Ecology, medicinal uses and domestication potentials of *Rauvolfia vomitoria* Afzel. (Apocynaceae) in Benin

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Summary

Rauvolfia vomitoria Afzel. (Apocynaceae) is one the most threatened medicinal plant species in Benin. *R. vomitoria* is a medicinal plants threatened in its habitat and overexploited by local people to treat psychoses and other psychiatric disorders. The distribution, ecology and conservation status of *R. vomitoria* are rarely investigated in Benin. This project seeks to understand the ecology and uses of *R. vomitoria* and discuss the threat faced by the species and potential for its domestications. *R. vomitoria* was found in the southern part of Benin, mostly in old fallows and in some of the remnant semi deciduous forests. Adult and seedlings densities did not vary across the distribution range.

However, there was a significant difference in population structure and morphology between regions, suggesting a recruitment bottleneck in the populations. *R. vomitoria* was widely used by local people to treat various diseases, mainly mental illness, malaria, jaundice, witchcraft and skin related diseases. These diseases are common in the study areas and witchcraft is particularly a serious aspect of the culture. This suggests a constant harvesting pressure on the species across its distribution range.

While they recognized that collecting distances are increasing over the years, local people suggested that *R. Vomitoria* is still available in their region and did not expect a short-term extinction of the species. However, given the shortening of fallows periods, *R. vomitoria* populations are at a higher risk of massive extinction than one would expect. We suggest that agro forestry system incorporate *R. vomitoria* as one way to ensure the long-term maintenance of the species across the landscape.

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1. Introduction

Indigenous people have been using medicinal plants for centuries. In Africa, 80% of the populations rely on traditional medicine for primary health care. In addition to non-commercial harvest of many plants for medicinal purposes, commercial harvest has increased the threat to extinction of many species. Unfortunately, we still lack information on the biology, distribution of even the common medicinal plants in many countries. Moreover, the impact of harvesting on the survival of the most endangered species has been long ignored while many of them are not domesticated and many species uncharacterized. *Rauvolfia vomitoria* Afzel. (Apocynaceae), although not even listed in the Red List of Benin plants (which is unrealistic), is probably one of the most endangered medicinal plants in Benin.

Nature conservation in African countries supposed that the rate of forest degradation, and more importantly, that the rate of species or sub-populations extinction decrease. Understanding indigenous knowledge and having local people involved into species conservation and management are prerequisites to expect long-term benefits. This project on *R. vomitoria* will set the tone for similar studies on many other endangered plant species (not only medicinal but also species used widely by people). This will provide the forestry department and other Non Governmental Organizations strongly involved into conservation of nature, the key information missing to justify many of their field activities, and also provide technical information for a better sustainable management plan of the species.

Here, we report the geographic distribution, the morphological variation, the ecology and uses of *R. vomitoria* and discuss the conservation status of the species and domestication potentials.

2. Methodology

2.1. Study areas

This study was conducted in the southern part of the Republic of Benin, located in West Africa, between latitudes 6º10N and 12º50N and longitudes 1ºE and 3º40'E. Benin lies between Nigeria on the east, Togo on the west, and is bordered by Burkina-Faso and Niger on the north. It has 125 km coastline on the south. Benin covers 114 763 km_2 with an estimated population of 6.7 millions, 61% of which live in rural areas (INSAE 2002; 2008). The population grows at a rate of 2.8 to 3.2 percent. The country has about 42 ethnic groups. The per capita GDP (in 2007) is $749 and the economy is mainly based on agriculture. Agriculture, mainly cotton farming, accounts for 40% of GDP and 70% of domestic exports and 75% of all employment (Bierschenk *et al.* 2003). The main food crops in Benin are maize, yams, cassava, cowpeas, sorghum and rice; the cash crops are cotton, groundnut and cashew (Igue *et al.* 2000; Vissoh *et al.* 2004). The majority of soils in Benin are poor and farmers often abandon their farms after only two or three years of cultivation and move to another areas to cultivate (USAID 2007). Given the poor soil fertility in Benin, increasing crops production necessitates increasing farm sizes and this leads to more land clearing. Land clearing for cotton and yam farming is one of the main causes of environmental degradation (Agbahungba *et al.* 2001; USAID 2007).

