrei* in sandy soil, not far from a river. Chemical screening of this specie showed the absence of toxic ions like oxalates, cyanides, nitrates and nitrites indicating that this mushroom could be edible. Its attracting odor could be due to the abundance of terpenes in its chemical composition.
Keywords: Ethnomycology, Mushroom, cynegetic, Zila bokilo, Tshopo province.

INTRODUCTION

More than a million persons depend deeply upon forests for their survival, so it is important to manage sustainably these complexes ecosystems [1-3].

The Congo basin with more than 300 millions hectares counts among wet dense forests in the world and provides survival to more than 20 millions people among whom, many depend up on natural resources to survive [4]. The Democratic Republic of the Congo (DRC) contains the majority of central African tropical forest and of Congo basin [5]. It possesses the second tropical forest block in the world after Amazonian forests [3, 6, 7].

Tshopo province is located in the dense and wet equatorial forest, so itsrural population depend upon this forest where they take the essential of their proteins, medication, energy, materials and gains. Among these natural products, are mushrooms. These latter constitute the second largest group of organisms in the biosphere after insects [8]. They constitute unique organisms in their gender which differ from other eucaryotes on the structure level, nutrition mode, growth and reproduction [9].

Mushrooms always create an interest and curiosity of men. Numerous species are appreciated foods since a long time. They have played a non-negligible role in the feeding of primitive human beings. Davina wasson considered mushrooms as being a gift from God fitting to make part of a feasting meal [10].

Apart from that aspect, mushrooms intervene in numerous human activities [11], this include hunting. Where as in demecology, hunting plays an important role in the mortality of species and consequently, have a negative impact on the future of exploited species. A non-controlled cynegetic activity may in the future, conducts to the reduction or the destruction of wild animals or certain species [12].

Considering the fact that the hunting as practiced in Tshopo province can conducted to reduction of wild animals population and that data on these mushrooms used as bait for hunting are scarce, this word was undertaken.

Indeed, the knowledge on edible wild mushrooms is of importance for the population of the Congo basin forests in general and that of the Tshopo province in particular in order to fight against poverty, under feeding and forest destruction. So, different works have been done on Congolese mushrooms in general and on those of Tshopo province in particular but the usage of mushrooms in the cynegetic art has not yet attracts the attention of many researchers and seems to be misknown.

This work intends to identify these mushrooms, to determine their nutritional value, to analyze them for their probable toxicity, to determine the animal species they can attracts and the tree species on which they can grow.

MATERIALS AND METHODS

Study area

Ethnomycological investigations have been carried out in some villages in Tshopo province (Fig-1) during about 66 months from January 2010 to July 2015.

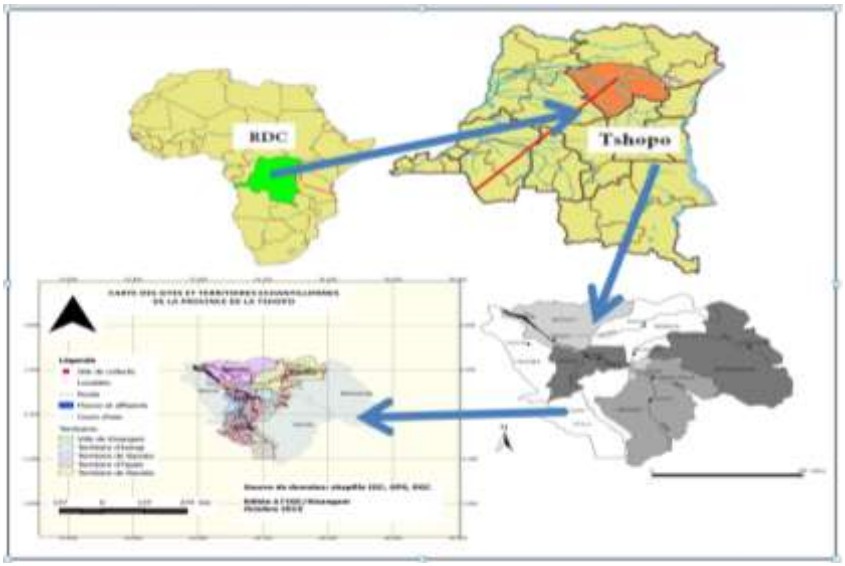

Fig-1: Data collection sites in Tshopo Province (IGC/Kisangani, october 2015).

