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Identification and Larvicidal Efficacy of Mosquito-Repelling Plants Used in Malaria Vector Control in South-East Benin

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Abstract

After identifying issues posed by some synthetic insecticides, the scientific community is now looking for other natural products with insecticidal and/or insect repellent characteristics. This study aimed to identify the plants of the Beninese flora known for their insecticidal and/or insect repellent effects and the indigenous techniques of their use and to evaluate the combined efficacy of extracts of a few on mosquito larvae for the control of malaria vectors in the South-East of Benin. A survey was conducted and the insecticidal effects of three combinations made from *Azadirachta indica*, *Cymbopogon nardus* and *Hyptis suaveolens* oils on *Anopheles gambiae* larvae were studied. The data were analyzed using R software, and Chi-square test was used to compare death rates. Twelve (12) plant species were identified. The combinations of *C. nardus* and *H. suaveolens* oils induced the highest mortality rates (90 ± 4.15% and 68 ± 9.14% with stages 2 and 3 larvae, respectively). The application of extracts from these plants to control the malaria vectors population would reduce the use of chemical pesticides, hence the need to promote and enhance the formulations of biopesticides based on plant extracts.

Keywords: Plant species, biopesticides, mosquito-repelling plants, vectors.

Introduction

Plants occupy important places in food and medicinal resources. People have used them for health care (OMS 1998), food, fuelwood, and lumber. Indeed, forest resources on the planet abound more than 300,000 plant species, among which more than 200,000 are recorded in tropical countries of Africa and have medicinal properties. These medicinal plants constitute precious resources in Africa for the populations who use them for their health care (Benlamdini et al. 2014). It is also important to note that numerous pathologies are treated in traditional ways in Africa and elsewhere in the world from the organs of local plants. We can cite the bites of poisonous snakes, diabetes in pregnant women (Ghourri et al. 2013), skin diseases and many other pathologies. For example, 80% of people bitten by poisonous snakes report a perfect recovery following traditional treatments (Grema and Koné 2003). Therefore, these plants are African antivenoms and offer many advantages over serums and vaccines since they are easily accessible, inexpensive, and stored at room temperature. In agriculture, market gardeners often use extracts from plant organs to protect crops and cereals against phytopathology and pests that directly affect market garden production (Diabaté et al. 2014). Extracts from neem leaves (*Azadirachta indica*) are used to effectively control cabbage aphids, flea beetles, and okra jassids. Papaya leaf extracts (*Carica papaya*) also have remarkable effects on okra aphids at all stages (Gnago et al. 2011).

Another example is malaria, which remains a genuine public health concern and a severe obstacle to the socio-economic development of Africa in the south of the Sahara (WHO 2019). Mosquito of the genus *Anopheles* bites to ensure its transmission to humans. Plants such as *Anacardium occidentale*, *Tephrosia vogelii*, and *Thevetia peruviana* can be used to control these mosquitoes (Agbizounon 2010, Mukhopadhyay et al. 2010, Akpo et al. 2017).

Today, these anti-mosquito plants belonging to the category of medicinal plants are progressively experiencing a chemical valorization. This valorization is due to various chemical studies related to the extraction of essential and vegetable oils and these oils are characterized to know the composition in useful substances to better guide their uses (Silou et al. 2004, Ndomo et al. 2009, Kando et al. 2017, Likibi et al. 2019, Ahouansou et al. 2019). However, the chemical recovery of plants is better oriented and better structured when knowledge of the relationships between plants and humans and their uses since the dawn of time is well documented. This knowledge about plants is generally held by few people, among whom there is a high illiteracy rate. This situation can lead to the disappearance of local knowledge, which is equivalent to an impoverishment of the genetic heritage, ultimately reducing the possibility of improving plant materials (Savadogo et al. 2016). Dupré (1991) underlined the link between practices, knowledge and resources. For this researcher, wisdom is only manifested when actualized in practice; the disappearance of these practices leads to the disappearance of knowledge about resources. With the importance of these declarations, States find themselves obligated to respect, preserve and maintain the knowledge, innovations and practices of indigenous and local communities that embody traditional lifestyles of interest for conservation and sustainable use of biodiversity (Le Goater 2007). Therefore, it is necessary to protect plant use knowledge and improve it in scientific dimensions to have a better oriented and rational use in applied entomology.

