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Larvicidal and Synergistic Properties of Fixed Oils Derived from Plants and their Capacity to inhibit Pupation and Emergence of Malaria Vector (*Anopheles gambiae*)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EM and DWW designed the study and wrote the protocol and the first draft of the manuscript. MB, AT, MWG, SZ and AZ collected and managed the data. HM and BBDS performed the statistical analysis; MN and AD supervised, reviewed, and edited the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Malaria vectors have evolved resistance to almost all WHO-recommended insecticides, which compromises vector control. This study aimed to evaluate the insecticidal activity of four doses of fixed oils from *Jatropha curcas* and *Ricinus communis* on *Anopheles gambiae* in western Burkina Faso. Biological tests of susceptibility to oil doses were carried out on two populations of the *An. gambiae* complex from August to October 2022 using the WHO standard protocol. The synergistic or antagonistic effects of the extracts' combinations were evaluated by comparing "sums of effects" to "effects of sums" according to the WHO protocol. R software enabled probit and ANOVA analyses. All oils showed larvicidal activity on susceptible strains of *An. gambiae,* Kisumu. The LC_{50} of the combination of the two oils was lower (54.09±1.03 ppm), followed by the oil of *J. curcas* (58.8±1.03 ppm) and that of *R. communis* (139.0±1.04 ppm) on the field strain of *An. gambiae*. *J. curcas* oil was more toxic on both strains, leading to 100% mortality at 48h and 72h of exposure. Synergistic insecticidal effects after 24h and additive effects after 48h of the combined oil at 50 and 150 ppm resulted in 41.75% to 91.66% mortality of larvae and reduced pupation from 2.66% to 0.00% and reduced the emergence of *An. gambiae* from 1.16 to 0.00%. *J. curcas* and *R. communis'* oils contain linalool, tannins, alkaloids, saponins, and terpenes. Applying *J. curcas* or *R. communis* oil or a combined oil at 50 and 150 ppm as a spray could constitute an effective strategy for integrated control of *An. gambiae* mosquitoes.

Keywords: Mosquito; larvicidal toxicity; synergy; Jatropha curcas; Ricinus communis.

1. INTRODUCTION

Mosquitoes are responsible for spreading several vector-borne diseases such as dengue, lymphatic filariasis, chikungunya, malaria, Japanese encephalitis and yellow fever [1]. The most common and dangerous mosquito-borne diseases include malaria, dengue fever and filariasis. They are caused respectively by *Plasmodium spp*. transmitted by *Anopheles spp*., alphaviruses and flaviviruses by *Aedes spp.*, and *Wuchereria bancrofti* by *Culex spp*. [2,1]. These are among the most common deadly diseases that cause millions of deaths each year around the world including Africa, the Caribbean, Europe, North and South America, and the Middle East [3]. Indeed, among these diseases, malaria, remains one of the deadliest vector-borne diseases. Its transcontinental exposure area includes Asia, Africa, and Latin and Central America, which represents 3.3 billion individuals affected, or approximately 45% of the world's population according to [4]. Children under five are the most vulnerable group affected by malaria; they accounted for 67% of malaria deaths worldwide [4]. Burkina Faso in 2021, 12,227,364 cases of malaria, including 4,867,506 in children, were recorded. The number of deaths linked to this disease was estimated at 4,355, including 2,930 children [5]. Despite control efforts, malaria remains a major public health concern. This parasitosis is notably carried out by *Anopheles gambiae* complex (*An. gambiae s. l.*), *An. funestus*, *An. nili* and *An. mouchetti* [6], with the *An. gambiae s. l.* complex as the major vector of malaria in Burkina Faso [7].

To counter the spread of vectors and therefore epidemics, the fight is essentially based on the use of insecticides. Mass chemoprophylaxis in the case of malaria is difficult for technical, economic, and chemoresistance reasons within Africa human population [8]. Vector control is therefore an essential element of the malaria control strategy. The key tools used in this fight are the pyrethroids based Insecticide-Impregnated Mosquito Nets (ITN) and indoor residual spraying (IRS) [REF]. Although, these current vector control methods are increasingly showing their limits with records of vector resistance development to insecticides and the increasing environmental damage caused by their usage [9-14]. It is therefore imperative to find alternative and/or complementary solutions to these vector control tools [15].

In the face of sustainability challenge arising from the use of synthetic insecticides, tropical plants

offer promising efficacy against vectors due to possession of active substances [16-17]. Numerous studies have reported the use of plant extracts as a bioinsecticide against the *Culex pipiens*, *Anopheles gambiae* and *Aedes aegypti* vectors of parasitic diseases [18-20]. The products from these tropical plants, which are locally available, biodegradable and sustainable, could be utilised as alternative bioinsecticides to these synthetic insecticides. This study focuses on the extracts of *Ricinus communis* L. (Euphorbiaceae), and *Jatropha curcas* L. (Euphorbiaceae), which are plants known for their medicinal and biofuel production properties [21,22,17]. This work aims to evaluate the larvicidal activity, the effects on pupation and emergence, and the synergy of the fixed oils of the grains of *R. communis* and *J. curcas*, on the larvae of *An. gambiae*.