The vegetation composition in Benin shows a gradient from the coast to the north, and is determined by annual rainfall, length of dry season and air humidity (Natta 2003; Adomou 2005). Benin comprises three ecological regions: the Guineo Congolean region, the Sudano-Guinean region and the Sudanian region. The mean annual rainfall varies from 900 to 1300 mm; the mean annual temperatures range from 26ºC, (amplitude of 5-10ºC) in 3 the Guineo-Congolean region to an extreme of 40ºC (amplitude 11-13ºC) in the Sudanian region. The mean annual rainfall and the rainy season duration decrease from the Guineo- Congolean region

(1200 – 1300 mm rainfall; 6 months rainy period) to the Sudanian region (900 – 1100 mm rainfall; 5 months).

2.2. Study species

Rauvolfia vomitoria Afzel. (Apocynaceae) (2n = 22, 66) is found mainly wild in Africa.

The plant is a shrub with up to 5 m height. The stem is glabrous, erect and stiff. The leaves are whorled; the leaf blade broadly ovate or ovate-elliptic, 5-12 cm long and 3-6 cm wide. The cymes are usually by four together. The corolla is greenish or pale green; the tube is subcylindric, with 6-12 mm long, inflated at throat and pubescent inside; the lobes are dolabriform, 1-2 mm long. The stamens inserted at corolla throat are disc-ring like, shorter than the ovaries. The ovaries are distinct with filiform styles, pubescent at the base; the pistil head is fleshy with a membranous basis. The fruit is a drupe, sometimes paired, ovoid or ellipsoid, 0.8-1.4 cm x 6-9 mm. Flowering: August-October, fruiting: October- December. (Leeuwenberg 2006; eFloras 2008)

Rauvolfia vomitoria has many different phytochemicals, with nearly half of them found in the roots, 20% of them found in the leaves and 30% found in the whole plant. However, *R. vomitoria* is known as a source of the medicinally important reserpine (an indole alkaloid) present in the roots. This alkaloid reduces high blood pressure and is used in treatment of mental illness. The root of the species is often harvested and may be used as a sedative, aphrodisiac or antispasmodic (Adjanohoun 1989; FAO 2008; Mulliken & Crofton 2008).

2.3. Distribution, density and population structure

In the different localities of south Benin, between the 6°10 and 7°30N, we interviewed forestry officers, traditional healers, and farmers to check on the presence of any known populations of the species in that region. Through preliminary studies and discussion with other botanists and conservationists in Benin, we surveyed the following localities where populations of *R. vomitoria* thrive:

Littoral – Dangbo – Pobe – Niaouli – Ahozon – Avegamey (Adomou 2005), Adakplamey – Ketou (Hountondji Yvon, personal communication), Tindji – Cove (Vodouhe Fifanou, personal communication). We surveyed forests, savannas and fallows of various ages throughout the suspected distribution region of *R. vomitoria* in south Benin. We installed three plots of 20 x 10 m in each identified population and estimate the density of plants greater than 50 cm height. We installed five 2 m x 1 m subplots at the four corners and the centre of each plots to estimate the density of seedlings (individuals < 50 cm height). We measured the diameter at base for each adult individual to establish the structure of each population and identified the vegetation type (farm, fallow, savannah, forest), canopy cover and disturbance level (root harvesting, land clearing).

2.4. Morphological diversity

We used ten most meaningful morphological traits recorded on each individual to check similarity of populations from specific sub-region (East, West). Morphological characters used were: trunk circumference, trunk height, number of branches per knot, number of verticillate leaves, number of inflorescences per knot, number of flowers per inflorescence, pedicel length, drupe diameter, number of twinned berries, number of seeds per drupe.