This study aimed to identify the plants of the Beninese flora known for their insecticidal and/or insect repellent effects and the endogenous techniques of their uses and to evaluate the insecticidal effects of three combinations made from *Azadirachta indica*, *Cymbopogon nardus* and *Hyptis suaveolens* oils on mosquito larvae for the control of malaria vectors in the South-East of Benin.

Materials and Methods

Study area description

The study was carried out from November 2018 to March 2019 in 18 villages from three municipalities. These are the municipalities of Abomey-Calavi and Sô-ava in the Atlantic Department and the municipality of Dangbo in the Department of Oueme (Figure 1). These municipalities were chosen because of the information gathered from the populations during the preliminary surveys. Indeed, at the end of the initial investigations, people exploit certain plant species to repel or kill the mosquitoes, vectors of diseases in these villages or city neighbourhoods.

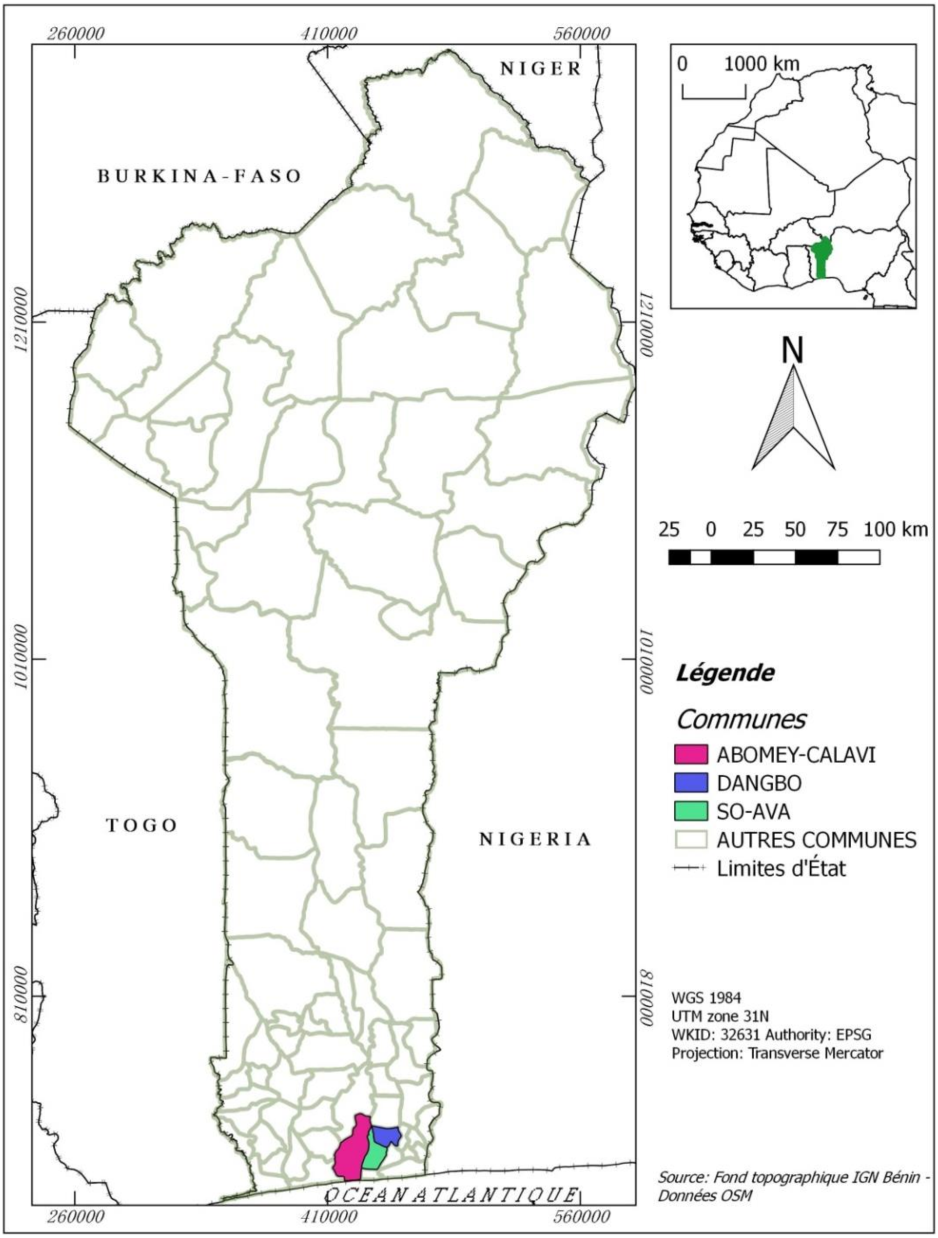

Figure 1: Map of Benin showing municipalities in which the work was carried out.

