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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research Vol. 13, Issue, 08, pp.18679-18685, August, 2021 DOI: https://doi.org/10.24941/ijcr.42048.08.2021

## **RESEARCH ARTICLE**

# ECTOMYCORRHIZAL FUNGI OF GENUS *RUSSULA* FROM ALÉDJO WILD LIFE RESERVE IN TOGO

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#### **ARTICLE INFO**

Kev Words:

### ABSTRACT

Article History: Received 25<sup>th</sup> May, 2021 Received in revised form 20<sup>th</sup> June, 2021 Accepted 23<sup>rd</sup> July, 2021 Published online 31<sup>st</sup> August, 2021

Ectomycorrhizal Fungi, *Russula*, Diversity, Alédjo Wildlife Reserve, Togo.

\*Corresponding author: Pondikpa NADJOMBE **Background:** The role of ectomycorrhizal fungi in tropical forest ecosystems is not to be overlooked. They promote plant growth and protect their roots from pathogens. Some species are commonly used by local populations in the diet and treatment of certain chronic diseases. To better assess the diversity of ectomycorrhizal fungi in Togo, a study was undertaken in the Alédjo Wildlife Reserve. The objective of this study is to contribute to a better knowledge of ectomycorrhizal fungi of the genus *Russula* from Alédjo Wildlife Reserve. **Methods:** For this purpose, fruit bodies were collected between 2016 and 2020 in the Alédjo Wildlife Reserve in 50 m x 10 m squares along 1000 m<sup>2</sup> transects established parallel in nine (9) different vegetations through woodlands and gallery forests. **Results:** Mycological surveys resulted in the collection of fifty-four (54) taxa of the genus *Russula*. Analysis of the collected specimens was done according to morpho-anatomical characters. All the specimens identified belong to seven (7) sections, twenty-one (21) subsections and thirty-two (32) species of the genus *Russula*, including seven (7) new species for Togo. **Conclusion:** This study showed that the diversity of ectomycorrhizal fungi in general and that of the genus *Russula* in particular is very high in Alédjo Wildlife Reserve.

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Citation: Pondikpa NADJOMBE, Hodabalo KAMOU, Boris Armel OLOU, Mèmbassolim SOGO, André De KESEL, Annemieke VERBEKEN and Kudzo Atsu GUELLY. "Ectomycorrhizal fungi of genus Russula from Alédjo Wild life Reserve in Togo", 2021. International Journal of Current Research, 13, (08), 18679-18685.

## **INTRODUCTION**

Among the most common ectomycorrhizal fungi in the forests of West Africa, there is Russulaceae family (1, 2, 3, 4) which includes the genera Lactarius, Lactifluus and Russula (5, 6, 7). The genus Russula has been the subject of several studies around the world and particularly in West Africa (3, 8, 9). The about 750 genus Russula has species worldwide (https://www.efta-online.org/genre/russula/). And as with many other groups of fungi, studies have been concentrated in the temperate zone and particularly in Europe. To date, in tropical Africa, there are about two hundred (200) species of the genus Russula described and identified (7, 10, 11). However, the majority of the species described, come from The Zambezian Endemism Centres (Burundi, Democratic Republic of Congo, Zimbabwe, Tanzania and Zambia) and

Mycological research in some of West African countries such as Benin (13, 14, 15), Burkina Faso (9, 16), Côte d'Ivoire (17), Niger (18), Senegal (1, 2) have mentioned the presence of *Russula* species in different vegetations. However, only Sanon *et al.* (9) studies that focused on the genus *Russula*. In Togo, Kamou *et al.* (19) have identified twenty-eight (**28**) species of *Russula* from Fazao-Malfakassa National Park and reported that several taxa have yet to be identified. Despite multiple work done in West Africa, on the genus *Russula* several species remain to be identified. Given the importance of ectomycorrhizal fungi around the world, it is necessary to continue mycological investigations on *Russula* species. This study is a contribution to a better knowledge and enhancement of the genus *Russula* from Alédjo Wildlife Reserve (AWR) in Togo.