2.5. Ethnobotany of *Rauvolfia vomitoria*

We surveyed farmers who have been using products of *R. vomitoria*, traditional healers and traders in various localities where *R. vomitoria* has been found according to our ecological survey. Thirty informants were interviewed per village and the sample comprised ten traditional healers, ten medicinal plant sellers, and ten medicinal plants users. We conducted semi-structured interviews with each of the informant about the uses of *R. vomitoria* in the local pharmacopoeia and other uses in the community. We specifically asked about the different uses of the plant, the recipes, the type of use, type of diseases treated. We also investigated the level of domestication of *R. vomitoria* and willingness to be involved in any domestication project in the future. We surveyed local and international markets to

estimate the market demand of *R. vomitoria* products, and the ecosystems harvested.

2.6. Statistical analysis

To test if adults and seedlings densities significantly differed between eastern and western regions, we used two samples Wilcoxon test. We used a Fisher's exact test to test if there is a significant difference in the size class distribution between the two geographical regions, because many cells in the contingency table had frequencies less than 5. Variations of all morphological traits were graphically examined and tests of normality performed. We used multiple variance analysis (MANOVA) followed by one-way ANOVA and a Student- Newman-Keuls post hoc test to compare variable means and to determine specific differences among populations. To summarize differences between eastern and western populations we used canonical discriminant analysis (CDA). Scores on canonical variables, plots of scores and squared distance between means (Mahalanobis distance) were used to explore the pattern of morphological dissimilarity among populations and between regions. Reduction of the original morphological dataset consisted in discarding not significantly different ($p > 0.05$) in the univariate analysis or not demonstrating Student-Newman-Keuls differences across groups, and additional variable reduction with the stepwise option in CDA.

3. Results and discussion

3.1. Distribution, density and variation in population structure

Rauvolfia vomitoria was found throughout the southern regions of Benin, between the 6°10 and 7°30N. *R. vomitoria* populations were found mainly in fallows, palm oil plantations and in semi-deciduous forest (Niaouli). The density of adults (Figure 1.A) and seedlings (Figure 1.B) of *R. vomitoria* was similar across the distribution range of the species. However, this lack of significant difference in population density masks a significant difference in population structure between the eastern and western populations (Figure 2). *R. vomitoria* populations in the eastern region lacked large individuals. In the western region where many individuals were found in dense forest and old fallows, the structure of the populations showed large

individuals but also a lack of individuals in the sapling stage. This suggests a bottleneck in recruitment of individuals from seedlings to sapling stages. This may be due to a high mortality of seedlings. Populations in the eastern regions were found in young fallows and this may explain the lack of large individuals. In both regions, *R. vomitoria* was found commonly on fallows and this suggests that the species may have developed an adaptive plasticity necessary to reduce the probability of extinction due to loss of original habitats.