Sampling and data collection

Qualitative and participatory surveys, based on interviews using questionnaire forms, were conducted in the three (3) municipalities in South-East Benin. People of both sexes were subjected to questions in each locality. The customary authorities were targeted, and the leaders of the various teams that carry out night activities in the area were the respondents. The survey took place with residents at their homes. Then we went to the bushes and markets to collect samples of the plants mentioned during the interview. The plants collected were used to constitute a herbarium. The choice of the villages or city districts of these municipalities was guided by the customs authorities and other people of advanced ages. However, we have done the work in neighbouring localities that these authorities did not recommend. The names of the plants, cited in local languages, are identified by their scientific names in the field or, if necessary, herbarium samples were collected to be determined at the National Herbarium laboratory of the University of Abomey-Calavi (UAC).

Extraction methods

Azadirachta indica, *Hyptis suaveolen* and *Cymbopogon nardus* were chosen because of information in the literature related to their insecticidal and/or insect repellent activities against crop pests and disease vectors. Plant organs were collected between October 2018 and December 2019. The essential oils of the leaves of *C. nardus* and *H. suaveolens* previously dehydrated by drying in the shade were obtained by hydrodistillation in the ‘’Laboratoire d’Etude et de Recherche en Chimie Appliquée’’ (LERCA) of the Polytechnic School of University of Abomey-Calavi. Vegetable oil from the seeds of *A. indica* was extracted by soxhlet in the same laboratory (Salghi 2011).

Constitution of plants extracts combinations

The different combinations of extracts are listed in Table 1. Concentration of extracts entering into each of the combinations was 100 ppm for *A. indica* oil (Nour et al. 2012), 97.33 ppm for *C. nardus* oil (Ahouansou et al. 2019) and 240.3 ppm for *H. suaveolens* oil (Conti et al. 2012). Insecticidal solutions consisted of lecithin, ethanol, the extract or combination, and distilled water. Lecithin and ethanol acted as adjuvants in these solutions. To disperse and stabilize 1 g of extracts in solutions, 0.1 ml of ethanol and 0.1 g of lecithin were used, respectively.

Bioassays

Assays were performed according to the protocol described by WHO (Abbott 1925). Five replicates were performed on stage 2 and stage 3 larvae of *An. gambiae*. The "Kisumu" strain of *An. gambiae* reared in the insectarium of the “Centre de Recherche Entomologique de Cotonou (CREC)” was used. Firstly, assays were carried out with the single extracts, and secondly with each of the three (3) combinations. The experimental design consisted of 15 cups each containing 100 ml of insecticidal solution for the test with the single extracts. For the assays with the combinations, 15 cups were used. Five other cups containing distilled water, lecithin, and ethanol served as controls. In each of the cups were introduced batches of 20 stage 2 larvae of the susceptible strain (Kisumu) of *An. gambiae* previously left on an empty stomach for 24 hours. These experiments were repeated on stage 3 larvae of the same strain. After 24 hours of exposure, dead larvae were counted and mortality rates were calculated.

Counting and statistical analysis of data

The examination of the interview guides was carried out manually. We used blank sheets which allowed us to collect a set of questions filled in during the survey, data such as the number of the survey, the number of plants listed, the different modes of applications and the names of organs used. The software used for data analysis is R studio version 3.6.3. This analysis is based on tests of the proportion of the variables sought according to the municipalities. Chi-square test was used to compare death rates. The significance level was 5%.

Table 1: Constitution of extracts combinations

	Cymbopogon nardus oil	*Hyptis suaveolens* oil
Azadirachta indica oil	C1	C2
Cymbopogon nardus oil	–	C3

– : No combination; C1: combination 1 ; C2: combination 2 : C3: combination 3.

