# Final Evaluation Report

| Your Details | |
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| **Full Name** | Badou Akotchaye Sylvestre |
| **Project Title** | Biodiversity assessment of Wild Edible Fungi and training of farmers: effective methods for in situ conservation in the Wari-Maro Forest |
| **Application ID** | 36954-B |
| **Date of this Report** | 28 / 01 / 2023 |

**1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.**

| Objective | Not achieved | Partially achieved | Fully achieved | Comments |
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| Identify some of the unknown Wild Edible Fungi (WEF) belonging to the genera Lactarius (L. ex Fr.) S.F. Gray., Lactifluus, Russula, Cantharellus, Psathyrella collected during our two previous projects and those which will be collected during the current project | | | | The specimens were photographed in situ, and field notes were taken on their macroscopic characters (Badou et al. 2018, 2021, 2022). They were dried for 24-48 hours in a field dryer at 40-60°C and immediately stored in airtight bags. Colour codes and names were taken from Kornerup & Wanscher's Methuen Handbook of Color (1978). The microscopic structures were examined with chemical reagents such as KOH, Congo red and Melzer. Observations, measurements and drawings of microscopic structures were carried out using a Leica DM 2700 M type optical microscope equipped with a digital camera and a drawing tube. The dimensions of the microscopic structures have been given as a range. Basidiospore sizes were presented in the following format: (a–) b– c– d (–e), where c is the mean, b = c - 1.96 * SD (5th percentile), and d = c + 1.96 * SD (95th percentile), "a" the extreme minimum value and "e" the extreme maximum value. "Q" is the length to width ratio which will be based on at least 50 spores and have been presented in the same format as basidiospore length and width (Badou et al. 2018, 2022). The structures of the pellis will be studied from a thin superficial scalp, taken either halfway between the centre and the edge of the cap (pileipellis) and/or halfway up the stipe (stipitipellis).<br>In addition, DNA was extracted from at least two representative specimens of each morphological species, |

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| | | | | according to Gardes & Bruns (1993) with a QIAGEN DNeasy Plant Mini Kit (QIAGEN Inc., Hilden, Germany), according to the manufacturer's instructions. The internal transcribed spacer region (ITS) of rDNA (Schoch et al. 2012), LSU (large ribosomal DNA subunit) and RPB2 (second largest subunit of RNA polymerase II) (Vetrovski et al. 2016), were amplified by PCR, using primer pairs ITS1–F (5'-cttggtcatttagaggaagtaa-3') / ITS4-B1 (5'-caggagacttgtacacggtccag-3'), LR0R (5'-cctttcaacaatttcacgt -3') / LR5 (5'-gggtccgagttgtaatttgt-3' ) and fRPB2-5F (5'-gaygaymgwgatcayttygg-3') / bRPB2-7R (5'-gaytgrttrtgrtcrgggaavgg-3') and will be sequenced using the Sanger method. |
| Determine the biochemical compounds of WEFs to detect WEFs with high nutritional values to advise them in the diet of children and the elderly (they constitute the most fragile layers of the local population the most exposed to diseases linked to malnutrition in rural areas) | | | | The biochemical analyses are already done. The total crude protein of each species was determined from the determination of total nitrogen, according to the Kjeldhal method (AOAC 1990). The lipid content of each species was determined according to the method described by AFNOR (1986), using a Soxhlet-type extractor. The extraction of the oils was obtained with hexane in a Soxhlet-type extractor (Unid Tecator, System HT2 1045, Sweden). The total sugar and reducing sugar contents of each species were determined using the phenol-sulfuric method as described by Dubois et al. (1956).<br>Finally, the energy value of each species was calculated using Atwater's specific coefficients (1899) for proteins, lipids and carbohydrates.<br>We have determined the biochemical compounds of Wild Edibles Fungi (WEF) to detect WEFs with high nutritional values to advise them in the diet of children and the elderly (they constitute the most fragile layers of the local population most exposed to malnutrition-related |