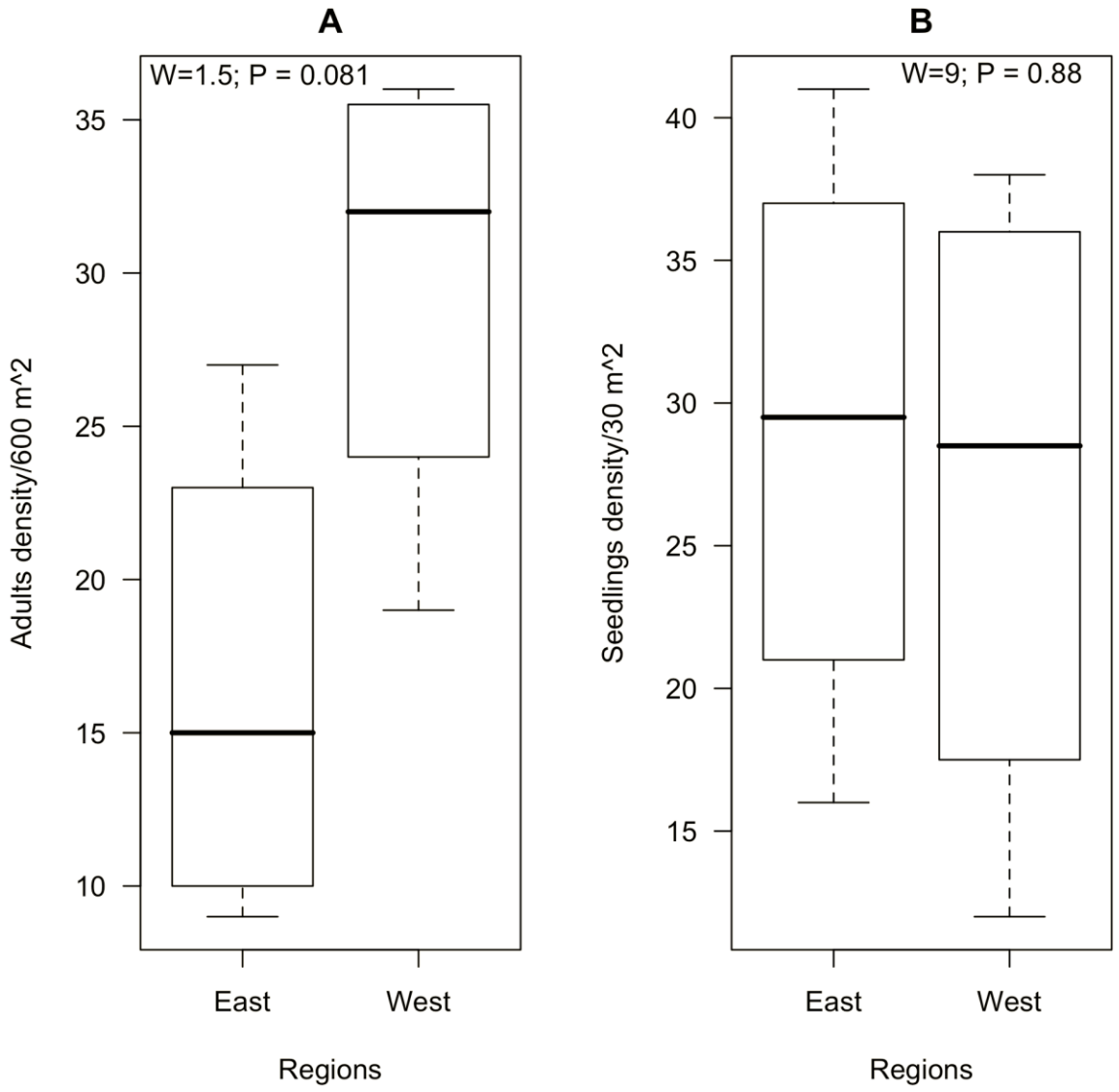

Figure 1: Densities of *Rauvolfia vomitoria* (A) adults and (B) seedlings populations did not vary significantly between the two geographical regions.

3.2. Morphological variation of *Rauvolfia vomitoria*

Multiple comparison of morphological traits indicated significant differences among populations (MANOVA, $p < 0.01$). Univariate analysis (ANOVA) also indicated significant variation of each trait among populations except for number of flowers ($p = 0.17$), number of twinned berries ($p = 0.41$) and trunk circumference ($p = 0.05$) which was nearly significant. Pairwise comparison of populations for each morphological trait resulted in regional subdivision (*post hoc* test, Student-Newman-Keuls; Table 1).