Results

Plant identification

Plants known for their insecticidal and/or insect repellent effects identified in Abomey Calavi, Sô-Ava and Dangbo districts of the South-East region of Benin are presented in Table 2.

Table 2 shows the listed species' organs and modes of application on mosquitoes. Analysis of the data in this table reveals that at the end of our survey carried out in districts of Abomey Calavi, Sô-Ava and Dangbo, 12 plant species are known for their insecticidal and /or insect-repellent effects were listed. From this table, it emerges that the leaves, seeds and fruit skin are the 3 organs of these species used by populations for mosquito control. This table also shows that fumigation, the deposit of plant organs in the corners of dwellings termed (DCD) and the trituration of leaves and liquid passage obtained on the body termed (TPB) are the 3 modes of application of these organs on mosquitoes according to the populations of the 3 study districts. This table shows that *Azadirachta indica* and *Lantana camara* are the 2 species recognized for their insecticidal and /or insect repellent effects in all the 3 study districts.

Table 2: Species listed with their organs and methods of application on mosquitoes

Species listed	Organs	Modes of application	Districts
Abrus precatorius	Leaves	Fumigation	Sô-Ava
Annona muricata	Leaves	Fumigation	Sô-Ava
Azadirachta indica	Leaves; Seeds	Fumigation	Abomey Calavi; Sô-Ava; Dangbo
Chromolaena odorata	Leaves; Seeds	Fumigation	Abomey Calavi; Sô-Ava
Citrus sinensis	Leaves; fruit skin	Fumigation	Sô-Ava; Dangbo
Crateva adansonii	Leaves	Fumigation; TPB	Sô-Ava
Cymbopogon nardus	Leaves	Fumigation	Abomey Calavi
Ficus polita	Leaves	Fumigation	Sô-Ava
Hyptis suaveolens	Leaves; Seeds	Fumigation; DCD	Abomey Calavi; Sô-Ava
Lantana camara	Leaves; Seeds	Fumigation	Abomey-Calavi, Sô-Ava; Dangbo
Senna occidentalis	Leaves; Seeds	Fumigation	Abomey Calavi
Senna siamea	Leaves	Fumigation	Abomey Calavi; Sô-Ava

TPB: Trituration of leaves and liquid passage obtained on the body; DCD: Deposit of plant organs in the corners of dwellings.

Availability of plant species identified by respondents

Of the twelve plants identified, 38.08% of 180 respondents indicated that *A. indica* is the most available species, followed by *H. suaveolens* (15.17%), *C. sinensis* (14.55%), *L. camara* (12.07%), *C. adansonii* (5.88%), *S. occidentalis* (4.64%) and *C. odorata* (3.4%). Species such as *A. precatorius*, *S. siamea*, *F. polita*, *A. muricata* and *C. nardus* are rare in the study area, and their percentages of availability are less than 3% (Figure 2)