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| | | | | diseases in rural areas). |
| Reforest the Wari-Maro Forest with WEFs specific partner trees | | | | This activity was made possible by the financial and technical contributions of SOS BIODIVERSITY NGO. We take this opportunity to thank them again. We trained 60 farmers on the different stages of nursery cultivation of forest species such as *A. africana*, *Isoberlinia* spp, etc. in the village of Wari-Maro. 3000 plants of *A. africana*; 1,000 plants of *Isoberlinia doka* Craib & Stapf.; 1,000 plants of *Isoberlinia tomentosa* (Harms) Craib & Stapf. and 2,000 plants of *Khaya senegalensis* (Desr.) A. Juss. Below are photos of reforestation activities in the natural habitats of the Wari-Maro Forest Reserve. |

**2. Describe the three most important outcomes of your project.**

**a). Harvesting wild edible fungi in natural habitats in the Wari-Maro Forest reserve**
The specimens were photographed in situ, and field notes were taken on their macroscopic characters (Badou et al. 2018, 2021, 2022).

**b). Identification and microscopic work in the MyTIPS laboratory**
The specimens were dried for 24-48 hours in a field dryer at 40-60°C and immediately stored in airtight bags. Colour codes and names were taken from Kornerup & Wanscher's Methuen Handbook of Color (1978). The microscopic structures were examined with chemical reagents such as KOH, Congo red and Melzer. Observations, measurements and drawings of microscopic structures were carried out using a Leica DM 2700 M type optical microscope equipped with a digital camera and a drawing tube. The dimensions of the microscopic structures have been given as a range. Basidiospore sizes were presented in the following format: (a–) b– c– d (–e), where c is the mean, b = c - 1.96 * SD (5th percentile), and d = c + 1.96 * SD (95th percentile), "a" the extreme minimum value and "e" the extreme maximum value. "Q" is the length to width ratio which will be based on at least 50 spores and have been presented in the same format as basidiospore length and width (Badou et al. 2018, 2022). The structures of the pellis will be studied from a thin superficial scalp, taken either halfway between the centre and the edge of the cap (pileipellis) and/or halfway up the stipe (stipitipellis).

Dr. Sylvestre A. BADOU is taking pictures of wild fungi in the forest reserve.


MSc. Ogouchinan Omo T'Ayo Inès Rodolphine in the forest reserve for the harvest of wild

Labelling of specimens with their herbarium number.


Treatment of specimens collected after the field.


Camera mount for taking high resolution technical photos.


Taking technical photos with a high-resolution camera.

Drying specimens with an electric dryer.

Left: Specimens dried and ready to be deposited in the herbarium. Right: The chemical reagents used to carry out the microscopic observations.


Microscopic observation by Dr. Sylvestre A. BADOU with the LEICA type microscope.


Taking pictures of the microscopic elements observed.

**c). Wild edible fungi collected**

We collected about 150 specimens of wild edible fungi dried and herbalised in the UNIPAR herbarium at the Research Unit in Tropical Mycology and Plant-Soil Fungi Interaction (UR MyTIPS) at the University of Parakou.


Left: Russula congoana Pat. Right: Cantharellus addaiensis Henn.

Top: Lactifluus gymnocarpoides (Verbeken) Verbeken. Bottom: Lactifluus flammans (Verbeken) Verbeken.

Lactarius saponaceus Verbeken.

Lentinus squarrosulus Mont.

Psathyrella tuberculata (Pat.) A.H. Sm.