2. MATERIALS AND METHODS

2.1 Study Sites

The study was carried out in two phases at the various sites presented on the map below (Fig 1). The plant collection phase took place in the capital city, Ouagadougou, as well as in Matourkou to the west of the country; the larvae collection phase took place in certain areas of Ouagadougou and Goudrin, a village on the outskirts of the capital.

2.2 Plant Material

The dried fruits of *R. communis* and *J. curcas* were collected in Ouagadougou and Matourkou respectively after identification by a botanical specialist. The fruits of both species were unshelled and from the seeds, oil extraction of *Ricinus* and Jatropha were fixed. To do this, the almonds were cleaned and dried in the shade under a ventilated rack at room temperature (37±2°C) to reduce their moisture content to 14%. One hundred grams of shelled almonds were ground in a Binatone BLG450 MK2 electric grinder from United Kingdom until a powder was obtained. This step was used to break the cell walls to release the fat contained in the almonds. The powders obtained from the almonds of each plant were used for the extraction of oils.

2.3 Extraction of Fixed Oils

The extraction of fixed oils (Fig 3) was carried out by maceration using a 1 L Erlenmeyer glass flask. In the laboratory, one hundred grams (100 g) of each plant powder was macerated in 800 ml hexane extractor with this Erlenmeyer glass flask at room temperature (37±2°C). To speed up the maceration, it was necessary to stir regularly using a mechanical stirrer for 24 hours. After 24 h, the macerate was filtered using cotton and a vacuum cleaner fitted with Whatman No. 1 paper. Each filtrate was separated in a glass flask and concentrated under vacuum on a rotary evaporator. Then it was completely evaporated in an oven at 40 ± 1°C for 6 h to obtain the final solution. This extracted solution was dried using a solvent desiccant to recover the purified fixed oil. The oils thus extracted were packaged in small glass bottles of 25 and 100 ml and stored in the refrigerator at 4°C.

2.4 Phytochemical Screening

The qualitative thin-layer chromatographic profile of the oil samples was established according to the experimental method reported by Wagner *et al.* [23]. A volume of 50 µL of each oil sample was dissolved in 450 µL of toluene. The mixture was homogenized using a vortex mixer. 5 µL of each oil solution was deposited using a microcapillary on a stationary phase of silica gel (G60 F254, Merck). The deposits of oil samples were separated by ascending elution over a 15 cm path, in a mobile phase composed of a mixture of toluene and ethyl acetate of analytical grade (97: 3; v/v). After elution, the chromatography plate was dried at room temperature in the laboratory (25°C), and then in a ventilated oven at 45°C for 15 min. The dried plate was sprayed with the sulfuric vanillin reagent and then placed in an oven preset at 110°C for 5 min. The phytochemicals in the oil deposits appeared in the form of colored spots characteristic of each group of phytochemicals investigated. The different tasks (spots) obtained were related to a given group of compounds according to the colour and the frontal reference (Rf). The frontal reference of the different tasks was determined according to the following formula: Rf = (d. substance) / (d. solvent) < 1; d. substance = distance traveled by the substance or spot; d. solvent = distance traveled by the migration solvent.

2.5 Collection of Larvae

The larvae of *Anopheles gambiae* was collected following the recommendations of the World Health Organization [24] in several neighbourhoods in the city of Ouagadougou (Wemtenga, Karpala, 1200 logements) and in

Goudrin, a village located about thirty km from Ouagadougou. Collection was carried out manually using ladles and buckets, which allowed the larvae to be trapped in their natural breeding sites (Fig 2A). Afterward, the larvae were transferred into 5 L volume cans with moderately closed caps to allow oxygenation of the larvae during transport to the insectarium.

2.6 Mosquito Rearing

In the insectarium the larvae were raised in tanks containing borehole water, covered with pieces of very fine mesh mosquito nets. They were fed with dog kibble throughout the larval stage, and watered with cotton soaked in glucose water as adults. After mating, the eggs hatched into larvae of the F1 generation. Only stage 3 and 4 larvae were used for testing. The breeding (Fig 2B) was maintained at a temperature of 27 ± 2°C, a humidity of 70 to 90% and a photoperiod of 12D - 12N.

2.7 Extracts Dilution

As the fixed oils are insoluble in water, it was necessary to dissolve them in the organic solvent Dimethyl sulfoxyde (DMSO) in order to prepare the stock solutions of the different extracts for laboratory tests. To do this, 20 ml of stock solution was initially prepared, i.e. 2 ml of solution for 18 ml of solvent, according to the [24] protocol. This preparation was kept in a screw cap bottle, with aluminium foil on the top. It was shaken vigorously to dissolve or disperse the material in the solvent. The stock solutions were stored at a temperature of 4°C to avoid their denaturation.

