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Larvicidal activity in the laboratory of aqueous extracts of lantana camara, *Bougainvillea spectabilis* and *Tephrosia villosa* against *Anopheles gambiae* SL vector of malaria in Cote d'Ivoire

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Abstract
Considering the pollution and resistance of chemical insecticides in *Anopheles gambiae* S.L., alternative control approaches have led to this study. Larvicidal activities of the leaves of *Lantana camara*, *Bougainvillea spectabilis* and *Tephrosia villosa* against the fourth instar larvae of *Anopheles gambiae* S.L. were evaluated. The susceptibility level and the homogeneity of wild strains were determined according to the method of Sinègre. The highest mortality rates were obtained from 15mg/ml with *Lantana camara* and *Bougainvillea* spectabilis. The larvicidal activity of *Lantana camara* on the wild strains was higher than *Bougainvillea* spectabilis. These wild strains have the same sensitivity as well as the KISUMU strain towards *Bougainvillea spectabilis* and *Lantana camara*. However, the wild population response to aqueous extracts was homogeneous against *Bougainvillea spectabilis* and heterogeneous against *Lantana camara*. *Lantana camara* and *Bougainvillea spectabilis* leaves were the most active aqueous extract on larvae of *Anopheles gambiae* S.L.

Keywords: Mortality rates, larvicidal, aqueous extract, *Anopheles gambiae*

Introduction
Malaria, the first of the parasitic endemics [1], remains one of the most serious public health problems. Despite treatment, malaria cases are still recorded because of the resistance of pathogens to drugs [2]. Recognizing this threat and awaiting the development of a vaccine, the World Health Organization recommends vector control as an important component of the global malaria prevention strategy [3]. Initially based on the destruction of breeding sites by draining swampy areas [4], malaria control evolved into the use of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) [5] with the advent of synthetic chemical pesticides. Thus, according to Mouchet and Golvan [6], the organophosphates come after the organochlorines, then the carbamates, and finally the pyrethroids. These chemical insecticides have occupied an important place in vector control strategies by reducing the number of malaria cases [7, 8]. However, although effective, the use of synthetic insecticides is not without danger for human beings and the environment.
Their intensive use leads to environmental pollution due to the significant accumulation of active ingredients in treated ecosystems [9] and their broad spectrum of action can lead to the eradication of non-target species [10]. And in the long term, synthetic insecticides lead to the development of resistance in main vector species [11, 12, 13]. Face to these realities, it is become essential to develop new alternatives for mosquito control.
Research could be directed towards natural chemicals from plants that are inexpensive and protect the environment [14]. Plants, sources of important natural compounds and with many therapeutic applications, can also be used as alternative insecticides. Plant extracts have been the subject of several studies to show their efficacy against *Anopheles gambiae* larvae [14, 15, 16, 17]. In this context, this study aims to evaluate the larvicidal efficacy of aqueous extracts of three plants against wild populations of *Anopheles gambiae*.

Materials and Methods

Criteria for the choice of plants

The plants (*Lantana camara*, *Bougainvillae spectabilis*, and *Tephrosia villosa*) were chosen based on their use in pharmacopoeia as a repellent by the local populations, bibliographical research on their insecticidal properties, and their abundance.

Plant collection and extraction

In 2015, *Lantana camara* and *Tephrosia villosa* leaves were collected in central Côte d'Ivoire (Bouaké) and *Bougainvillae spectabilis* leaves were collected in the South of the country (Abidjan). Harvests were made with pruning shears and transported to a laboratory in packages with sufficient ventilation to dissipate the heat produced by fermentation of the leaves [18]. In the laboratory, the leaves have been rinsed, cut, and spread out on plastic film in an airy room to be dry for two weeks at room temperature out of direct sunlight. Once dried, the leaves have been ground. The powder obtained was used to prepare the total aqueous extract according to the method of Zihiri *et al*. [19]. Indeed, 100g of powder have been dissolved in one liter of distilled water, and then mixed in a blender type MIDEA® during six cycles of two minutes each. The homogenate obtained was first wrung out in a clean cloth square, then filtered twice on hydrophilic cotton, and finally on Wattman paper (Ø: 3 mm). The filtrate obtained was dried at 60°C in a proofer (SELECTA®). The powder obtained was stored until use.