### MATERIALS AND METHODS

Study area: Alédjo Wildlife Reserve is located in ecological zone II defined by Ern (20), and more precisely straddles on Tchaoudjo (Central region) and Assoli (Kara region) districts. This Reserve is located on Togo Mountains fault line, covers an area of 765 ha and is positioned between 9°11 and 9°17 North latitude and 1° and 1°24 East longitude (21). The Alédjo Wildlife Reserve is located in the semi-humid climatic zone with a unimodal regime. The total annual rainfall ranging between 1200 mm and 1600 mm over seven (7) months from April to October (22, 23). The temperature of the locality rangs between 17°C and 38°C with an annual average of about 28°C (Figure 2). The main vegetations are woodlands dominated by Isoberlinia tomentosa (Harms) Craib & Stapf, Isoberlinia doka Craib & Stapf, Uapaca togoensis Pax, Monotes kerstingii Gilg, gallery forests dominated by Berlinia grandiflora (Vahl) Hutch & Dalz and Uapaca guineensis Müll.

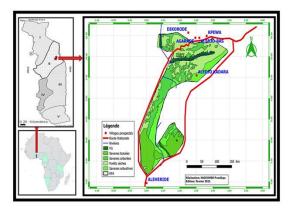


Figure 1. Location of Alédjo Wildlife Reservevegetations

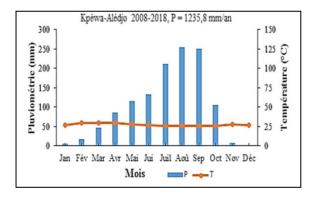


Figure 2. Umbrothermal curve

The average population density is thirty-two (**32**) habitants per  $km^2$ , which is low compared to the national average of 116.7 habitants per  $km^2$ . The most represented ethnic groups in the study area are the Tém who share their living space with Kabyè, Nawdba and Lamba ethnic groups, Tchamba migrants, and transhumant Fulani. Agriculture is the main activity around which other forms of activities revolve. It is practiced by more than 80% of the active population (23).

**Data collect :** Fungi have been collected exclusively in woodlands and in the gallery forests of Alédjo Wildlife Reserve following random sampling along the 250 m x 20 m transects established within nine (9) vegetations namely: woodlands dominated by *Isoberlinia tomentosa and Isoberlinia doka* (FCI), woodlands dominated by *Uapaca* 

togoensis (FCU), woodlands dominated by Monotes kerstingii (FCM), woodlands dominated by I. tomentosa, I. doka and M. kerstingii (FCIM), woodlands dominated by U. togoensis and M. kerstingii (FCUM), woodlands dominated par I. tomentosa, I. doka and U. togoensis (FCIU), woodlands dominated by I. tomentosa, I. doka, U. togoensis and M. kerstingii (FCIUM), gallery forests dominated by *B. grandiflora* (FGB) and gallery forests dominated by Berlinia grandiflora and Uapaca guineensis (FGBU). Mushrooms have been pictured using an Olympus x D Image Carte (the publishing house: OLYMPUS IMAGING AMERICA INC). The fruiting bodies have been then described following description sheet established by Ndong et al. (24) and macroscopic characters have been identified. The specimens have been dried with their herbarium numbers at 60°C using an electric dryer dehydrator from the D-rrex - Food Dehydrator St-ckli - White/Grey - 3 stainless steel grills for 24 hours. At the end of the drying, the dried samples are packed in the Minigrip plastic bags with their herbarium numbers to be stored at the herbarium of the University of Lomé (TOGO).

**Species identification:** Species identification has been made based on macroscopic and microscopic description. Documents such as the Systematics and Nomenclature of the Illustrated Flora of Central Africa, Paper 15, 16, 17, *Russula* I, *Russula* II, *Russula* III of Buyck (25), Buyck (10), Buyck (26), Buyck (11), Singer (27), the monographic flora of the genus *Lake* (28), the works of Sanon *et al.* (9), Ndong *et al.* (24), De Kesel *et al.* (13), Heim (29), Beeli (30) and the website (www.indexfungorum.org) have been used as well as our personal observations have been used for identification.