2.8 Larvicidal Test, Monitoring of Pupation and Emergence

The biological tests concerned larvae, pupae and adults of Kisumu or *An. gambiae* (Fig 3). They were carried out at the "Centre National de Recherche et de Formation sur le Paludisme" (CNRFP) in the bioassay room at 27 ± 2°C with a relative humidity of 80 ± 10%. The photoperiod in the room was 12 h of light and 12 h of darkness. Stage 3 and 4 larvae were isolated using a pipette and morphological criteria then sorted (25 larvae per cup) and kept under observation for 30 min. During this time, larvae that showed movement difficulties or an abnormal appearance were replaced.

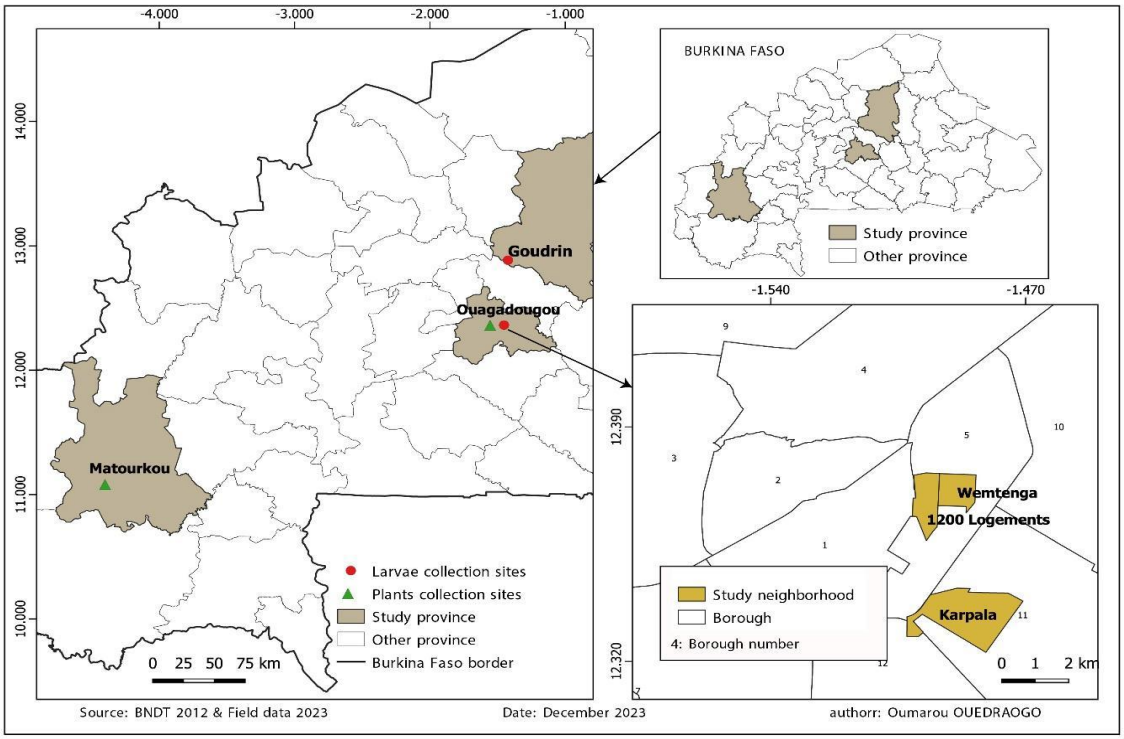

Fig 1. Map of plants and larvae collection sites

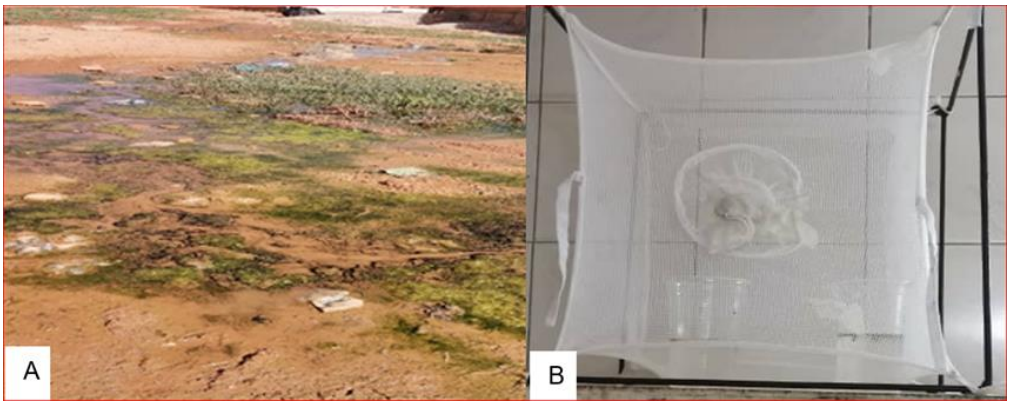

Fig 2. Collection sites (A) and breeding tank for mosquito adults (B)

The tests were carried out in transparent plastic cups with a capacity of 500 ml initially containing 200 ml of drilling water. Then the volume of water equivalent to the volume of stock solution that should be added to each cup according to the concentration was removed, and then finally the appropriate volume of the stock solution of *J. curcas* or *R. communis* oil was added to each cup to obtain the desired final concentration in a total volume of 200 ml.