Phytochemical screening

Phytochemical screening was carried out according to the protocol described by Evans [20] and Ghani [21]. The presence of several phytochemicals (saponins, tannins, flavonoids, alkaloids, steroids, and terpenoids) was tested based on the appearance of specific coloration or precipitates characteristic of the secondary metabolite.

Collection and processing of larvae

Anopheles gambiae larvae (wild strains) were collected from rice fields in the town of TIASSALE (124 km from Abidjan) and transported to the laboratory for rearing. Stage III and IV larvae of the F0 and F1 generations were used for larvicide tests. *Anopheles gambiae* larvae (KISUMU), provided by the INHP (National Institute of Public Hygiene) insectarium, were used as a sensitive reference strain.

Larvicide tests

The method was based on the WHO [22] standardized sensitivity tests. For each plant, a series of six disposable cups containing respectively 100ml of extract of concentrations of 5, 10, 15, 20, 25, and 30 mg/ml was prepared. Then, twenty-five of third and fourth instar larvae were placed in each of the cups. Control cups without extract were run in parallel. Four replicates were performed for each concentration and the control cup. Mortality was observed after 24 hours of exposure. Three trials were performed for each test.

Data analysis

Data were processed with BioStat Pro V5 software. A two-factor analysis of variance followed by a Turkey multiple comparisons test was used to assess differences in mean mortality rates at a probability of $p<.05$. From average mortality rates, the lethal concentrations (LC50 and LC90) of each aqueous extract in the two populations were determined using the log-probit model [23]. These LC50 and LC90 values were used to estimate the level of sensitivity of wild larvae to the extracts compared to the sensitive strain (KISUMU) according to the method of Sinègre *et al*. [24]. The coefficient "K" or Resistance ratio to 50 (RR50) which is the ratio LC50 of the wild strain/CL50 of the sensitive strain and the coefficient "P" which is the ratio LC90/CL50 of the wild strain, allow ruling respectively on the rate of resistance and the homogeneity of the harvested larval populations (Table 1).

Table 1: Methods of interpretation of the results given by the sensitivity tests according to Sinègre and al [23].

LC50 wild/CL50 KISUMU "K" Coefficient	Wild LC90/Wild LC50 "P" Coefficient	Interpretation
K ≤ 2,5	P≤ 2,5	Homogenous sensitive
	2,5<P≤ 5	Heterogeneous with partial tolerance
	5<P	Heterogeneous with partial resistance
2,5<K≤ 5	P≤ 2,5	Homogenous tolerant
	P> 2,5	Heterogeneous with partial resistance
5<K	P≤ 2,5	Homogenous résistant
	P> 2,5	Résistant Heterogeneous

Results

Phytochemical study

The qualitative analysis revealed the presence of the same chemical groups in the three aqueous extracts of the plants (Table 2). These extracts contain saponins, alkaloids, catechic tannins, and polyphenols including flavonoids. On the other hand, they are devoid of gall tannins, quinones, sterols, and terpenes.

Table 2: Chemical composition of different aqueous plant extracts.

Phytochemical compound	Aqueous plant extract		
	Bougainvillae spectabilis	***Lantana camara***	***Tephrosia villosa***
Saponins	+	+	+
Polyphenols	+	+	+
Flavonoids	+	+	+
Galic tannins	-	-	-
Catechic tannins	+	+	+
Alkaloids	+	+	+
Sterols and terpenes	-	-	-
Quinones	-	-	-

(+): Présent; (-): Absent.

Larvicidal activity of the different plant extracts on the laboratory strain of *Anopheles gambiae* "KISUMU"

Depending on their concentration, the aqueous plant extracts had variable effects on the exposed larvae (Table 3). The exposure of the larvae "KISUMU strain" to the aqueous extracts of *Lantana camara*, *Bougainvillae spectabilis* and *Tephrosia villosa* generated mortality varying from 46.9 ± 2 to 100% at concentrations ranging from 5 mg/ml to 30 mg/ml. The aqueous extracts of *Lantana camara* caused 100% mortality at the minimum concentration of 5 mg/ml while those of *Bougainvillae spectabilis* and *Tephrosia villosa* generated this mortality, respectively, at 15 mg/ml and 25 mg/ml.