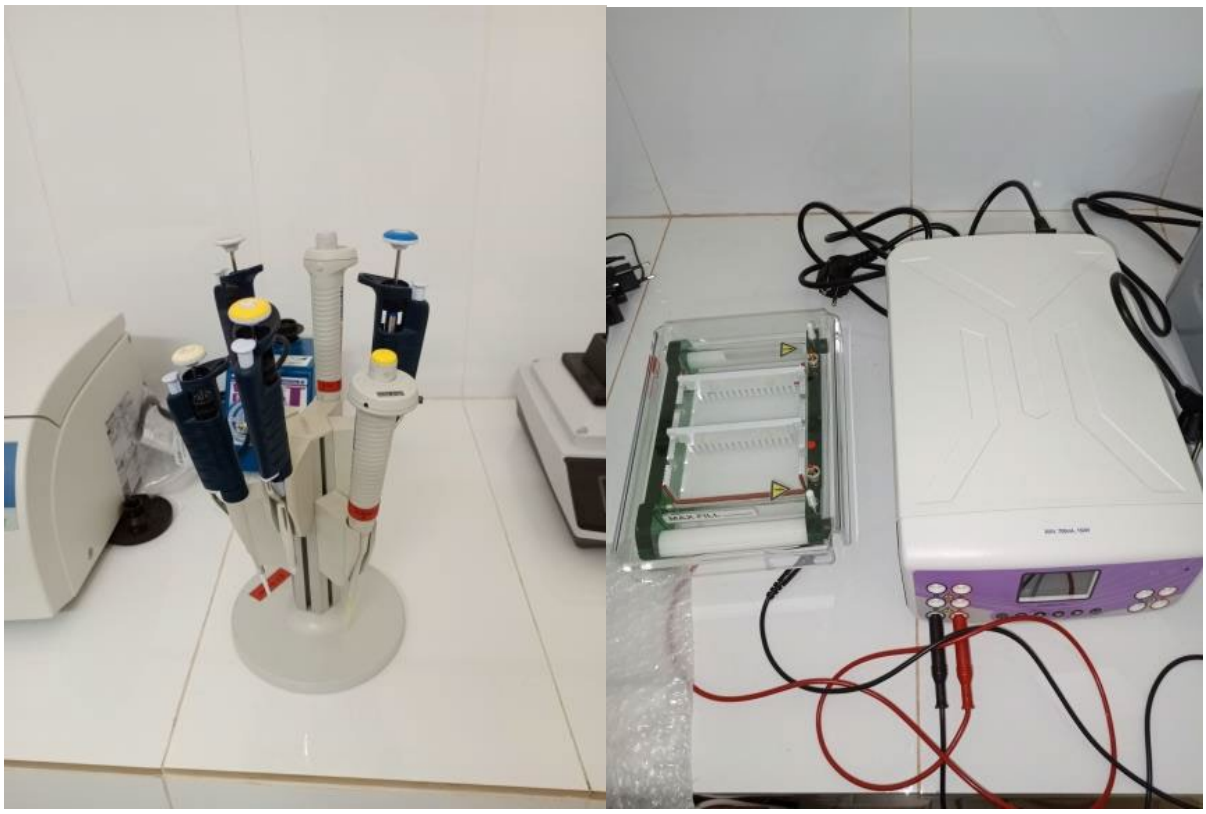
Left: Russula oleifera Buyck. Right: Lactifluus sudanicus Maba, Yorou & Guelly.

Left: Lactarius densifolius verbeken & karhula. Right: Lactarius edulis Verbeken & Buyck.

Left: Lactifluus luteopus (Verbeken) Verbeken. Right: Pleurotus tuber-regium (Rumph. ex Fr.) Singer.

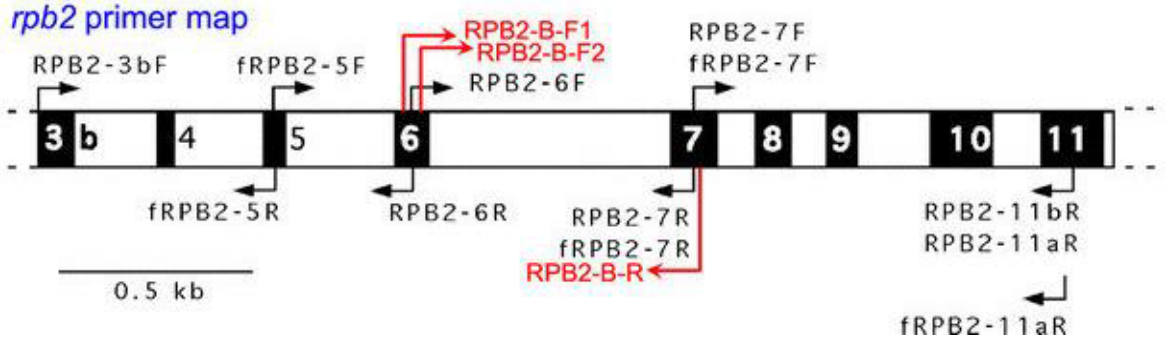
**d). DNA Extraction and Molecular Analysis**