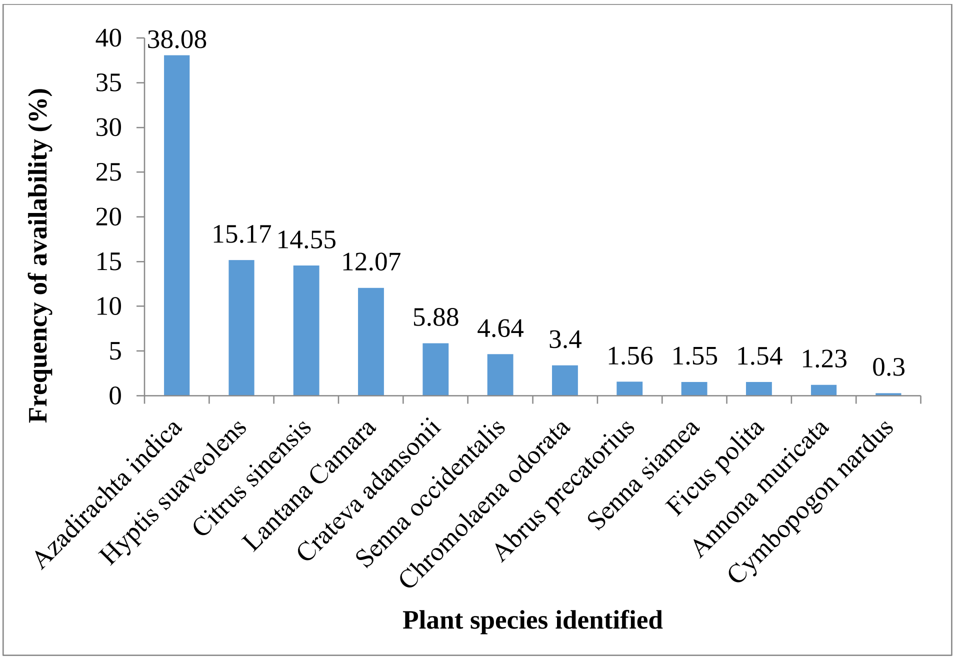

Figure 2: Plant species surveyed and their percentages of availability by respondents.

Images, local and common names of listed species

After identifying the 12 plant species, their images were taken and their local and common names were also recorded. Figures 3–14 show their pictures, local names and common names.

Local and common names of *A. precatorius*
Viviman (Goun)
Omisimisi, oyuologbo (Yoruba and Nago)
Rosary pea (English)
Pois rouge (French)

Figure 3: Leaves, stems and fruits of *A. precatoris.*

Local and common names of *A. muricata*
Yovowouinglé (Fon)
Yovonyglwe (Goun)
Soursop (English)
Corossolier (French)

Figure 4: Leaves, stems and fruits of *A. muricata.*

Local and common names of *A. indica*
Kininuti (Fon and Goun)
Dogonyaro, Egui lili (Yoruba and Nago)
Neem (English and French)

Figure 5: Leaves and seeds of *A. indica*.

Local and common names of *C. odorata*
Agatou (Fon and Yoruba)
Bitter bush (English)
Herbe de laos (French)

Figure 6: Leaves of *C. odorata*.

Local and common names of *C. sinensis*
Yovozin (Fon and Goun)
Orsan (Yoruba)
Sweet orange (English)
Oranger (French)

Figure 7: Branches and leaves of *C. sinensis.*

Local and common names of *C. adansonii*
Wontonzuzuen (Fon and Goun)
Tanya (Yoruba)
Sacred barna (English)
Poire à l'ail sacré (French

Figure 8: Branches and leaves of *C. adansonii*.

Local and common names of *C. nardus*
Timan (Fon)
Tii (Yoruba)
Lemongrass (English)
Citronnelle (French)

Figure 9: Leaves of *C. nardus.*

Local and common names of *F. polita*
Voman, Avleman (Fon and Goun)
Odan (Yoruba)
Heart leaved fig (English)
Figuier (French)

Figure 10: Leaves of *F. polita*.

Local and common names of *H. suaveolens*
Azongbidi (Fon)
Zansoukpêman (Goun)
Disibu (Yoruba)
Bush tea (English)
Gros baume (French)

Figure 11: Leaves and flowers of *H. suaveolens.*

Local and common names of *L. camara*
Hlaciayo (Fon)
Ewonagogo (Yoruba)
Cherry pie (English)
Lantanier (French)

Figure 12: Leaves,stems and flowers of *L. camara.*

Local and common names of *S. occidentalis*
Kinikiniba (Fon)
Ganyitogle (Goun)
Abo rere (Yoruba and Nago)
Coffee senna (English)
Faux Kinkéliba (French)

Figure 13: Leaves and fruits of *S. occidentalis.*

Local and common names of *S. siamea*
Kenu (Fon)
Kassia (Yoruba and Goun)
Cassia tree (English)
Cassia du Siam (French)

Figure 14: Branches and leaves of *S. siamea.*

Efficacy of plants extracts

The larval mortality rate in the control solution was 0% regardless of the larval stage (Tables 3, 4 and 5). With *A. indica* oil, this rate was 14.28 ± 4.60% and 3 ± 3.34% for stage 2 and stage 3 larvae, respectively (Tables 3 and 4). The mortality rates of larvae exposed to *C. nardus oil* were 64.89 ± 6.82% and 31.96 ± 9.28% with stage 2 and stage 3 larvae, respectively (Tables 3 and 5). With *H. suaveolens* oil, the mortality rates were 44.21 ± 7.45% and 7.92 ± 5.26% for the stage 2 and stage 3 larvae, respectively (Tables 4 and 5).