**Statistical analysis:** Minitab version 16.0 has been used to express the similarity between habitats. Diversity parameters have been calculated: determination of alpha diversity by calculating species richness (N0), Shannon diversity index (H), Piélou's equitability (E), Simpson index (D). The Shannon index, Piélou equitability index, Simpson index and Jaccard index have been calculated according to the following formulas:

- Shannon Index (*H*), H= ()  $Log_2$  () according to Magurran (2004) (*H*, in bits),

Where s = sum of species, Ni = number of people of considered species; N = total number of species surveyed.

- Piélou Equitability Index (E),  $E = \frac{H'}{Log2 (N0)}$  (Magurran 2004).

(E) measures the variation in abundance of different fungal species surveyed. Its value rangs between 0 and 1: the value tends towards 0 when very few species have a higher abundance and 1 when all species have the same abundance.

**Simpson Index (D),**  $D = \Sigma$  (pi<sup>2</sup>) according Odum (1976), Simpson Index value increases as the number of species decreases.

**Jaccard Index** (1901), Ij = 100C/A + B - C *in* Roux & Roux, (1967) with Ij = Jaccard index, A = number of species in group 1, B = number of species in group 2, C = number of species common to both groups. If Ij is greater than 50% then woodlands and gallery forests share almost the same species.

## RESULTS

**Diversity of** *Russula* **species :** Thirty-two (**32**) species have been identified and divided into seven (**7**) sections and twenty-one (**21**) subsections. Species identified during previous surveys (2007 - 2008; 2010 - 2012; 2013 - 2015) have been also taken into account. Table I shows the distribution of species by section and subsection. *Russula* section Heterophyllae (Fries) is the most represented in the Alédjo Wildlife Reserve with seven (**7**) subsections and eleven (**11**) species followed by the section of Fistulosaev (Heim and Singer) Buyck with five (**5**) subsections and eight (**8**) species. Afterwards comes the section of the Polychromae (Mayor) with three (**3**) subsections and five (**5**) species, followed by the section of Crassotunicatae Singer (Singer) with three (**3**) subsections and three (**3**) species.

# Table I. Distribution of *Russula* species by sections and subsections

SECTIONS	SUBSECTIONS	SPECIES		
	Sebsteriors	R. afronigricans Buyck		
COMPACTAE	Nigrigantinae Bataille	<i>R. pellucida</i> (Goossens &		
(Fries)		Heim) Buyck		
CONSTANTES	~ ~.	<i>R. congoana</i> aff Patouillard		
(Singer)	Sardoninae Singer	<i>R. purpureomutabilis</i> Buyck		
(* <b>6</b> 7	Aureotactinae Heim ex	R. acriannulata Buyck		
CRASSOTUNICA	Buyck			
TAE	Oleiferinae Buyck	R. oleifera Buyck		
Singer (Singer)		R. roseovelata Buyck		
	Roseovelatinae Buyck			
	Brunneodermatinae	R. cellulata Buyck		
	Buyck	<i>R. fissurata</i> Sanon & Buyck		
	-	R. liberiensis Singer		
FISTULOSAE (Heim & Singer) Buyck	Brunneofloccosinae Buyck	R. brunneofloccosa Buyck		
	Concolorinae Buyck	R. discopus Heim		
	Fistulosinae (Heim &	R. subfistulosa aff Buyck		
	Singer) Buyck			
	Meleaginae Buyck	R. diffusa var. diffusa Buyck		
		R. meleagris Buyck		
	Cyanoxanthinae Singer	R. atrovirens Beeli		
	Echinospematinae Buyck	R. echinosperma Heim& Gilles		
	1 5	R. viscidula Buyck		
	Guayarenses Singer	R. cyclosperma Buyck		
		R. annulata aff Heim & Buyck		
	Hétérophyllinae (Maire)	R. flavobrunnea var.		
HETEROPHYLLA	Schaeffer	violaceotincta Buyck		
E (Fries)	Semerier	R. flavobrunnea var		
		aurantioflava Buyck		
	Ilicinae (Romagnesi)	R. albofloccosa Buyck		
	Buyck			
	Mimeticinae Buyck	R. alveolata Heim		
	Pseudoepitheliosinae	R. hydropica Buyck		
	Buyck	R. pseudocarmesina Buyck		
PLORANTES	Archaeinae (Heim &	R. parvulospora Buyck		
(Bataille)	Romagnesi) Buyck			
	Aurantiomarginitinae	R. aurantiomarginata Buyck		
DOLVCUDOV	Buyck	R. ochrocephala Buyck		
POLYCHROMAE	Integrinae (Maire &	R. testacea Buyck		
(Maire)	Romagnesi) Singer	D D 1		
	Luteomaculatinae Buyck	R. compressa Buyck		
		R. fulvoochrascens Buyck		