Based on ANOVA distribution, larval mortalities varied from plant to plant ($F= 1110.26$; $p<.05$) and from dose to dose ($F= 2,286.8$; $p<.05$). However, paired multiple comparisons showed that larval mortality no longer differs significantly from dose 10 and 15 mg/ml, respectively for *Bougainvillae spectabilis* and *Tephrosia villosa* extracts ($p> 0.05$) regardless of dose (Table 3). *Bougainvillae spectabilis* and *Lantana camara* extracts also generated the same larval mortality rates from dose 15 mg/ml onwards at p>0.05.

Table 3: Mortality percentages of *Anopheles gambiae* Kisumu larvae after 24H of exposure to different concentrations of aqueous plant extracts.

Plant	Concentration (mg/ml)	24hr % Mortality (Mean±SD)	LC50 [UCL–LCL] (mg/ml)	LC90 [UCL–LCL] (mg/ml)
Lantana camara	Control	0±0	1,9 [1,3 - 2,5]	2,6 [2,1 - 4,7]
	5	$100±0^{A, a}$		
	10	$100±0^{A, a}$		
	15	$100±0^{A, a}$		
	20	$100±0^{A, a}$		
	25	$100±0^{A, a}$		
	30	$100±0^{A, a}$		
Bougainvillea spectabilis	Control	0±0	5,2 [5 - 5,4]	8,5 [8,1 - 8,9]
	5	$46,9±2,5^{B, c}$		
	10	$94±1^{A, b}$		
	15	$100±0^{A, a}$		
	20	$100±0^{A, a}$		
	25	$100±0^{A, a}$		
	30	$100±0^{A, a}$		
Tephrosia villosa	Control	0±0	5,3 [4,5- 6,1]	12,3 [11 - 13,9]
	5	$47,5±0,5^{B, d}$		
	10	$83,3±1,6^{B, c}$		
	15	$92,8±1,7^{B, b}$		
	20	$98±1^{B, a}$		
	25	$100±0^{A, a}$		
	30	$100±0^{A, a}$		

Mean ± Standard Error; Mean mortality rates of a plant followed by the same lowercase letter do not differ significantly at $p> 0.05$ (ANOVA test followed by Tukey's HSD test). Mean mortality rates of the same concentration followed by the same uppercase letters do not differ significantly at $p> 0.05$ (ANOVA test followed by Tukey's HSD test). LC50 and LC90: Lethal concentrations able to kill 50 and 90% of larvae, respectively; LCL: Lower confidence limit; UCL: Upper confidence limit;

Larvicidal activity of different plant extracts on the wild strain of *Anopheles gambiae* "TIASSALE"

With the wild strain of *Anopheles gambiae*, the plant extracts had variable effects on the larvae (Table 4). Mortality ranged from 37.8 to 100% for *Bougainvillae spectabilis*, from 57 to 100% for *Lantana camara* and from 9.7 to 57.8% for *Tephrosia villosa*. Mortality of 100% was achieved at 20mg/ml and 30mg/ml for *Bougainvillae spectabilis* and *Lantana camara*, respectively. These mortalities varied significantly according to the extracts ($F=4841.04$; $p<.05$) and between the doses ($F=1390.86$; $p<.05$). Multiple comparisons showed that, *Bougainvillae spectabilis* extract and *Lantana camara* extract generated the same larval mortality rates from a concentration of 10mg/ml at $p> 0.05$. Dose by dose comparisons also showed that mortality was no longer significantly different from 15mg/ml, regardless of the extract $p> 0.05$ (Table 4). The lowest larval LC50 and LC90 were respectively 4.2 mg/ml and 11.1 mg/ml of *Lantana camara* extract, 6.13 and 11.7 mg/ml of *Bougainvillae spectabilis* extract; and 20 and 88.6 mg/ml of *Tephrosia villosa* extract (Table 4).

Table 4: Mortality percentages of wild *Anopheles gambiae* larvae after 24H of exposure to different concentrations of aqueous plant extracts.