The province of Tshopo has 199.567 square kilometers and is limited in the north by the provinces of Haut Uele and Bas Uele, in the south by the provinces of Maniema and Kasai Oriental and in the west by the province of Equateur. It is situated on 2° 13 and 2°S, 22°33 and 28°E for longitude. The altitude varies from 336 to 524m with an average of 451 meters [13]. This province of DRC is situated in central Congo basin and possess a typically equatorial hot and wet climate of AF type according to Köppen classification [14, 15]. Phyto-geographically it is locates in the *Guinéo-Congolese* region and is covered by equatorial forest with large biodiversity [16-20].

MATERIAL

The ethnomycological investigation led us to find a mushroom used as bait by hunters called "zila bokilo" in local language. This bait mushroom is often found in the forest dominated by *Gilbertiodendron dewevrei* species on sandy soil, not far from a stream. It grows on the roots of the host tree *Gilbertiodendron dewevrei* (Limbali). The majority of harvested specimens were taken from the ground; they are hypogeous. "Zila bokilo" can be found at depth reaching 50 cm in the soil. So machete, hoe, spade even knife are used for the mushroom extraction.

Usually, the mushroom extracted from the soil does not have a long duration (more than 3 days). However, if it is not injured, it is kept with earth all around for at most one week. By decomposing, the species gives off a very pleasant smell.

The mushroom constituted our biological material. It was kept in alcohol at 80% and transported to the laboratory.

A digital SONY 12.1 MEGA brand camera was used for taking pictures of some samples and a map 60 csx GARMIN brand GPS for taking geographical coordinates.

METHODS

A pre-elaborated questionnaire has served to collect data from 90 hunters. The latters have been chosen without taking into account the age and the socio-professional category. All of them were male. This questionnaire was about local names, the organs used as well as their use. In addition, individual interview was used before preliminary prospection.

In general, according to the hunters, the trap containing the mushroom as bait is put on the trail

where small mammals and other wild animals pass. The animals are attracted by the pleasant odor released by the bait thanks to their developed sense of smell.

The harvested biological material was identified at the herbarium of the Faculty of Science of Kisangani University by comparison with the mushroom specimens kept at the Mycology laboratory and with the mushroom catalogs.

It can be noticed that for the interviewed hunters this biological material (Zila bokilo) is a fruit or a seed. So, to identify this ethnospecie in order to see if it is a fruit or a mushroom, two culture media were prepared one with peptone agar and another with dextrose starch agar.

The chemical screening in other to determine toxic ions like oxalate, cyanides, nitrates, nitrites and detection of mycochemicals groups were done according to well-known procedure [21-24].

RESULTS AND DISCUSSION

Figure-2 shows our biological material

Fig-2: The mushroom in soil and harvested

This vegetal species used by hunters as bait has a weigh average of 15 g, spherical shape (diameter comprised between 3 and 5 cm), sometimes irregular and bumpy black, covered with prominent pyramidal warts. Firm flesh, at first clear, then black-purple and traversed by white veins, sinuous and very tight, blushing in the air, a very powerful and aromatic odor. This description is similar to that of Polesse [11] for a mushroom called *Tuber nigrum*.

Many authors have asserted that there are a hundred species of *Tuber*. So, at this stage, it cannot be confirmed that our biological material "Zila bokilo" is really *Tuber nigrum* species, only DNA analyses would confirm if it is the correct species. However, the comparison with the mushroom specimens kept at the Mycology laboratory and with the mushroom catalogs, indicates that the species under consideration, called Zila bokilo (in Lingala), Mbushi or Simiakilo (in Mbole), Shimba okilo (in lotetela) was assumed to be a mushroom of T*uber sp*, not a fruit or a seed as alleged by interviewed people. In fact, *Tuber sp* belongs to *Tuberaceae* family, *Tuberales* order, *Ascomycetes* Class, *Ascomycotina* Sub- branch, *Euascomycota* branch, *Mycota* Reign.