The different concentrations used were 25, 50, 100, 150, 250, 350, 400 ppm. For each concentration including the control, four replications were used. In the controls, 25 larvae were introduced into each cup containing 249 ml of drilling water and 1 ml of DMSO. Téméphos was used as a positive control. The larvae were monitored for 72 h to observe mortality, pupation and emergence. Dead larvae and moribund larvae (those which only moved slowly after stirring the water) considered dead were counted for the evaluation of larval mortality 24 h, 48 h and 72 h after their contact with the fixed oils. This method was carried out according to the standard protocol of the WHO [24].

Surviving larvae from the larvicidal trial treated with an extract at a concentration of LC_{50} were monitored daily to determine the pupation period and adult emergence rate of the treated larvae. The average Harley index was used to compare the effect of different extracts on larval growth and survival rates [25]. The experiment was repeated three times. To examine the synergistic or antagonistic effects of the extract mixtures, the mean mortality values for the combined treatments was compared to those for the single treatments. The effects are classified as follows:

- Additive if the difference is not significant,
- Synergistic if the effect of the combined extracts is significantly greater than the sum of their separate effects,
- Antagonistic if the effect of the extract combinations is significantly lower than the sum of their separate effects.

2.9 Statistical Analysis

The data obtained from the bioassays of fixed oils were entered using Excel software. When larval mortality between 5 and 20% was noted in the controls, corrected mortality (Mc) was obtained according to the Abbott formula (*F1*) [26] where X is the percentage of observed mortality and Y the percentage of control mortality.

$$Mc = \frac{(X - Y) \times 100}{100 - Y} \qquad (F1)$$

The data obtained were analyzed using R software. The lethal concentrations 50 and 90 (LC_{50} and LC_{90}) which result in 50% or 90% mortality of the larvae were determined with their confidence intervals using the probit logistic regression model. They were expressed in ppm in cases where they could be determined. The different CL values were subjected to an analysis of variance (ANOVA) followed by the determination of the differences existing between the CL_{50} on the one hand and between the CL_{90} on the other hand using Fisher's Least Significant Difference (LSD) test at the significance threshold of 5%. The mortality rate (M) was determined by the number of dead larvae (D), moribund larvae (Mo), and total number of larvae (L) according to the following formula (*F2*).

$$M = \frac{(D + Mo)\, X100}{L} \qquad (F2)$$

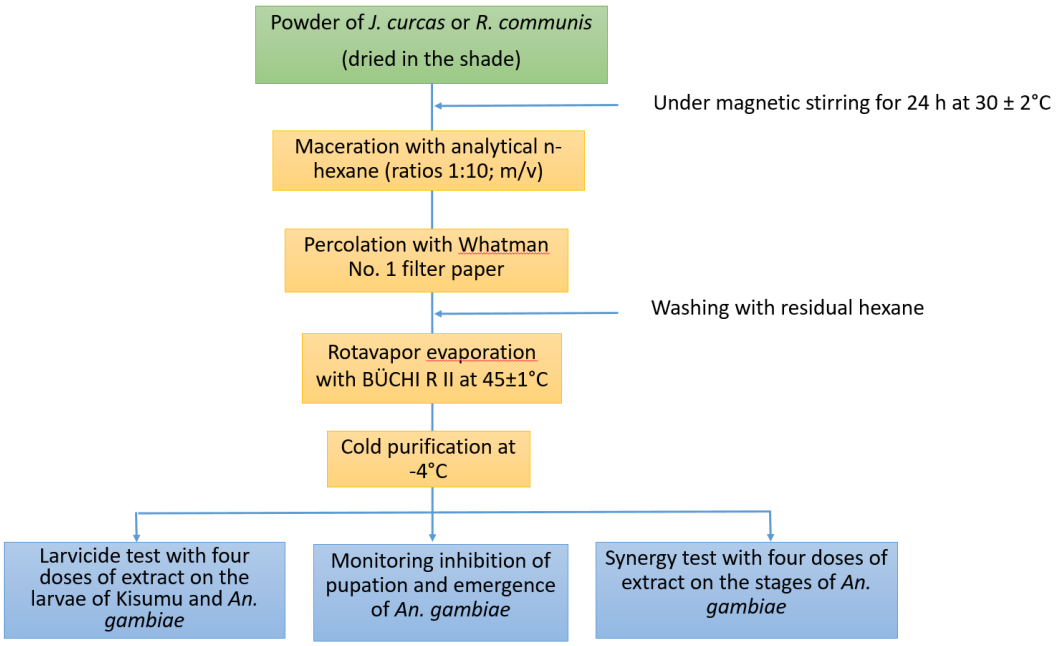

Fig 3. Diagram of the experimental design, starting with the extraction of active substances and continuing with the biological tests on mosquitoes according to the standard protocol of the World Health Organization [24].