Plant	Concentration (mg/ml)	24hr % Mortality (Mean±SD)	LC50 [UCL–LCL] (mg/ml)	LC90 [UCL–LCL] (mg/ml)
Lantana camara	Control	0±0	4,2 [3,5 - 5,1]	11,1 [9,7 - 12,8]
	5	$57±1,5^{A,b}$		
	10	$89,5±2,2^{A,b}$		
	15	$94,9±1,2^{A,a}$		
	20	$98,1±0,9^{A,a}$		
	25	$99±0,9^{A,a}$		
	30	$100±0^{A,a}$		
Bougainvillea spectabilis	Control	0±0	6,13 [5,5 - 6,7]	11,7 [10,7 - 13,2]
	5	$37,8±3,3^{B,b}$		

	10	$79,3\pm2^{B,b}$		
	15	$96,8\pm1,4^{A,a}$		
	20	$100\pm0^{A,a}$		
	25	$100\pm0^{A,a}$		
	30	$100\pm0^{A,a}$		
Tephrosia villosa	Control	0 ± 0	20 [17,5 - 23,4]	88,6 [62,8 – 152]
	5	$9,7\pm3^{C,b}$		
	10	$24,2\pm0,7^{C,b}$		
	15	$50,6\pm1,9^{B,a}$		
	20	$53,5\pm0,5^{B,a}$		
	25	$55,8\pm2^{B,a}$		
	30	$57,8\pm0,7^{B,a}$		

Mean ± Standard Error; Mean mortality rates of a plant followed by the same lowercase letter do not differ significantly at $p > 0.05$ (ANOVA test followed by Tukey's HSD test). Mean mortality rates of the same concentration followed by the same uppercase letters do not differ significantly at $p > 0.05$ (ANOVA test followed by Tukey's HSD test). LC50 and LC90: Lethal concentrations able to kill 50 and 90% of larvae, respectively; LCL: Lower confidence limit; UCL: Upper confidence limit;

Determination of the susceptibility of the wild strain "Tiassale"

The resistance Ratio 50 (RR50) or "K" coefficient of the wild populations was 1.18; 2.21 and 3.77, respectively for *Bougainvillae spectabilis*, *Lantana camara* and *Tephrosia villosa* extracts (Table 5). These values indicate that this strain was susceptible to *Bougainvillae spectabilis*, partially tolerant to *Lantana camara* and resistant to *Tephrosia villosa*. The values of the "P" coefficient of Anopheles from Tiassalé with respect to extracts of *Bougainvillae spectabilis* (1.91), *Lantana camara* (2.64), and *Tephrosia villosa* (4.43) indicate that this wild population of *Anopheles* is homogeneous in its response to exposure to *Bougainvillae spectabilis* but heterogeneous to those of *Lantana camara* and *Tephrosia villosa*.

Table 5: K and P coefficients and interpretation of the sensitivity of the wild type strain to the medium versus the laboratory strain

Plants	K	P	Interpretation
Bougainvillae spectabilis	1,18	1,91	Homogenous sensitive
Lantana camara	2,21	2,64	Heterogeneous with partial tolerance
Tephrosia villosa	3,77	4,43	Heterogeneous with partial resistance

Discussion

The phytochemical screening revealed the presence of various groups of secondary metabolites. Saponins, alkaloids, polyphenols including flavonoids, and catechic tannins were present in the aqueous extracts of the three plants. These results are similar to those obtained from phytochemical studies conducted on aqueous extracts of *Lantana camara* [25], *Bougainvillae spectabilis* [26], and *Tephrosia villosa* [27]. However, other studies revealed the absence of flavonoids in the extract of *Bougainvillae spectabilis* [28] and *Lantana camara* [29]. This study also revealed the absence of quinones, gall tannins, and Sterol and terpenes in the three extracts while the presence of quinone in the aqueous extract of *Lantana camara* was reported [30]. These differences in the composition of the extracts with those of the other studies could be related to the biotope. Sunshine, climate, soil composition, altitude..., could influence the secretions of the essences biochemical from one plant to another.

The results of the larvicidal tests showed that all extracts had a toxic activity on both strains of *Anopheles gambiae*. All extracts caused 100% mortality of *Anopheles gambiae* "KISUMU" larvae. This rate of 100% was also reached with the extracts of *Lantana camara* and *Bougainvillae spectabilis* against the wild strain of *Anopheles gambiae* "TIASSALE". The correlation between concentration and mortality of *Anopheles gambiae* larvae shows that the higher the dose is, the higher the larvicidal activity is. These results are in agreement with those of Koua *et al.* [32] on the effect of the aqueous extract of *Persea americana* on *Anopheles gambiae*. The author states that the mortality of *Anopheles gambiae* larvae increases with the concentration.