The name Zila bokilo means « Brother –in-law, wait a bit » i.e. true promise given to a considered family member to wait a little when going to set a trap with the mushroom in order to catch safely a gave to give to him. This gives him the impression to be welcomed in a short time.

Several authors have affirmed that the vernacular denominations take into account the form of the mushroom, its odor or its use. These denominations may find their inspiration in a legend or a traditional tale. Indeed, local names give important indices on edible mushrooms usages and importance for populations; their study may be important and instructive [25-28]. Table-1 give list of animals captured by hunters with *Tuber sp.* mushroom as bait

Table-1: List of the animals captured with *Tuber sp.* mushroom as bait.

N°	Vernacular name	Scientific name	Order	Family
1	Koto	*Cephalophus dorsalis* Gray ,1846	Artiodactyla	Bovidae
2	Mboloko, Antilope	*Cephalophus monticola* Tunberg	Artiodactyla	Bovidae
3	Ndjiko, porc-epic	*Atherurus africanus* Gray, 1842	Rodentia	Hystricidae
4	Motomba, Rat de Gambie	*Cricetomys emini* wroughton, 1910	Rodentia	Cricetidae
5	Esende, ecureil	*Funisciurus anerythrus*,Thomas, 1890	Rodentia	Sciuridae
6	Lotimo, squirrel	*Anomalurus derbianus* Gray, 1842	Rodentia	Anomaluridae
7	Sombo, Potamochère	*Potamochoerus porcus* L., 1758	Artiodactyla	Suidae
8	Makako, singe, monkey	*Cercopithecus sp.*	Primates	Cercopithecidae
9	Kola, ecargot, snail	*Achantina sp.*	Mollusca	Achatinidae
10	Koba, tortue, tortoise	*Kinixys erosa*, Bell, 1827	Testudines	Testudinidae
11	Libobi, chacal	*Canis adustus* Sundevall, 1847	Carnivora	Canidae

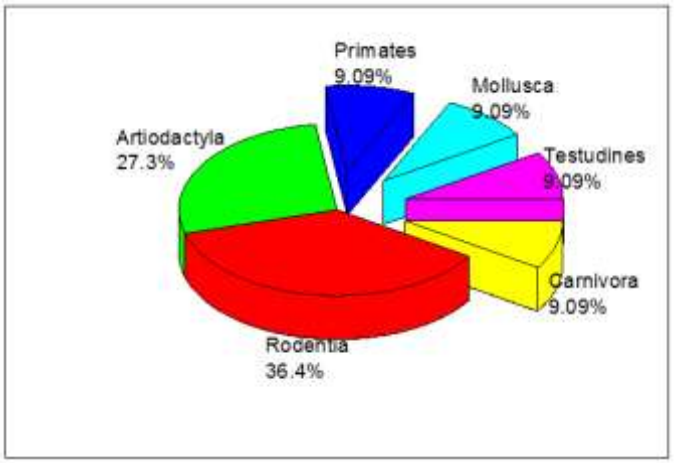

Fig-2: percentage of animal orders

Obtained results indicates that rodents (36.4%) are more attracted by the mushroom, among them is *Cricetomys eminii* or Gambian pouched rat which is a mammal most captured from this bait. Nebesse [29] reveals that rodents constitute a group which undergoes a strong cynegetic pressure, mainly Gambian pouched rat.

In the list above, it can be found mycophagous animals such as: monkey (*Cercopithecus sp*), turtle (*Kinixys erosa*), snail (*Achantina sp.*), Thomas's rope squirrel (*Funisciurus anerythrus)*. It is curious to note that a carnivore such as the jackal (*Canis adustus*) is attracted and fond of this mushroom.

It can also noticed that the animal of the Artiodactyla order such as the bush pig (*Potamochoerus porcus*), bay duiker (*Cephalophus dorsalis*) and blue duiker (*Cephalophus monticola*) constitute the second group (27.3%) of animal attracted this bait mushroom. Several authors report that research, as well as the harvest of *Tuber spp*. is usually done using truffle dog (carnivore); pig (Artiodactyla) or flies [29-35].