3. RESULTS AND DISCUSSION

3.1 The Yield of Fixed Oils

This efficient extraction method reveals that both plants are rich in fixed oils. The extraction yielded more fixed oil from *J. curcas,* richer than *R. communis*. The yield of fixed oil was lower in *R. communis* with an index of 0.40 compared to 0.71 for *J. curcas* (Fig 4).

3.2 Phytochemical Composition of Fixed Oils

Phytochemical analysis showed several compounds involved in insecticidal activity in the mosquito larvae tested. The screening revealed linalool, tannins, alkaloids, saponins, and terpenes in *J. curcas* oil and linalool, tannins, alkaloids, and saponins in *R. communis* oil, but with frontal reference (Fr) variables (Table 1).

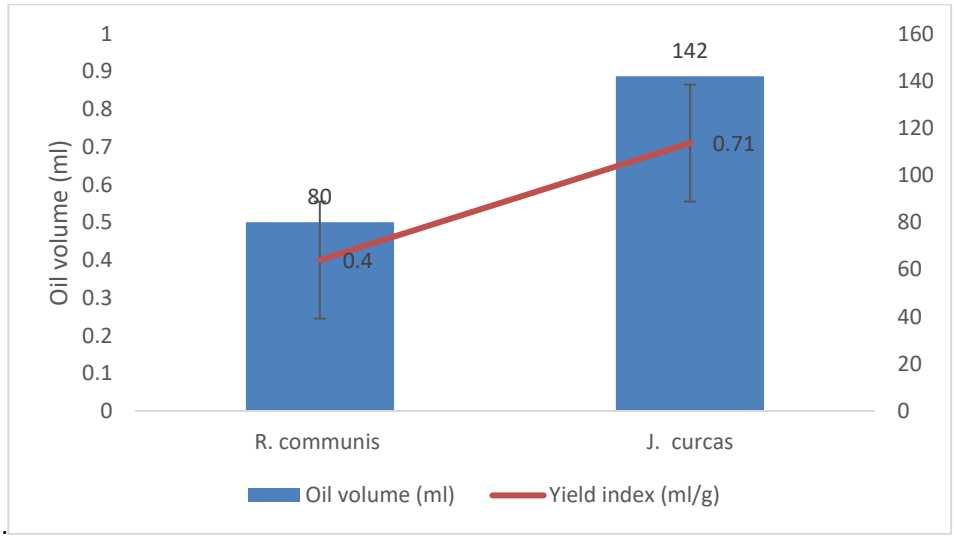

Fig 4. Yield of vegetable fixed oils

Table 1. Phytochemical compounds of *J. curcas* and *R. communis* fixed oils

Oil species	Fr	Color	Observation	Oil species	Fr	Color	Observation
	0.17	blue	Nd		0.06	blue	Nd
	0.30	blue	Linalol		0.17	blue	Linalol
	0.87	blue	Nd		0.43	blue	Nd
J. curcas	0.79	yellow	Tannins	***R. communis***	0.67	yellow	Tannins
	0.77	light yellow	Alkaloids		0.88	light yellow	Alkaloids
	0.27	white-foamed	Saponins		0.15	white-foamed	Saponins
	0.94	blue-purple	Terpènes		0.43	blue	Nd

Fr: frontal reference

Table 2. Lethal concentrations (LC_{50} and LC_{90}), their 95% confidence intervals, and regression parameters of the larvicidal activity of oils and their combination against susceptible strain Kisumu 24 h post-treatment

Oils	LC_{50} (ppm)	LCL_UCL	LC_{90} (ppm)	LCL_UCL	Df	Slope	Ki^2
J. curcas	58.8 ± 1.03	55.0-63.0	137 ± 1.06	124.0-155.0	46	5.98	35.8
R. communis	99.0 ± 1.04	99.0-132.0	343.0 ± 1.06	286.0-434.0	46	4.65	132.0
Combination JC _ RC	48.0 ± 1.04	44.6-51.5	125 ± 1.06	112.0-143.0	46	5.27	35.3

LC: lethal Concentration; LCL: lower confidence limit; UCP: upper confidence limit; df: degree of freedom; SE: standard deviation

Table 3. Lethal concentrations (LC_{50} and LC_{90}), their 95% confidence intervals, and regression parameters of the larvicidal activity of oils and their combination against *An. gambiae* 24 h post-treatment

Oils	LC_{50} (ppm)	LCL_UCL	LC_{90} (ppm)	LCL_UCL	Df	Slope	Ki^2
J. curcas	58.8±1.03	55.0-63.0	137±1.06	124-155	46	5.98	35.8
R. communis	139 ±1.04	123-156	352 ±1.05	304-425	46	5.46	114
Combination RC_JC (1/2)	54.09±1.03	50.9-59.3	126 ±1.06	112-146	46	6.06	57 .6