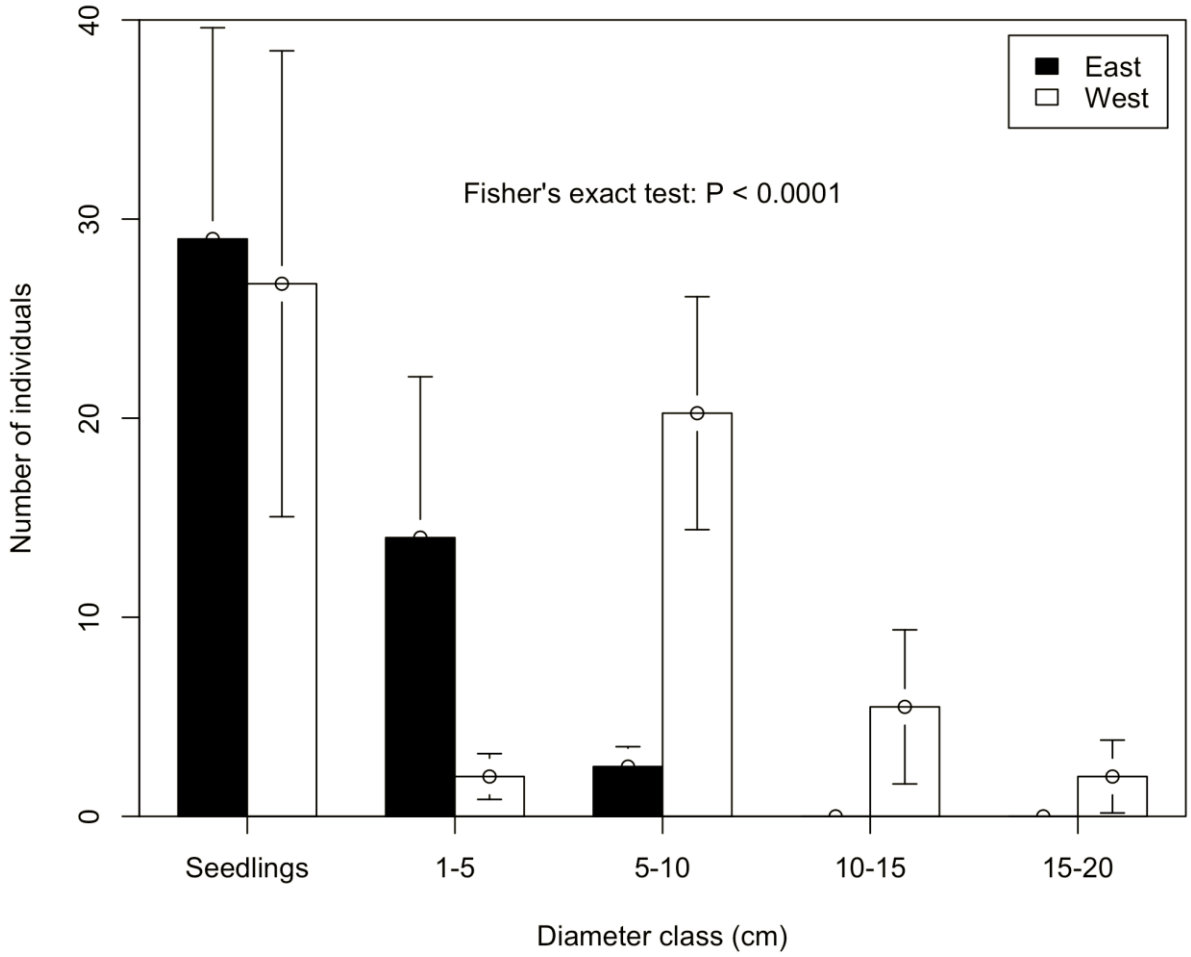

Figure 2: Population structures of *R. vomitoria* across regions. Populations in the eastern region lack large individuals and western populations surprisingly have low recruitment in the sapling life stage. Error bars represent standard deviations.

In the CDA 88.7% of the total variation were explained by the first two canonical functions with high correlation coefficient (Table 2). Populations tended to be separated by number of branches per knot, number of verticillate leaves, number of inflorescences, trunk height and circumference and pedicel length. Those traits were highly correlated to canonical functions. Most individuals were allocated to their respective populations with 52 to 75% group membership in populations. Canonical discriminant analysis based on regional subdivision indicated 100% of good allocation of all populations (Figure 3).

Table 1. Means and standard deviations of ten characters measured for populations of *R. vomitoria* in South-West and South-East of Benin.