With the KISUMU strain, the lethal concentrations (50 and 90) were 1.9 and 2.6 mg/ml for *Lantana camara*, 5.2 and 8.5 mg/ml for *Bougainvillae spectabilis* and 5.3 and 12.3 mg/ml for *Tephrosia villosa*, while those of the wild strains were 4.2 mg/ml and 11.1 mg/ml for *Lantana camara*, 6.13 and 11.7 mg/ml for *Bougainvillae spectabilis* and 20 and 88.6 mg/ml for *Tephrosia villosa*. Studies have shown that aqueous extracts of plant leaves have toxic effects on wild larvae of *Anopheles gambiae*. Notably, *Ocimum gratissimum* and *Solenostemon monostachyus* [31] with LC50 respective of 200 and 232 ppm, *Persea americana* [32] with LC50 of 317.61 µg/ml on the fourth instar larvae. On the other hand, *Lantana camara* also had a larvicidal effect on third instar larvae, with an LC50 of 1.96 g/ml [33]. The ethanolic extract of *Tephrosia villosa* had an LC50 greater than 500 µg/ml on third instar larvae [34].

The results of this study show a low activity of the extracts used compared to those of the others. This difference could be explained by the extraction technique. Furthermore, the larvicidal activity of the plants recorded in this study can be explained by the presence of secondary metabolites revealed by the phytochemical study, which, according to the literature, are highly toxic to mosquito larvae. Indeed, saponosids, tannins, terpenes, flavonoids are endowed with insecticidal properties [35]. For example, saponins can affect the development of insects. Their interaction with cholesterol causes a disruption of the synthesis of ecdysteroids [36]. However, with the concentration of 15 mg/ml, the mortality of the larvae of the wild strain did not vary significantly for all extracts. This indicates that this value is the optimal concentration for all three aqueous extracts.

Among the three extracts, the lowest lethal concentrations were obtained with the extracts of *Lantana camara* and

Bougainvillae spectabilis. This result shows that *Lantana camara* and *Bougainvillae spectabilis* are the most toxic extracts on the larvae on both strains.

The difference in activity of the plant extracts while the phytochemical study reveals the same composition for all of them can be explained by a qualitatively and quantitatively variable presence of metabolites. This variability is influenced by the composition of the soil, the geographical position and the solar radiation on which they are dependent [37]. It could also explain the divergence of the results of this study with those of other authors. Solvents and extraction techniques could also be the cause. Generally, the solubility of secondary metabolites is higher in organic solvents than in water. Mahmoudi *et al*. [38] recorded higher levels of total polyphenols in ethanolic, acetonic and methanolic extracts. Merghem [39] revealed a high solubility of alkaloids in organic solvents compared to water.

The LC50 of the wild strain appeared to be one, two and four times higher than that of the reference Kisumu strain compared to *Bougainvillae spectabilis* (K=1.18), *Lantana camara* (K=2.21) and *Tephrosia villosa* (K= 3.77) respectively. P coefficients above 2.5 show the heterogeneity of wild populations for their response to exposure to *Lantana camara* and *Tephrosia villosa*. In contrast, P values below 2.5 indicate homogeneity of wild larvae in their response to exposure to *Bougainvillae spectabilis*. Thus, taking into account K and P, these results show that the wild larvae were partly tolerant to *Lantana camara* and partly resistant to *Tephrosia villosa*. However, they were fully susceptible to *Bougainvillae spectabilis*.

These results indicate the presence of populations resistant to the aqueous extract of *Tephrosia villosa* in the TIASSALE region. This presence could therefore compromise larval control against *Anopheles gambiae* in this region. Aqueous extracts of *Lantana camara* and *Bougainvillae spectabilis* are the most promising in the quest for an alternative larvicide and should be further explored.

Conclusion

This study confirmed the larvicidal activity of the plants against *Anopheles gambiae*. The results show that the aqueous extract of the leaves of *Lantana camara* and *Bougainvillae spectabilis* could be used in malaria control. The efficacy demonstrated by these plants in the laboratory could be continued in small-scale field trials.

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