Efficacy of plants extracts combinations

The efficacy of plants extracts combinations are presented in Tables 3, 4 and 5. The mortality rates were 85.71 ± 4.82% and 45.45 ± 9.80% for stage 2 and stage 3 larvae, respectively with the combination 1 (C1) (Table 1 and Table 3). A significant difference was observed between the mortality rates caused by the combination 1 (C1) and the mortality rates caused by the two mono-treatments (χ^2 test, $p < 0.0001$) whatever is the larval stage.

Table 3: Efficacy of plant extracts combination 1 on stages 2 and 3 larvae of *An. gambiae*

Extracts & Combinations	Larval stages	N tested	N dead	**Mortalities (%)**	P value
AiO_100 ppm	L2	112	16	14.28	<0.0001
AiO_100 ppm + CnO_97.33 ppm	L2	98	84	85.71	
AiO_100 ppm	L3	100	3	3	<0.0001
AiO_100 ppm + CnO_97.33 ppm	L3	99	45	45.45	
CnO_97.33 ppm	L2	94	61	64.89	<0.0001
AiO_100 ppm + CnO_97.33 ppm	L2	98	84	85.71	
CnO_97.33 ppm	L3	97	31	31.96	0.0731
AiO_100 ppm + CnO_97.33 ppm	L3	99	45	45.45	
Control	L2	107	0	0.00	
	L3	104	0	0.00	

N: Number; AiO: Azadirachta indica oil; CnO: Cymbopogon nardus oil.

The combination 2 (C2) induced mortality rates of 18.69 ± 5.22% and 9.17 ± 5.41% with the stage 2 and stage 3 larvae, respectively (Table 4). A significant difference was observed between the mortality rate of stage 2 larvae caused by the combination 2 (C2) and the mortality rate of stage 2 larvae caused by *H. suaveolens* oil alone (χ^2 test, p < 0.0001). With stage 3 larvae, this difference was not significant (p = 0.9382).

Table 4: Efficacy of plant extracts combination 2 on stages 2 and 3 larvae of *An.gambiae*

Extracts & Combinations	Larval stages	N tested	N dead	**Mortalities (%)**	P value
AiO_100 ppm	L2	112	16	14.28	0.2739
AiO_100 ppm + HsO_240.3 ppm	L2	107	20	18.69	
AiO_100 ppm	L3	100	3	3	0.1189
AiO_100 ppm + HsO_240.3 ppm	L3	109	10	9.17	
HsO_240.3 ppm	L2	95	42	44.21	<0.0001
AiO_100 ppm + HsO_240.3 ppm	L2	107	20	18.69	
HsO_240.3 ppm	L3	101	8	7.92	0.9382
AiO_100 ppm + HsO_240.3 ppm	L3	109	10	9.17	
Control	L2	107	0	0.00	
	L3	104	0	0.00	

N: Number; AiO: Azadirachta indica oil; HsO: Hyptis suaveolens oil.

Regarding the combination 3 (C3), the mortality rates were 90 ± 4.15% and 68 ± 9.14% with stage 2 and stage 3 larvae, respectively (Table 5). A significant difference was observed between the mortality rates caused by the combination 3 (C3) and the mortality rates caused by the two mono-treatments (χ^2 test, p <0.0001) whatever the stage.

Table 5: Efficacy of plant extracts combination 3 on stages 2 and 3 larvae of *An. gambiae*

Extracts & Combinations	Larval stages	N tested	N dead	**Mortalities (%)**	P value
CnO_97.33 ppm	L2	97	31	31.96	<0.0001
CnO_97.33 ppm + HsO_240.3 ppm	L2	100	90	90	
CnO_97.33 ppm	L3	97	31	31.96	<0.0001
CnO_97.33 ppm + HsO_240.3 ppm	L3	100	68	68	
HsO_240.3 ppm	L2	95	42	44.21	<0.0001
CnO_97.33 ppm + HsO_240.3 ppm	L2	100	90	90	
HsO_240.3 ppm	L3	101	8	7.92	<0.0001
CnO_97.33 ppm + HsO_240.3 ppm	L3	100	68	68	
Control	L2	107	0	0.00	
	L3	104	0	0.00	

N: Number; CnO: Cymbopogon nardus oil; HsO: Hyptis suaveolens oil.