	West	East
Trunk circumference	26.79 ± 13.30	12.35 ± 2.91
Number of branches per knot**	3.26 ± 0.25	2.62 ± 0.70
Number of verticillate leaves**	4.00 ± 0.00	3.44 ± 0.73
Number of inflorescences**	3.14 ± 0.35	2.71 ± 0.52
Number of flowers ns	69.27 ± 12.19	71.26 ± 36.38
Number of twinned berries ns	2.22 ± 1.91	1.52 ± 0.81
Number of grains per fruit**	1.77 ± 1.66	1.97 ± 1.10
Trunk height**	35.42 ± 19.22	27.33 ± 26.97
Berry diameter*	0.64 ± 0.22	0.59 ± 0.28
Pedicel lengthns	0.43 ± 0.24	0.43 ± 0.34

** $p < 0.001$; * $p < 0.05$; . $p = 0.05$; ns: $p > 0.05$

Table 2. Percentage of variation explained and canonical correlation of the six canonical discriminant functions used in the analysis.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	2.324	62.1	62.1	0.836
2	0.996	26.6	88.7	0.706
3	0.316	8.5	97.2	0.49
4	0.054	1.5	98.7	0.227
5	0.031	0.8	99.5	0.172
6	0.02	0.5	100	0.14

Morphological variation is congruent with population structures of *R. vomitoria*.

Size factors such as trunk circumference and height heavily account for the discrimination of eastern and western populations. In addition yield parameters such as number of whorled leaves, number of branches at knot, number of seeds per drupe vary from eastern to western localities. Harvesting pressure on the eastern populations (the population structure analysis indicated lack adults) might have led to some adaptive response at plant level through the increase of some leaf and fruit traits. Further investigations on the genetic variation of the species in its distribution range and analysis of correlation between genetic and morphological trait would shed light on the impact of harvest pressure on the genetic makeup of *R. vomitoria*.

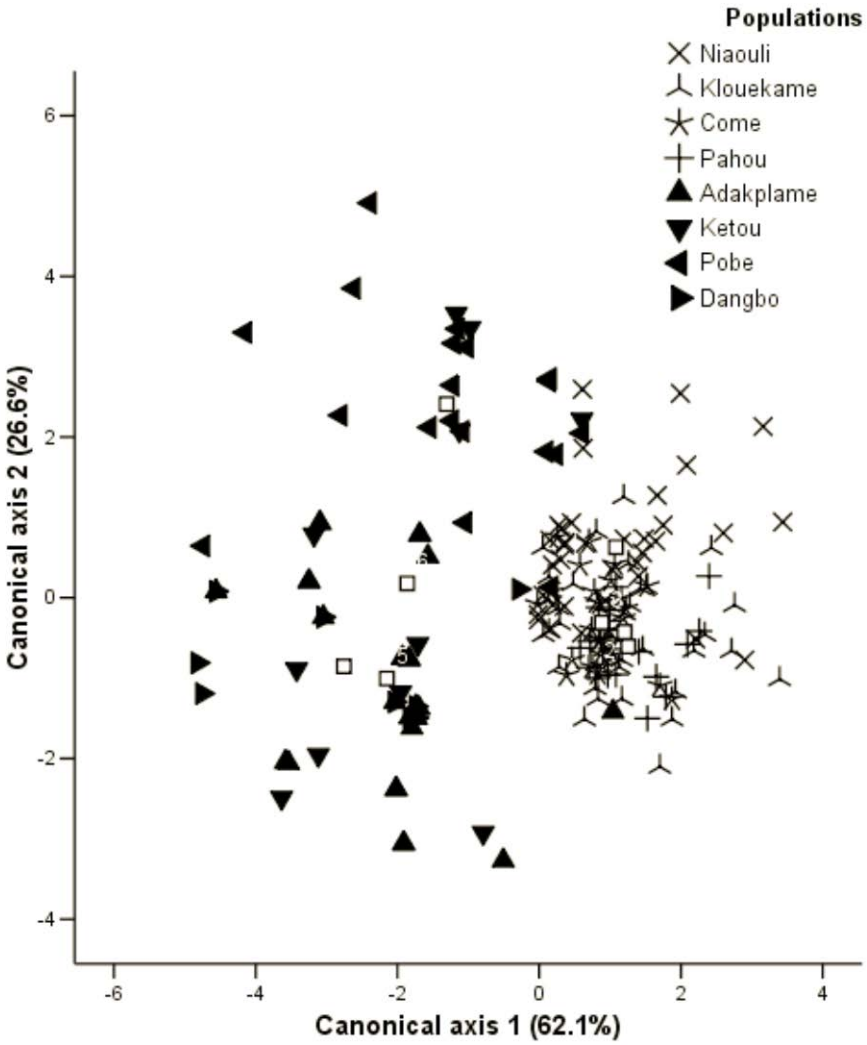

Figure 3. Morphological variation of *R. vomitoria* populations based on two canonical functions. Solid triangles indicate populations from the eastern region; cross-like signs indicate the western region. Group centroids are indicated by empty squares.