LC: lethal concentration; LCL: lower confident limit; UCP: upper confident limit; df: degree of freedom

Table 4. Dynamic of larval mortality of susceptible and field strain of *An. gambiae* after 24, 48 and 72 h of exposure

Treatment	Conc.	Kisumu mortality (%)	*An. gambiae* mortality (%)		
		24 h	24 h	48 h	72 h
J. curcas	50ppm	44.00±2.00 ^{bc}	45.43±0.12 ^{b}	77.66±9.61 ^{ab}	100±0.00 ^{a}
	150ppm	97.00±1.00 ^{ab}	93.33±2.2 ^{a}	100±0.00 a	100±0.00 ^{a}
R. communis	50ppm	20.00±1.63 ^{c}	24.5±10.99 ^{c}	46.00±7.77 ^{bc}	64±5.40 ^{ab}
	150ppm	44.00±1.63 ^{bc}	44.9±2.04 ^{b}	70.66±7.03 ^{ab}	100±0.00 ^{a}
Combination Jc_Rc	50ppm	47.33±1.42 ^{bc}	46.7±2.2 ^{b}	73.33±6.22 ^{ab}	100±0.00 ^{a}
	150ppm	97.00±0.71 ^{ab}	95.03±1.19 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
DMSO		0,00 d	0,00 ^{d}	0,00 ^{c}	0,00 ^{b}
Water		0,00 d	0,00 ^{d}	0,00 ^{c}	0,00 ^{b}
Temephos1.25ppm		100,00 ^{a}	100,00 ^{a}	NA	NA
F		163.27	163.27	5.41	4.61
P-value		< 0.001	< 0.001	< 0.001	0.008
Significance		***	***	***	**

*Means in the same column, assigned with the same letter, are not significantly different at 5% threshold (Tukey test); **: highly significant; ***: very highly significant, DMSO: dimethyl sufoxide; Ppm: part per million; Con.: concentration*

Table 5. Synergistic and additive effect of combined oils on larval mortality and pupation and adult emergence of *An. gambiae*

Treatment	24 h Mortality (%)		48 h Mortality (%)		72 h Mortality (%)		Nymphosis (%)	Emergence (%)
	50ppm	150ppm	50ppm	150ppm	50ppm	150ppm	50ppm	50ppm
Sums of effects (ƛ1)	35.91 ^{b}	71.83 ^{b}	78.91 ^{a}	97.58 ^{a}	97 ^{b}	100 ^{a}	2.66 ^{b}	1.16 ^{a}
Effects of sums (ƛ2)	41.75 ^{a}	91.66 ^{a}	82.41 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	0.00 ^{a}	0.00 ^{b}
δ (%)	+ 16,26	+ 27,61	+ 4,44	+ 2,48	+ 3,09	00,00	- 100,00	- 100,00
F	12.10	151.7	1.007	3.59	20.89	1,00	22.00	15.40
P-value	0.002	2.41e-11	0.33	0.07	0.0001	0.33	0.0001	0.0007
Significance	**	***	NS	NS	***	NS	***	***
Insecticidal effect	Synergic	Synergic	Additive	Additive	Synergic	Additive	Synergic	Synergic

*Means in the same column, assigned with the same letter, are not significantly different at 5% threshold (Tukey test); **: highly significant; ***: very highly significant; ppm: parts per million; δ (%): synergistic and additive capacity*

3.3 Susceptibility of Kisumu Larvae to Fixed Oils

All oils and combinations demonstrated larvicidal activity in susceptible strains of *An. gambiae*. This insecticidal activity varied depending on the fixed oils. The lethal concentration 50 (LC_{50}) of the combination of the two oils (*J. curcas* and *R. communis*) was the lowest (48.0 ± 1.04 ppm), followed by the oil of *J. curcas* (58.8 ± 1.03 ppm) and *R. communis* (99.0 ± 1.04 ppm). The LC_{50} therefore varied from 48.0 ppm to 99.0 ppm (Table 2). According to the ANOVA test, *R. communis* oil was the least toxic to this strain.

3.4 Susceptibility of *An. gambiae* Larvae to Fixed Oils

The LC_{50} and LC_{90} values of *R. communis* oils and the combination are slightly higher on *An. gambiae* than on the susceptible ones. However, these values remain invariable for *J. curcas*. The LC_{50}s gradually varied from 54.09 ppm (combination) to 139.0 ppm (*R. communis*) while the LC_{90}s varied from 126 to 352 ppm in the same order (Table 3). These results show a higher activity with the combination of the two oils on *An gambiae* than the two taken separately.