In fact, dogs are specially trained to become truffle hunters. They are educated and bred to find out truffles in the soil thanks to their odor organs regularly initiated. This truffle collecting technique is the most spread. As to the pig, it likes naturally truffles and discovers them due to its odor organ. The used pigs are generally females because the truffles emit a substance which resembles to the pheromone from the male saliva [36, 37]. These two animals have an odor organ very developed and are attracted by the powerful aroma from truffles, pigs can feel them at more than 6 meters. According to several authors, the truffle is a sub earthly mushroom. It is a mycelium fruit and may be collected by different ways: with the pigs, with dogs as well as the flies [31-33]. The animal uses its legs, digging the soil to collect mushrooms.

But in this survey our informants indicates that no one has bred the pig or the dog to look for the *Tuber sp* mushroom. Whereas, in many countries of the world among which France and Chine, truffle dealer's recourse to the pigs and dogs to collect them. This, even if, they consider in their area that, a hunter who found a place where a truffle is hidden finds wealth. He cannot show to anyone except to a person close to him; for instance his son, or close friend.

The majority of collected *Tubers sp* specimens have been extracted from *Gilbertiodendron dewevrei (*Limbali) with which it lives in symbiosis. According to our informants, it is also possible to find *Tuber sp* in

roots of other essences such as *Brachystegia laurentii (Fabaceae); Chrysophyllum africanum (Sapotaceae); Cleistopholis patens (Annonaceae) ; Guibourtia demeusei (Fabaceae); Julbernardia seretii (Fabaceae); Uapaca Sp (Phyllanthaceae), ……* But, the *Tuber* which grows on roots of these last species are not used as bait by hunters because, according to them, only mushrooms from roots of *Gilbertiodendron dewevrei* have good smell and are the best bait for animals.

Several authors assert that this host species *Gilbertiodendron dewevrei* is ectomycorhizian [25-28]. The *Tuber sp*., thanks to vegetative, the mycelium, needs to associate in symbiosis to a tree to exchange nutritive elements. Ectomycorhizian mushrooms are typically constituted by macro mushrooms and include several edible species which are collected in the nature such as truffles [37-39]. Some ectomycorhizian mushrooms produce their fructification organs under the soil [40].

According to some authors [27, 41-43], the tree species may form mycorhizes with several mushrooms and one mushroom may associate with several species of trees. So the improvement of nutritive capacity of poor soils by ectomycorhizian associations could explain the earth confinement of certain species in tropical forests and henceforth the coexistence of certain Caesalpinioideae taxons [44]. Fitter and Moyerseon, [45] reveal that the Caesalpinioideae could have co-evoluated with ectomycorhizian mushrooms in adaption of particular places. The ectomycorhizians have been more intensely studied on temperate tree species but there had been too interesting discoveries on tropical ectomycorhizians in Africa [46]. Gillet [47] assert that *Gilbertiodendron dewevrei* is to be developed as well in poor soils as hydromorph soils thanks to interactions with the ectomycorhizes. He also indicated that *Gilbertiodendron dewevrei* forests are sensitive to forest cuttings done in the system of extensive cultivation that allows secondary species penetration. They could be transformed in poor forest or in degraded savannahs after cutting and cultivation period if forest cuttings are important and frequent.

In their studies, Eyi ndong et *al.,* [25] have shown the ectomycorhization of Caesalpiniaceae family trees (notably the *Gilbertiodendron dewevrei), Euphorbiaceae (*mainly *Uapacca spp)* and *Gnetaceae* (uniquely *Gnetum africanum)* by the mushrooms. But they did not cite and collect *Tuber sp*. in their work.

Truffle lands are generally lands with stones and well drained. Many authors indicated that the truffles grow better in this type of soil. Some researchers have found that for the efficient truffle development, two important factors are taken into account: the climate chiefly of Mediterranean type and the soil, generally with calcary. Most of soils from Tshopo province are acids. They are in general of ferralitic nature, sandy and clayish deeply washed by rainy waters. Many authors [48, 49] have revealed that the mycorhizes are found on soils extremely poor in soil nutrients and increase their survival chance. The truffle presences in soils constitute one of the solutions for our forests and at the same time for the well being of riverside populations in these forests. That is why it must be exploited sustainably. According to above results, we think that the truffles could grow on all kinds of soils, acid as well as basic.