A total of 55 specimens were used for DNA extraction. DNA was extracted from at least two representative specimens of each morphological species, according to Gardes & Bruns (1993) with a QIAGEN DNeasy Plant Mini Kit (QIAGEN Inc., Hilden, Germany), according to the manufacturer's instructions. The internal transcribed spacer region (ITS) of rDNA (Schoch et al. 2012), LSU (large ribosomal DNA subunit) and RPB2 (second largest subunit of RNA polymerase II) (Vetrovski et al. 2016), were amplified by PCR, using primer pairs ITS1–F (5'-cttggtcatttagaggaagtaa-3') / ITS4-B1 (5'-caggagacttgtacacggtccag-3'), LR0R (5'-cctttcaacaatttcacgt -3') / LR5 (5'-gggtccgagttgtaatttgt-3' ) and fRPB2-5F (5'-gaygaymgwgatcayttygg-3') / bRPB2-7R (5'-gaytgrttrtgrtcrgggaavgg-3') and will be sequenced using the Sanger method.



DNA extraction.






**ITS, RBP1, RPB2 and LSU**
PCR.

**e). Biochemical analysis of wild edible fungi**
We selected a total of 20 wild edible fungi specimens for biochemical analysis. The samples chosen for the biochemical analyses concern the most consumed edible fungi species in the Wari-Maro region.

The biochemical analyses are already done. The total crude protein of each species was determined from the determination of total nitrogen, according to the Kjeldhal method (AOAC 1990). The lipid content of each species was determined according to the method described by AFNOR (1986), using a Soxhlet-type extractor. The extraction of the oils was obtained with hexane in a Soxhlet-type extractor (Unid Tecator, System HT2 1045, Sweden). The total sugar and reducing sugar contents of each species were determined using the phenol-sulfuric method as described by Dubois et al. (1956).

Finally, the energy value of each species was calculated using Atwater's specific coefficients (1899) for proteins, lipids and carbohydrates.

We have determined the biochemical compounds of Wild Edibles Fungi (WEF) to detect WEFs with high nutritional values to advise them in the diet of children and the elderly (they constitute the most fragile layers of the local population most exposed to malnutrition-related diseases in rural areas).

**f). Training of farmers in nursery cultivation of forest species and reforestation of natural habitats in the Wari-Maro Forest Reserve**
This activity was made possible by the financial and technical contributions of SOS BIODIVERSITY NGO. We take this opportunity to thank them again.

We trained 60 farmers on the different stages of nursery cultivation of forest species such as *A. africana*, *Isoberlinia* spp, etc. in the village of Wari-Maro. 3000 plants of *A. africana*; 1,000 plants of *Isoberlinia doka* Craib & Stapf.; 1,000 plants of *Isoberlinia tomentosa* (Harms) Craib & Stapf. and 2,000 plants of *Khaya senegalensis* (Desr.) A. Juss. Below are photos of reforestation activities in the natural habitats of the Wari-Maro Forest Reserve
https://www.facebook.com/765197430295377/posts/pfbid021j8K8MNBK7sQB5rbeVKC5PwEVmbjjEtaN5wZAff8GuJZxCBvDtJXYvqEKz3iVBTel/

https://www.facebook.com/765197430295377/posts/pfbid0wjVWCVaCEx8MntUWe2ddVCJWSVXb1oDLy6i4oTjS8NwnkibKPShon7CgNDYydvk2l/

Installation of the straw hut with young farmers from the Wari-Maro region who will house the plant.

Cleaning the site and preparing the soil for potting.

Farmers in training can water in the river to water the soil.

Watering the soil to make it homogeneous.

Preparation of the bags to receive the potting soil.

Soil treatment by farmers.