3.3. *Rauvolfia vomitoria* as a non-timber forest products

Rauvolfia vomitoria was used by local people to treat a wide range of diseases from mental illness, jaundice to fertility related diseases (Figure 4.A; Table 3). The most common diseases free-listed by informants were mental illness and witchcraft. There was not significant difference in the diseases listed by consumers and traditional healers (Fisher's exact test; $p = 0.1159$; Figure 4.A). However, only healers listed that they use *R. Vomitoria* to treat sterility, postpartum and menstrual pains. In contrast to traditional healers, consumers listed in addition that *R. vomitoria* was used to facilitate kids teething and lower blood pressure. This suggests a limited difference in knowledge across the various groups of users of *R. vomitoria*, although each user groups has some specialty.

The list of diseases free-listed by informants suggests a potentially high value of *R. vomitoria* for local people. Malaria is a common disease in tropical Africa and harvesting medicinal plants to treat or prevent the disease is of paramount benefit. Our findings that *R. vomitoria* is used to treat witchcraft is also important given that mystical and magical related diseases are common in Benin. However, it was not possible to identify the mechanism of fighting these diseases. Informants suggested that fighting magical diseases is about protecting the spirit of the patient through the use of plants chemicals.

The pharmacopoeia recipes used to treat these diseases vary according to traditional healer. Regardless of the type of informant, roots and leaves were the most commonly used organs used in these recipes (Fisher's exact test; $p = 0.5837$; Figure 4.B). Although only in few cases, that the whole plants are used, harvesting roots often lead to the death of the whole plant.

Rauvolfia vomioria is harvested from fallows of various ages nearby their village or farms. Most informants suggested that the species is still available and not threatened to extinction although some informants reported that the distance to the collecting sites of the species has increased over the years. They listed land clearing and the shortening of fallows period in the region as the main reason explaining the scarcity of the species.