The first indicator for the presence of the truffle is the burnt marker [50]. This indicator is known in our area of research as many informants have stated that "Zila bokila" mushroom is often found in a forest dominated by *Gilbertiodendron dewevrei* on sandy soil, not far from river beds. From there, it is developed at the tree root level where it lives in symbiosis. One the land where it is hidden, one can observes the presence of mycelium of grey coloration or sometimes whitish. Its aspect is comparable to a burning of an organic material under the tree, this being the best indicator for hunters. This experience is inherited from their ancestors.

According to our informants, nobody has eaten this mushroom, apart from its use for hunting, they don't know other uses. In fact, a mushroom, though searched and eaten elsewhere can be misknown and neglected in some areas. Indeed, in China, the *Tricholoma matsutake* had less interest locally in Sichuan before Japanese request which stimulated its exportation in 1980's and seemed to have incited its consumption locally.

Till now, to determine if a mushroom specie is edible, there is no practical and simple test than its consuming in an area or a country. The habit often varies from one area to another and in some cases, there are single changes in the tradition [25]. So, the determination of scientific name of a mushroom gives a good indication on its uses, in some cases, only the genus name is enough. But, the only guide on the edibility is to know a person who as eaten a particular type and has survived. Local practices and preferences constitute then another source of useful information [25].

The use of *Tuber sp* in this study is compared of that of others studies (Table-2) in the same field.

Table-2: comparison of results obtained with the Bibliographical data.

Species	Uses	Consulted documents						
		A	B	C	D	E	F	G
Tuber sp (zila bokilo)	Cynegetic (hunting)	-	-	-	-	-	-	+
Tuber nigrum	food (gastronomic)	-	+	+	+	+	+	-

Legend : A : Bola, 1986 B : Polesse, 2000 C : Egli *et al*., 2002 D : FAO, 2006 E : Raven et al., 2008 F : Eyi Ndong, 2011 G: Present work

As it can be seen from this table, according to consulted literature, a part from our work, *Tuber sp* is only used as food in gastronomy. Apparently, these authors are not interested in cynegetic aspect.

De Kesel et *al*., [28] asserted that the rejection of a mushroom by the result of ethnomycological method is not an absolute guarantee of its toxicity. Indeed, a Madagascar specie *Phallus indusiatus* Vent, with a bad smell, is considered as toxic whereas it is known edible in China. This species is for medicinal use [46]. It is the reason why the chemical screening of toxic substances was done for our mushroom. Obtained results are given in table 3.

Table-3: Chemical screening of toxic substances and mycochemical groups.

Substances toxiques				mycochemical Group	
oxalate	cyanides	nitrates	nitrites	Terpenes and sterols	saponines
-	-	-	-	+	+

Legend: +: presence - : absence

This table show that toxic ions like oxalate, cyanides, nitrates and nitrites, are not present in our mushroom, but it contains abundant mycochemical Group terpenes, sterols and saponines. This indicates that "Zila bokilo" would be none toxic justifying the its use as food elsewhere. The strong smell and aroma of this mushroom would be due to terpenes and probably to a thioether, the bis (méthylthio) methane, used in the preparation of truffle oil, a food product imitating the truffles aroma [33].

CONCLUSION

This study has allowed us to survey about a vegetal specie called "zila bokilo" used by hunters in Tshopo province as bait hunting. The analysis reveals that zila bokilo is not a fruit or a seed as considered by many of our informants but an ectomycorhizian comparable to a truffle and identified as *Tuber sp*. This latter developed in mycorhize on the roots of *Gilbertiodendron dewevrei* not far from river beds and is used thanks to its attractive odor. As this important mushroom is found on the roots of a truffle tree, it depends on the survival this specie of tree and of tropical forest. The use of this mushroom by indigenous people must be done sustainably without destroying host tree and the forest. This work has shown that *Tuber sp* could be no toxic and then considered as an edible mushroom. This can increase its economic potential. This work is a contribution to the knowledge of useful mushrooms of DRC in general and of Tshopo Province in particular. The DNA analyses would give molecular characteristics of this mushroom and give precision on the specie.

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