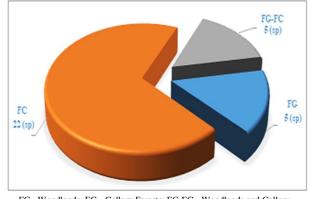
New quotes: Mycological investigations in most cases lead to new species. A total of seven (7) species of the genus *Russula* either 21% of identified taxa have been first found in Togo thank this study. These are *Russula atrovirens*, *Russula discopus*, *Russula echinosperma*, *Russula hydropica*, *Russula pellucida*, *Russula pseudocarmesina* and *Russula viscidula*. These species belong to three (3) sections and five (5) subsections. *Russula* section Heterophyllae is the most represented with three (3) subsections and five (5) species.



a: Russula atrovirens, b: Russula discopus, c: Russula echinosperma, d: Russula hydropica, e: Russula pellucida, f: Russula pseudocarmesina g: Russula viscidula

New quotes of the genus Russula from AWR

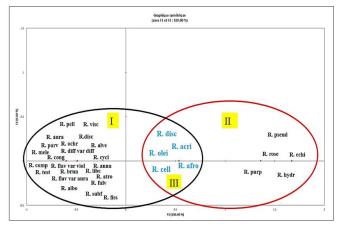
**Specific wealth of different vegetations :** From thirty two (**32**) species identified, 68.75% of species are specific to woodlands, 15.62% species specific to gallery forests and 15.62% species are common to woodlands and gallery forests (Figure 3). The majority of species have been harvested from woodlands. These results show that woodlands of Alédjo Wildlife Reserve are four times richer in species of the genus *Russula* than gallery forests.



FC - Woodlands; FG - Gallery Forests; FC-FG - Woodlands and Gallery Forests; sp – species

Figure 3. Specific spectra of different vegetations

The representation of species on axis 1 and axis 2 of the Factor Analysis of Components (AFC) shows variability: the species are dispersed on the two axis and divide in three groups (Figure 4).



I. Woodland species; II. Gallery forest species; III. Species common to woodland and gallery forests.

Figure 4. Representation of species in absence on the factor 1-2 plan of AFC

This figure shows that the diversity of *Russula* species is higher in woodlands (I) than gallery forests (II). However, some species are common both woodlands and gallery forests (III). Table II shows the distribution of the different species according to vegetations.

#### Table II. Distribution of different species according to vegetations

SPECIES	COLLECTION N°	VEGETATIONS
R. albofloccosa Buyck	NAD47	VEGETITIONS
R. alveolata Heim	NAD89, NAD145	-
<i>R. annulata</i> Heim	NAD4	-
<i>R. atrovirens</i> Beeli	NAD2, NAD17, NAD56	
<i>R. aurantiomarginata</i> Buyck	NAD61	-
R. brunneofloccosa Buyck	NAD35	
R. compressa Buyck	NAD28, C1892, C2223	
R. congoana Patouillard	C2161, NAD83	
R. cyclosperma Buyck	NAD71, NAD72	
R. diffusa var. diffusa Buyck	NAD11	
<i>R. fissurata</i> Sanon & Buyck	NAD46, NAD15	
R. flavobrunnea var	NAD90	Woodlands
aurantioflava Buyck		
R. flavobrunnea var.	NAD50	
violaceotincta Buyck		
R. fulvoochrascens Buyck	NAD20	
R. liberiensis Sing	NAD88	
R. meleagris Buyck	NAD 95, C1818b	
R. ochrocephala Buyck	NAD19	
R. parvulospora Buyck	NAD8	
R. pellucida (Goossens &	NAD52	
Heim) Buyck		
R. subfistulosa Buyck	NAD63, NAD67	
R. testacea Buyck	NAD53, C1884, C2217	
R. viscidula Buyck	NAD29	
R. echinosperma Buyck	NAD5	
R. hydropica Buyck	NAD12	
R. pseudocarmesina Buyck	NAD1	Gallery forests
R. purpureomutabilis Buyck	NAD36	
R. roseovelata Buyck	NAD44	
R. acriannulata Buyck	NAD9, C1907, C2176,	
	NAD137	
R. afronigricans Buyck	NAD87	Woodlands
R. cellulata Buyck	NAD76, C2152, C2219,	and
	NAD38	Gallery forests
R. discopus Heim	C2199, NAD55	4
R. oleifera Buyck	C2162, C2305, C2035	