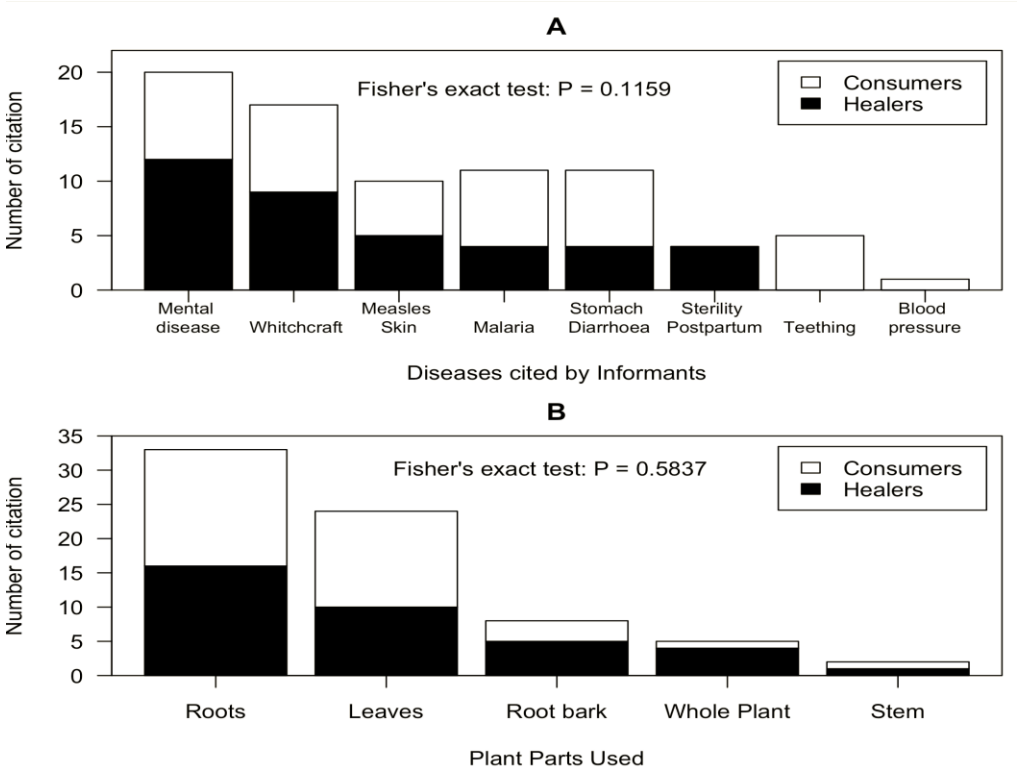

Figure 4. *Rauvolfia vomitoria* was used to treat a wide range of diseases, (A) from mental illness to sterility and postpartum or menstrual pains. (B) Roots and leaves were the most commonly used part of *R. vomitoria* used by informants.

There was no large scale domestication effort at local people level. However, we found cases of *R. vomitoria* plantations in traditional gardens by four traditional healers (Figure 5) and in few cases, we found farmers who spared the species on their farms during land clearing or in their gardens (Figure 6). We only found a handful of people involved in the trade of the species in the study region. Most of the products are harvested directly from the wild. According to informants, the relative availability of the species made its trade non profitable.

Table 3: Recipes and *Rauvolfia vomitoria* organs used for the main disease groups

Diseases	Organs used	Recipes
Mental illness	Roots	Dried roots (without bark) finely grinded.
Stomachache	Flowers	Consume R. vomitoria's dry flowers powder.
Measles and Skin related	Leaves and roots	Patient takes steaming bath with infusion of *R. Vomitoria leaves and roots.*
Malaria	Leaves and stem	Infusion with leaves of *R. vomitoria* and *Argemone mexicana.*
Insomnia	Leaves	Put nine (for men) or seven (for women) leaves of R.vomitoria under patient pillow respectively if the patient
Diarrhoea	Leaves	Soaked leaves

Figure 5. *Rauvolfia vomitoria* conserved by a traditional healers in his private garden.

Figure 6: *Rauvolfia vomitoria* spared in a maize + palm oil agro forestry system.

4. Conclusions and implication for conservation

Populations of *R. vomitoria* differed in their characteristics between the two regions of South Benin based on their population structure and morphological traits. These differences suggest that populations of *R. vomitoria* may exhibit a genetic structure linked to morphology or habitats. The high density found in some sites and assurance from local people that the species is still available and may not be threatened to extinction mask a gradual shift in the conservation status of the species. With the shortening of fallows periods and an increase in land clearing expected in the southern Benin in coming years, there is a serious threat to extinction of *R. Vomitoria* and companion species that occurs on fallows such as *Voacanga africana*. We suggest a large scale conservation effort in the region starting by raising awareness of the local people on the ecology of the species (most preferred habitats), the various utilisations of the species and contribution to primary healthcare, and the threat faced by the species given the observed and expected shortening of fallows in the region. In parts of Benin and many other West African countries few species such as *Vittelaria paradoxa, Parkia biglobosa,* and many medicinal plants are spared in traditional agro forestry system and this contribute to their *in situ* conservation.

Similar strategy, although still at its infancy, was developed by few local people for *R. vomitoria*. The species was found in palm oil plantations where it can thrive over the years and be better conserved given that plantations may last for many years.

Acknowledgments

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Appendices: Field pictures from the research team

Team members discussing about the floral organization of *Rauvolfia vomitoria*

Mature and immature fruits of *Rauvolfia vomitoria*

Rauvolfia vomitoria mature fruits harvested by the research team

Measuring *Rauvolfia vomitoria* size in the field using a calliper.

Rauvolfia vomitoria in an old fallow. This is the most common habitat

Rauvolfia vomitoria harvested in a palm oil plantation