The analysis of variance (Table 4) indicates very significant variations in mortality rates ($Pr < 0.00001$; $F \geq 4.61$). The oils in combination were the most active, with mortality rates of 47.33% on the Kisumu strains 46.7% on the field strain at 50 ppm, and 97% on Kisumu at and 95.3% on *An. gambiae* at 150 ppm. This is followed by *J. curcas* oil, with 44% mortality on Kisumu and 45.43% on *An. gambiae* at 50 ppm after 24 h of exposure and *R. communis* oil at 50 ppm (20% mortality on Kisumu and 24.5% on *An. gambiae*), and at 150 ppm (44% mortality on Kisumu and 44.9% on *An. gambiae*). Total mortalities (100%) of *An. gambiae* larvae were obtained with *J. curcas* oil and that of the combination at 50 ppm in 72 h and 150 ppm in 48 h. With *R. communis* oil, total mortality was only obtained at a concentration of 150 ppm at 72 h.

3.5 Synergistic Activity of Fixed Oils on *An. gambiae*

The analysis indicates synergistic and additive insecticidal effects of the combination of *J. curcas* and *R. communis* oils depending on the exposure time (Table 5). Doses of 50 and 150 ppm caused synergistic effects on the larvae of *An. gambiae* after 24 h of exposure ($ƛ1 < ƛ2$; $P < 0.001$; $F \geq 12.10$) and additive effects after 48 h of exposure ($ƛ1 \approx ƛ2$; $P \geq 0.07$; $F \geq 1.007$) against the larvae of *An. gambiae*. After 24 h, the dose of 150 ppm was more synergistic (δ: + 27.61%), followed by the dose of 50 ppm (δ: + 16.26%), respectively resulting in 91.66% and 41.75% mortality of the larvae of *An. gambiae*. The analysis showed a synergistic effect on pupation ($ƛ1 > ƛ2$; $P < 0.001$; $F: 22.00$) reducing it from 2.66% to 0.00% and on the emergence of adults ($ƛ1 > ƛ2$; $P < 0.001$; $F: 15.40$) which goes from 1.16 to 0.00%.

3.6 Discussion

Linalool, tannins, alkaloids, saponins and terpenoids from the hexanic *J. curcas* and *R. communis* extracts from this study are known for their larvicidal properties against mosquitoes [27-31]. These extracts demonstrated larvicidal properties depending on the plant and the concentration used. The mortalities observed due to *R. communis* oil and the combination reveal lower lethal concentrations (LC_{50}) on the Kisumu strain than on *An. gambiae*. As for *J. curcas* oil, it gave high mortalities on the susceptible strain and the field strain with almost invariable concentrations. Some extracts showed more toxicity than others. Indeed, the hexanic extracts of *J. curcas* and the combination were more toxic againt mosquito larvae than the hexanic extract of *R. communis* on the larvae of the two strains tested. This assumes that these two plants contain different toxic compounds even if in the phytochemical screening we find the same major chemical groups. *J. curcas* oil has low LCs compared to those of *R. communis* on *An. gambiae*. These results, including the LC_{50} value of *J. curcas* being doubly lower than the LC_{50} value of *R. communis*, show that these two oils give very highly significant mortality between them depending on the concentrations. Thus, *J. curcas* is more effective than *R. communis* against the larvae of *An. gambiae*. According to Rahuman *et al.* [32] the extract of *J. curcas* with petroleum ether was particularly active against *Ae. aegypti* (Diptera: Culicidae) (LC_{50} 8.79 ppm, LC_{90} 35.39 ppm) and against *Cx. quinquefasciatus* (LC_{50} 11.34 ppm, LC_{90} 46.52 ppm). These results are largely different from our results from the LC_{50} point of view. However, their LCs are lower than ours regardless of the oil of *J. curcas*, *R. communis,* or their combination. This could be explained by the use of the solvent, which does not extract the

same toxic compounds as hexane. The larvicidal properties of hexanic extracts from several plants have already been demonstrated [33-38]. The authors recorded a high insecticidal effect of the extracts of the leaves of *R. communis* on another harmful insect *Tribolium castaneum (*Coleoptera: Tenebrionidae), with a mortality of 90.9% at a concentration of 2.5% after 24 h of exposure [35]. Similarly, *R. communis* in the larvae of *Aedes albopictus* presented LC_{50} and LC_{90} of the order of 149.58 ppm, 268.93 ppm and 155.58 ppm, 279.93 ppm for the second and third instar larvae respectively after 48 h [39]. Our results of the hexanic extract of *R. communis* seeds after 48 h of treatments are almost similar to the results of [35]. This could be explained by the fact that the extraction of leaves or seeds of *R. communis* gives the same compounds toxic to insect pests.