The dendrogram analysis distinguishes five (5) groups: The majority of woodland species are found in woodlands dominated by *Uapaca togoensis*, *Isoberlinia* spp. and *Monotes kerstingii* (FCUIM) and gallery forest species are mostly found in gallery forests dominated by *Berlinia grandiflora* and *Uapaca guineensis* (FGBU) (Figure 5).

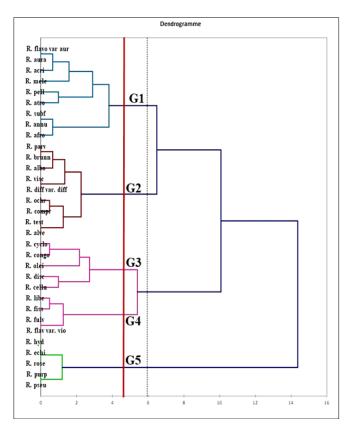
**Group G1:** woodland species dominated by *Isoberlinia* spp., *Uapaca togoensis* and *Monotes kerstingii*.

Group G2: woodland species dominated by *Isoberlinia* spp., woodland species dominated by *Isoberlinia* spp., *Uapaca* togoensis, Woodland species dominated by *Isoberlinia* spp., *Uapaca* togoensis and Monotes kerstingii.

**Group G3:** woodland species dominated by *Isoberlinia* spp. and *Monotes kerstingii*, woodland species dominated by *Uapaca togoensis* and *Monotes kerstingii*.

**Group G4:** woodland species dominated by *Isoberlinia* spp., woodland species dominated by *Monotes kerstingii*, woodland species dominated *by Uapaca togoensis* and *Monotes kerstingii*, woodland species dominated by *Isoberlinia* ssp., *Uapaca togoensis* and *Monotes kerstingii*.

**Group G5:** gallery forest species dominated by *Berlinia* grandiflora and Uapaca guineensis.



# Figure 5. Distribution of species identified according to different vegetations

The species richness of woodlands dominated by *I. doka, I. tomentosa, U. togoensis* and *M. kerstingii*, woodlands dominated by *I. doka, I. tomentosa* and *U. togoensis*, and woodlands dominated by *I. doka, I. tomentosa* and *M. kerstingii*, is significantly higher than that of other woodlands and gallery forests.

#### Table III. Distribution of species according to vegetations

Vegetations	Np	N0	N0mP	Н	E	D	Frequent species
FCI	25	15	10±3,0 9	3,45	0,53	21	R. alveolata, R. compressa, R. congoana, R. fulvoochrascens, R. ochrocephala, R. oleifera, R. testacea.
FCM	25	08	8±2,16	1,2	0,18	140, 2	R. atrovirens, R. cellulata R. fulvoochrascens, R. pellucida.
FCU	25	13	10±2,0 4	3,7	0,57	12,7 1	R. alveolata, R. annulata, R. aurantiomarginata, R. brunneofloccosa, R. congoana.
FCIM	25	18	11±2,7 6	2,54	0,45	52,1 5	R. afronigricans, R. aurantiomarginata, R. cellulata, R. congoana, R. cyclosperma.
FCIU	25	20	15±3,1 9	2,1	0,48	19,3 4	R. albofloccosa, R. atrovirens, R. compressa, R. cyclosperma, R. flavobrunnea var aurantioflava, R. oleifera, R. testacea.
FCUM	25	16	13±2,9 8	4,2	0,68	9,2	R. atrovirens, R. fissurata R. fulvoochrascens, R. liberiensis, R. subfistulosa.
FCIUM	25	23	19±3,5 3	5,02	0,7	7,3	R. afronigricans, R. albofloccosa, R. brunneofloccosa, R. fissurata, R. flavobrunnee var. violaceotincta, R. fulvoochrascens, R. liberiensis, R. ochrocephala, R. testacea, R. subfistulosa.
FGB	15	05	5±1,85	0,9	0,13	95,7 2	R. cellulata, R. oleifera, R. pseudocarmesina.
FGBU	20	09	9±2,58	2,2	0,27	58,3 5	R. afronigricans, R. cellulata, R. echinosperma, R. oleifera, R. pseudocarmesina.