Filling of bags (pots) by farmers to receive the seeds of forest seedlings.

Seeds ready for sowing.

Sowing seeds in pots filled with soil by trained farmers.

Here are the seeds in germination.

Here are the plants after 3 months spent in the nursery and ready to be reforested in natural habitats.

Transport of seedlings for reforestation with the help of farmers.

Natural habitats in which the plants have been reforested.

Actual reforestation of plants in the natural habitats of the Wari-Maro Forest Reserve.

Left: The Village Chief of Wari-Maro planting a tree in the forest. Right: Dr. Sylvestre A. BADOU (Project Manager) planting a tree in the natural habitats of the Wari-Maro Forest Reserve.

The most significant achievements of this work are:

- Taxonomic and molecular clarification for the identification of wild edible fungi.

- The biochemical analyses of the specimens in order to know their nutritional values to better fight against infant malnutrition and elderly people.

- Training farmers in the cultivation of partner forest trees with wild edible fungi in nurseries. This training will allow them to have an income-generating activity secondary to agriculture and they will now be able to produce forest seedlings that they can resell to the local community, to NGOs and to the state. Thanks to this, they will be able to improve their living conditions on the socio-economic level. Likewise, they will participate in the restoration of natural habitats and biodiversity in general and wild edible fungi.

**3. Explain any unforeseen difficulties that arose during the project and how these were tackled.**

During the execution of our project, we did not encounter any problems. All activities went well with the active participation of local actors.

**4. Describe the involvement of local communities and how they have benefitted from the project.**

The local populations have been a great help for the success of the project activities on the ground. They made themselves very available to follow the training in the cultivation in the nursery of the partner forest trees of the wild edible fungi. More specifically, they participated in all the production activities of forest seedlings. In addition, they facilitated our access to the sites which hosted the young plants in cultivation and the access to the sales rooms which served as places of meeting and exchange between the project team and the local populations. They have been a great capital for the execution of reforestation activities in the natural habitats of the Wari-Maro Forest reserve.

The project provides local populations and forest authorities with identification keys for each species of wild edible fungi. A catalogue grouping the photo of each species of wild edible fungi. Similarly, a manual relating to each wild edible fungi with their nutritional values (in proteins, carbohydrates, lipids, vitamins and trace elements) is available for forest managers and above all also to help in the fight against hunger and element deficiencies valuable nutrients for children and the elderly. Farmers have been trained in the cultivation of forest trees in nurseries. This new income-generating activity will now allow them to improve their living conditions and they (farmers being conservation ambassadors in the Wari-Maro region) will produce forest seedlings to participate in the restoration of natural habitats. and biodiversity. Our project, through the training of farmers in the cultivation of forest trees in nurseries, promotes participatory and community conservation.

**5. Are there any plans to continue this work?**

We do plan to continue this work by continuing:

- The training of farmers from the other remaining villages in which we have not been able to intervene in the Wari-Maro region such as: Agbassa, Abéokouta, Igbèré and Olougbè in the cultivation in nurseries of forest trees that are partners of wild edible fungi.

- Raising awareness and informing farmers about the laws, standards and rules governing the conservation and sustainable management of natural habitats and biodiversity in Benin.

- Enlighten local actors on their roles as ambassadors for the conservation of natural habitats and biodiversity in the Wari-Maro region.

**6. How do you plan to share the results of your work with others?**

- Data and results on biochemical analyses, descriptive taxonomy and molecular phylogeny on wild edible fungi from the Wari-Maro region will be disseminated to my colleagues and other researchers around the world through: The hourly cost of pro deo assistance that I perform with Prof. Dr. Ir. Nourou SOULEMANE YOROU at the Faculty of Agronomy of the University of Parakou.

- The monthly scientific seminars and communication organised by our laboratory will be used to make known our acquired knowledge.

- The annual scientific conferences and colloquia organised by the University of Parakou.

- Similarly, also the national, sub-regional and international conferences on the conservation of biodiversity and

- Publications and scientific works that will be generated.