Discussion

The surveys carried out in Abomey-Calavi, Dangbo and Sô-Ava made it possible to identify some plants of Beninese flora known for their insecticidal and/or insect-repellent effects and the endogenous techniques of their use for malaria vectors control. Bioassays were carried out to evaluate the insecticidal activities of three combinations made from *Azadirachta indica* oil, *Cymbopogon nardus* oil and *Hyptis suaveolens* oil on *An. gambiae* (Kisumu) larvae.

Our surveys revealed that *A. indica* and *L. camara* are the species cited by populations in the three municipalities of the study area, but *A. indica* is the most mentioned. Its effectiveness explains this in controlling malaria vectors (Agbizounon 2010, Nour et al. 2012). In addition to its effectiveness, the high rate of use of *A. indica* by populations is also linked to the abundance of its leaves during all seasons. This position of the most cited species among the listed plant species, occupied by *A. indica* is also confirmed by the oil of its seeds, which constitutes a promising larvicide for controlling mosquitoes resistant to synthetic insecticides (Agbizounon 2010).

Our investigations also showed that *C. nardus* is the least cited plant of the twelve (12) identified plant species. The low rate of use of this species by the populations of the study area is thought to be due to the lack of knowledge of its insecticidal and/or insect repellent properties. These results are similar to those of Savadogo et al. (2016), who showed that many localities do not know the biopesticide virtues of certain plants. In the twelve (12) identified species, *H. suaveolens* is the second most cited species after *A. indica*. According to residents of the study locations, these two plants are used to control mosquitoes that carry human diseases effectively. These results corroborate the findings of Savadogo et al. (2016), who confirmed that *A. indica* and *H. suaveolens* are part of the thirty plant species used by the Mossé for biopesticides in Burkina Faso.

During the surveys, many inhabitants of the localities visited did not have any knowledge of phytopesticides. This observation would be linked to the rise of synthetic pesticides, which have played a major role in the abandonment of phytopesticides. This is explained because phytopesticides are not easily accessible, while synthetic pesticides are practically available in all markets.

The repetition of fumigation in the three study municipalities shows that these areas' populations exert more fumigation to apply local plants to mosquitoes. These results corroborate those of Savadogo et al. (2016), who confirmed that the Mossé of Burkina Faso uses more fumigation to apply local plants against mosquitoes and ticks.

The results revealed that the leaves are the most cited along with the seeds as local plant organs used for mosquito management. This shows that the leaves and seeds contain molecules that exert insecticidal and/or insect repellent effects on insects. This observation was also made by Mukendi (2011), who showed that the leaves and seeds are effective insecticides against aphids, thrips and insects that perforate the stems of maize.

Based on the results of our investigations, we have recorded a total of twelve (12) plant species from eleven (11) families and eleven (11) genera. This number of plant species exploited by populations for mosquito control reflects the importance of plant diversity in vector control. Thus, according to Dupré (1991), local knowledge constitutes wealth from the point of view of biological diversity. From a genetic point of view, they are considered existing conservatories of variability.

Bioassays carried out to evaluate the insecticidal effects revealed that larval mortality rates generated by the combinations of *A. indica* oil and *C. nardus* oil (C1) and *C. nardus* oil and *H. suaveolens* oil (C3) were significantly higher than individually tested mortality rates. This observation shows that the effects between these combined extracts are non-antagonistic. These non-antagonistic effects can be explained by the fact that the compounds of the mixtures and their chemical properties have improved the efficiency of the molecules determining the insecticidal effects of extracts (Kindozandji et al. 2020).

Larval mortality rates caused by combination of *A. indica* oil and *H. suaveolens* oil (C2) were significantly lower than the death rates from the single extracts. This observation indicated that there are antagonistic effects between these combined extracts. These results are similar to those of Narkhede et al. (2017).

Conclusion

This study identified some plant species that populations exploit for mosquito management. Three modes of application of these species on mosquitoes were recorded: the Deposit of plant organs in the Corners of the Dwellings (DCD), Fumigation (F) based on these plant organs and Trituration of the leaves and liquid Passage obtained on the Body (TPB). Twelve species in total have been recorded. The combinations of *C. nardus* and *H. suaveolens* oils induced the highest mortality rates (90 ± 4.15% and 68 ± 9.14% with stage 2 and stage 3 larvae, respectively). Beyond these application modes, this work has also made it possible to know the most exploited plant organs.

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Competing interests

All authors declare that they have no competing interests.

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