Np = number of plots, N0 = species richness, N0mp = average species richness per plot, H = Shannon index, E = Pielou equitability index, D = Simpson index It ranges on average between 5 and 9 species per  $500 \text{ m}^2$  plot in woodlands, and in gallery forests it ranges from 3 to 5 species on average. Table III shows the most frequent species and the specific diversity values of the main vegetations.

## DISCUSSION

Alédjo Wildlife Reserve despite its small area contains a mosaic of vegetations that provide habitat for fungal development. Previous floristic studies have reported an exponential diversity of plant and animal species (31). This diversity has also been reported in mushrooms (32, 33). Of these fungi, only the diversity of Lactarius and Lactifluus have been assessed by Maba et al. (33). However many species remain to be described. A total of forty-one (41) Russula taxa have been describe of which thirty-two (32) have been identified up species level. These identified species belong to seven (7) sections and twenty-one (21) subsections. The Compactae section (2 species), the Constantes section (2 species), the section of the Crassotunicatae (4 species), the section of Fistulosae (8 species), Heterophyllae section (11 species), Plorantes section (1 species) and Polychromae section (5 species). Based on these results, one could say that the Alédjo Wildlife Reserve is full of a wide variety of Russula species, many of which have been identified. This diversity could be explained by the domination of ectomycorrhizal tree in vegetation of this Reserve. Moreover, Alédjo Wildlife Reserve is located in the northern part of Atacora chain in Togo and benefits from a microclimate that favours the fruiting of mushrooms.

However, Kamou et al. (19) recorded twenty-eight (28) species of the genus Russula from Fazao-Malfakassa National Park over an area of 192,000 ha. In Burkina Faso twenty-two (22) species of the genus Russula have been identified in woodlands and gallery forests of Dan and Kou, including four new ones (9). In Benin, nine (9) species have been described by De Kesel et al. (13), Yorou et al. (14), Boni et Yorou (15). In Côte d'Ivoire Vanié Léabo et al. (17) surveyed thirty-six (36) species of Russula in the Comoé National Park over an area of 11500 Km<sup>2</sup>. Thoen and Bâ (34) recorded eleven (11) species of Russula in southern Senegal in Afzelia africana and Uapaca guineensis vegetations. Ba et al. (3) recorded thirtythree (33) species of the genus Russula in Guinea Forest. Several of these species have been reported in several tropical African countries. These include R. congoana var congoana in Burkina (35) and Benin (14), R. bururiensis in Tanzania, DRC (Haut-Katanga), Burundi (Mosso-Malagarai) and Congo Brazzaville (36); R. compressa in Burundi (37), DRC Haut-Katanga (38), DRC Kipopo (39), Tanzania (37) and Benin (14); R. roseovelata, R. sublaevis, R. flavobrunne avaraurantioflava, R. flavobrunnea var. violaceotincta, R. aurantiomarginata, R. fulvoochrascens in DRC Upper Katanga and Kipopo (39; 40), R. ochrocephala in Senegal, DRC (38), R. oleifera in Burundi Mosso-Malagarasi (41), Benin (14, 42), R. alveolata and R. ochrocephala in Senegal (38), in the RCA (40). Species such as Russula cellulata, Russula compressa, Russula congoana, Russula ochrocephala and Russula oleifera are found in almost all countries in tropical Africa. The frequency of these species in several countries and vegetations can only be explained by their great plasticity in adapting to different environments and associating with different forest species. Moreover, these can be easily recognize in the field.