**7. Looking ahead, what do you feel are the important next steps?**

For the continuation of the project in the Wari-Maro region, it is important:

- We plan to intensify eco-medicinal surveys on wild fungi among local populations in the Wari-Maro region to know their level of knowledge of local populations on the ecology of wild fungi. Also, know the medicinal uses that local populations make of wild fungi to better develop inclusive sustainable conservation strategies.

- Establish patterns of diversity, spatial and temporal distribution of wild fungi found in the natural habitats of the Wari-Maro Forest Reserve during our previous projects which are listed on the IUCN Red List (Vulnerable, Critically Endangered, etc.) of Benin compiled by Yorou & De Kesel (2011).

- Strengthen the self-confidence of local actors so that their integration into solutions to find the best practices for sustainable in situ conservation of wild fungi in the Wari-Maro Forest reserve is more effective and inclusive.

- We also plan to carry out an extensive chemical analysis of specimens of wild fungi that traditional healers use to cure certain diseases in the Wari-Maro region. These analyses will allow us to know the nature of the bioactive substances that these wild fungi possess to better value them in the future.

- Perform a land use study that will show what types of habitats, current habitats will change in the future (either the next 20 or 50 years) using remote sensing. This will make it possible to know what types of natural habitats will transform the current natural habitats in which wild edible fungi are available and to make a forecast of the evolution of the diversity of wild edible fungi in the future of the Wari-Maro Forest Reserve to know the appropriate conservation and sustainable management strategies that we can adopt and apply.

- Sensitise and inform local actors (farmers, local populations and leaders of village groups) on the laws and standards in force on the conservation and sustainable management of natural habitats and biodiversity.

- Continue the training of farmers from other villages in the Wari-Maro region where our project could not intervene on the cultivation of forest trees in nurseries to be more involved in the restoration of natural habitats and to the biodiversity.

- Carry out further reforestation of natural habitats using partner trees of wild edible fungi.

- Encourage local populations (especially children and the elderly) to consume more wild edible fungi with high nutritional values through information and

education sessions to effectively fight against famine and child malnutrition and elderly people who represent the most sensitive strata in the Wari-Maro region.

**8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?**

Yes, we have used the Rufford Foundation logo in our various mid-term reports that we have produced. We sent this publicity material to the foundation after the execution of our activities. Similarly, we used the Rufford Foundation logo in our article published in the journal Mycological Progress (https://doi.org/10.1007/s11557-021-01756-y) and in the journal African Journal of Ecology (https://doi.org/10.1111/aje.12948) .

**9. Provide a full list of all the members of your team and their role in the project.**

To achieve the objectives of our project, we called on the following skills:

**Dr. BADOU Akotchayé Sylvestre**, He has 8 years of experience in collecting data on the characterization of the natural habitats of macro fungi. He ensured the taxonomic identification (morphological description and microscopic observations) and the molecular analysis of the specimens.

**Yaou Marc**, He has been an agronomist specializing in Natural Resource Management since 2017. He has trained farmers in the cultivation in nurseries of partner trees of wild edible mushrooms (Isoberlinia doka Craib & Stapf., I. tomentosa, A. africana and P. erinaceus) and the reforestation of natural habitats.

**Ogouchinan Omo T'Ayo Inès Rodolphine**, she has three years of experience in biochemical analysis and the extraction of bioactive substances from plants, fungi and others. She coordinated activities related to the biochemical analysis of wild edible fungi at the Laboratory of Pharmacognosy and Essential Oils (L.A.P.H.E), University of Abomey-Calavi (Benin).

**Olodo Francine**, she has four years of experience in collecting mycological data in different forest reserves in Benin. It ensured the collection of wild edible fungi in the natural habitats of the Wari-Maro Forest reserve.

**10. Any other comments?**

It is important and crucial to do further biochemical and molecular analyses of wild edible fungi to increase the number of specimens evaluated. Similarly, it is also essential to inform and raise awareness among local populations of the laws and standards available on the conservation and sustainable management of natural habitats in Benin and West Africa.

This activity was made possible by the financial and technical contributions of SOS BIODIVERSITY NGO. We take this opportunity to thank them again.