Regarding the insecticidal activity, *J. curcas* presents a much higher mortality compared to *R. communis* at 150 ppm and in 24 h. *J. curcas* caused a mortality of 97% on Kisumu and 93.33% mortality on the field strain of *An. gambiae.* On the other hand, at the same exposure time *R. communis* caused a mortality of 44% on Kisumu and 44.9% on *An. gambiae*. In 48 h of exposure, still with 150 ppm, *J. curcas* gives 100% mortality on *An. gambiae* and *R. communis* causes 70.66% mortality on *An. gambiae*. In 72 h of exposure to 150 ppm, *R. communis* caused 100% mortality. Rawani *et al.* [33] showed that the non-polar extracts, precisely those obtained with hexane and chloroform, are more toxic to the larvae of *An. gambiae* than the polar extract obtained with ethanol. Hexane extracts of *Cissus populnea*, *Cochlospermum planchonii*, and *Phyllanthus amarus* have high toxicity on the larvae of *An. gambiae*. Their LC_{50} varies between 80 and 180 ppm depending on the part of the plant used [33]. The LC_{50} values obtained in our study were likely to be lower due to a different chemical composition of the plants used. In addition, the method of obtaining the extracts is different since our extracts were obtained with maceration under mechanical stirring with hexane while those of [33] were done by maceration with an initial use of ethanol to extract the compounds. The chemical composition of the extracts is likely to be the reason for the difference in mortality observed. The works of [40] evaluated the insecticidal activity of *R. communis* extracts by contact in *Melanaphis sacchari* (Hemiptera: Aphididae), where they recorded a mortality of 96% after only 72 h. Our experiment with *R. communis* oil after 72 h gave 100% mortality in almost all concentrations except the lowest (50 ppm), however, our results are more effective compared to theirs. This is perhaps due to the difference in the insect species and the mode of contact. In other words, we can hypothesize that *Melanaphis sacchari* is more resistant to the product compared to *An. gambiae*. *R. communis* also exerts identical toxicity in several species of harmful insects such as *Spodoptera frugiperda* (Lepidoptera: Noctuidae) [41], *Atta sexdens rubropilosa* (Hymenoptera Formicidae) [42] and *Cx. quinquefasciatus* (Diptera: Culicidae) [43].

A combination (1/2) of these two oils gave an LC_{50} of 54.9 ppm and an LC_{90} of 126 ppm on *An. gambiae* larvae. The combination showed lower LC_{50} and LC_{90} than each of the two oils taken separately. This shows that there is a highly significant difference in mortality between the combination and these oils taken individually. At 150 ppm and in 24 h, the combined oil caused 97% mortality on Kisumu and 95.03% on *An. gambiae* larvae; in 48 h of exposure the combination resulted in 100% mortality on *An. gambiae*. We only deduce that the combination of these two oils has a synergistic insecticidal effect. A comparison of the effect of the combination of the two oils and the sum of the effects shows that the combination is much more toxic than the sum of the effects. In 24 h of observation we notice that the mortality is highly significant between these oils ($F = 12.1$ and P-value = 0.002). The pupation rate shows a very highly significant difference ($F = 22$; P-value = 0.0001), as does the emergence rate ($F = 15.4$; P-value = 0.0007). From these results, we deduce that there is a synergistic effect in the combination of the two oils in this study.

The insecticidal effect of *J. curcas* extract could originate from the sterols and terpene alcohols it contains [44,45] tested hexanic extracts of *J. curcas* against *Ochlerototatus triseriatu* and *An. gambiae* at a concentration of 250 µg/ml. The results were spectacular, wtih total mortality observed after 24 h [45]. The evaluation of the toxicity of the treatment on the environment was not carried out, nor was the identification of the active substance. The authors have not assessed the environmental impact or identified the active substance. These authors tested the same products on caterpillars of *Helicoverpa virescens* (Lepidoptera: Noctuidae) and *Helicoverpa zea* (Lepidoptera: Noctuidae), which are cotton pests. The extracts were ineffective on

H. virescens but active on *H. zea* with a 60 to 70% reduction in caterpillar weight after 15 days of feeding at a concentration of 250 µg/ml. Some authors have highlighted the active compounds contained in extracts from polar solvents. Indeed, methanolic (polar) extracts of *R. communis* contain alkaloids such as 3-carbonitrile-4-methoxy-N-methyl-2-pyridone and 3-carboxy-4-methoxy-N-methyl-2-pyridone which are very toxic compounds on *An. gambiae s.l.* [46]. Terpenoids appear to exist freely in non-polar extracts while in polar extracts, hydrolysis is required to reveal them. The greater toxicity of hexanic extracts could be explained by the presence of terpenoids, which are compounds obtained mainly with non-polar extracts and whose toxicity against larvae is no longer needed to be demonstrated as they are the majority compounds of essential oils and fixed oils [47,48]. Their toxic effect would be added to that of other compounds, thus leading to higher mortality.

4. CONCLUSION

This work aimed to evaluate the larvicidal activity, the effects on pupation and emergence, and synergies of fixed oils from the grains of *R. communis* and *J. curcas*, on the larvae of *An. gambiae*. The oils and their combination have high larvicidal properties on *An. gambiae*, the malaria vector. *J. curcas* oil, rich in terpenes, is more toxic than that of *R. communis*. The combination of these two oils has a synergistic and additive effect on the larvae. Both oils are rich in chemical compounds involved in the insecticidal activities obtained. The application of *J. curcas* or *R. communis* oil or their combination at 50 and 150 ppm as a spray could constitute an effective strategy for vector control and sanitation of the living environment in an integrated management approach to reduce the proliferation of mosquitoes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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