A total of seven (7) new species for Togo, namely R. atrovirens, R. discopus, R. echinosperma, R. hydropica, R. pellucida, R. pseudocarmesina, R. viscidula are noted and nine (9) described taxa that could probably be new species. In Burkina four (4) new species have been reported by Sanon et al. (9) which are new for science. These are Russula fissurata sp. nov.in the subsect. Brunneodermatinae, R. oculata sp. nov in the subsect. Mamillatinae, R. sankarae sp. nov in subsect. *R*. Aureotactinae. turpis sp. nov. in subsect. Aurantiomarginatinae, while R. oleifera becomes the type species of the new sub-section Oleiferinae of sect. Ingratae Aurantiomarginatinae/ Brunneodermatinae / Mamillatinae. In addition to the Russula species described by Kamou et al. (19), the number of Russula identified in Togo amounts to forty-three (43) species; which represents about 8.6 to 10.75% of Russula species described in tropical Africa. These two (2) protected areas located on the Atacora chain share a large number of Russula species (24 species). These are R. acriannulata, R. afronigricans, R. albofloccosa, R. alveolata, R. annulata, R. aurantiomarginata, R. brunneofloccosa, R. cellulata, R. compressa, R. congoana, R. cyclosperma, R. diffusa, R. fissurata, R. flavobrunnea, R. fulvoochrascens, R. liberiensis, R. meleagris, R. ochrocephala, R. oleifera, R. parvulospora, R. purpureomutabilis, R. roseovelata, R. subfistulosa, R. testacea; either 75% of the species identified in AWR. This can be explained not only by the similarity of vegetations and climate but also by the orography. The diversity of *Russula* in woodlands is higher with twenty-two (22) specific species than in the gallery forest vegetations where there are five (5) specific species with five (5) common species to both vegetations. This can only be explained by the high diversity of ectomycorrhizal trees in woodlands. The number of new species reported belong practically to gallery forests. This richness could be explained on the one hand by the high density of vegetation along the rivers and on the other hand by the humidity level which is however maintained in the gallery forests than in the woodlands. It should be noted that the number of Russula species identified in the AWR is only a fraction of the species of the genus Russula that could be available in this Reserve.

### CONCLUSION

Mycological investigations in Alédjo Wildlife Reserve have shown that this Reserve constitutes an important stock of ectomycorrhizal fungi that are still very little known. This study identified thirty-two (**32**) species of *Russula* including seven (**7**) new quotes and a significant number of unidentified species have been recorded in woodlands dominated by *Isoberlinia* spp., *Uapaca togoensis*, *Monotes kerstingii* and gallery forests dominated by *Berlinia grandiflora* and *Uapaca guineensis*. The Alédjo Wildlife Reserve is much diversified with a predominance of the Russulaceae and more precisely the genus *Russula*. Because of their functional importance in forest ecosystems and in daily life, both food and economic, fungi in general and the different ectomycorrhizal groups in particular deserve special attention.

### ACKNOWLEDGMENTS

The authors first thank the people living along Alédjo Wildlife Reserve and the guides for their collaboration, which has been very useful in prospecting the area and acquiring the necessary informations. They then thank the Togo Ministry of the Environment and Forest Resources (MERF) for authorizing this research in the Alédjo Wildlife Reserve. They also thank the Curator of the Alédjo Wildlife Reserve for his technical support. Finally, the authors thank the officials of the National Botanical Garden of Belgium for their technical and material support in the identification of species.

**CONFLICT OF INTEREST:** The authors declare that they have no conflicts of interest in relation to this article.

**AUTHORS' CONTRIBUTIONS:** All the authors were involved in all stages from conception to writing of this article, including analysis and data processing, as well as the contribution to the necessary reagents.

**SOURCES OF SUPPORT:** European Union/Project AMCC<sup>+</sup>/2020

ETHICAL CONSIDERATION: Not Applicable.

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