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Original Article

Adulticidal and post-exposure reproductive effects of crude n-hexane leaf extract of *Ocimum suave* Willd. on *Anopheles gambiae* s.l.

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Abstract

Background: Insecticide resistance increases the need for complementary mosquito-control agents. This study evaluated the adulticidal and post-exposure reproductive effects of a crude n-hexane leaf extract of *Ocimum suave* Willd against field-derived *Anopheles gambiae* s.l.

Methods: The extract was formulated in olive oil at nominal concentrations of 0.05%, 0.25%, 0.75%, 1.00%, 4.00% and 7.50% (v/v) and evaluated using the WHO tube-bioassay framework. Twenty-five non-blood-fed females aged 3-5 days were tested per tube, with four exposure replicates and two olive-oil controls per concentration. A parallel assay evaluated mosquitoes pre-exposed to 4% piperonyl butoxide (PBO).

Results: Extract-only 24-h mortality increased from 24% at 0.05% to 70% at 7.50%, while PBO pre-exposure increased mortality to 56-91% across the corresponding concentrations. All 60-min knockdown values remained below 25%; therefore, KDT50 and KDT95 were not estimable within the observation period. Exposed females produced fewer eggs than control females (1.33 ± 0.44 versus 4.39 ± 0.74 eggs), and a negative-binomial model estimated an incidence-rate ratio of 0.304 (95% CI: 0.147-0.627; p = 0.0013). No hatching was recorded among eggs produced by exposed females.

Conclusion: The concentration-related adult mortality, PBO-associated enhancement and model-supported post-exposure reproductive response support further chemical standardization and vector-control evaluation of *O. suave* crude leaf extract.

Keywords: *Anopheles gambiae s.l.; Ocimum suave; Crude n-hexane extract; Adult mortality; Piperonyl butoxide; Reproductive response*

1. Introduction

Insecticides remain central to the control of malaria vectors and other medically important arthropods. They are deployed through long-lasting insecticidal nets, indoor residual spraying, household aerosols and insecticide-treated materials. Innovations such as insecticide-impregnated fabrics and polymer wall linings have further extended the range of treated surfaces used to reduce contact between people and disease vectors [1,2].

These interventions remain essential components of vector control; however, sustained insecticide use across public health, agriculture and households has increased selection pressure on mosquito populations and contributed to the global expansion of insecticide resistance [3]. In Delta State, commercial aerosols are widely used for household mosquito control [4], and many formulations are dominated by pyrethroids. Repeated and poorly coordinated household use may add to the selection pressure already imposed by public-health and agricultural applications. Although the principal insecticide classes, pyrethroids, carbamates, organo phosphates and organochlorines, remain important, declining efficacy in some vector populations, potential side effects and acquisition costs reinforce the need to develop complementary control options [5,6].

Plant-derived biocides are increasingly investigated as complementary agents because they provide structurally diverse compounds with potential ovicidal, larvicidal, pupicidal, repellent and adulticidal activities. Essential oils, solvent extracts, powders, ashes and growth-regulating preparations have demonstrated activity against mosquitoes and other insect pests [7,8]. Reports of larvicidal activity from plant oils, powders and ashes have established botanicals as a productive source of candidate vector-control agents [9-12]. This search remains relevant to malaria control because the disease continues to impose a substantial burden, particularly in sub-Saharan Africa [13]. The continuing burden, together with the expansion of insecticide resistance, makes it important to examine complementary agents at more than one mosquito life stage. Effective botanical products could broaden the range of tools available for integrated vector management, especially when both their adulticidal activity and their effects on mosquitoes surviving exposure are characterized under controlled conditions. Evaluation of mortality, knockdown and subsequent reproductive performance can therefore provide a more complete account of botanical activity than a single acute endpoint.

Biological mosquito control encompasses bacterial larvicides, entomopathogens, predators, larvivorous fishes, insect growth regulators and plant-derived products [14-16]. Backswimmers, dragonflies and larvi vorous fishes can reduce larval populations, while *Lysinibacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* have been incorporated into larval-control programmes. Larvivorous fishes and insect growth regulators have also produced substantial reductions in immature mosquito populations under experimental conditions [17-20]. Botanical studies have likewise reported toxicity of plant oils, ashes and extracts against mosquito larvae and adults [10-12]. Much of this evidence has focused on larval control, whereas adult contact activity and the subsequent reproductive performance of surviving females remain less extensively documented. *Ocimum suave* is locally available and has demonstrated biological activity in related mosquito studies, supporting its further evaluation as a botanical resource [21,22]. Nevertheless, adulticidal evidence for *O. suave* remains limited, and the reproductive consequences for females surviving adult exposure are poorly understood. The present study therefore assessed the concentration- and time-related adult responses of *An. gambiae* s.l. to crude n-hexane *O. suave* leaf extract, evaluated the influence of PBO pre-exposure, and examined egg production and hatching following adult exposure. By integrating immediate knockdown, 24-h mortality and post-exposure reproductive observations, the study provides a connected assessment of lethal and continuing biological effects.

2. Materials and Methods

2.1 Experimental site

The experiment was conducted in the Exposure Room of the Entomology Unit, Delta State University, Abraka, Nigeria. Mosquitoes were reared in the insectary at 28 ± 2 °C and 80 ± 5% relative humidity under a 12:12 h light: dark photoperiod.

2.2 Plant collection and preparation

Ocimum suave leaves were collected in Aniocha South Local Government Area, Delta State, Nigeria. A taxonomist in the Department of Botany, Delta State University, Abraka, authenticated the plant material. The leaves were collected in clean bags, air-dried at room temperature for 14 days and ground to a fine powder. The powdered material was extracted in the Pharmaceutical Laboratory using an n-hexane Soxhlet procedure [21]. The recovered crude leaf extract was stored in an airtight vial at 4 °C until formulation and bioassay.

2.3 Mosquito collection, rearing and identification

Anopheles larvae were collected during the early morning from puddles, tyre marks, ditches and stagnant-water habitats across Aniocha North and South Local Government Areas. Collections from the different habitats were pooled and transported in clean plastic buckets to the Entomology Unit in sectary. Larvae were maintained in covered rearing trays until emergence. Adults were transferred to holding cages and provided with sugar solution. Females were separated from males using antennal morphology and identified with the key of Coetzee [23]. Because identification was morphological, the mosquitoes are reported as *Anopheles gambiae* sensu lato (s.l.). For each nominal concentration in the main bioassay, 150 females were allocated among six WHO tubes containing 25 mosquitoes each: four exposure tubes and two concurrent control tubes. Females were 3–5 days old and were acclimatized in holding cages for 24 h before testing.

2.4 Adult contact bioassay

The crude n-hexane *O. suave* leaf extract was formulated in extra-virgin olive oil at nominal concentrations of 0.05%, 0.25%, 0.75%, 1.00%, 4.00% and 7.50% (v/v). Three stock formulations were first prepared by mixing 5.0, 25.0 and 75.0 mL of crude extract with 95.0, 75.0 and 25.0 mL of olive oil, respectively, to obtain 5%, 25% and 75% (v/v) stocks. For the lower test concentrations, 1.0 mL of each respective stock was diluted with 99.0 mL of olive oil to obtain 0.05%, 0.25% and 0.75%. The 1.00%, 4.00% and 7.50% formulations were prepared directly by mixing 1.0, 4.0 and 7.5 mL of crude extract with 99.0, 96.0 and 92.5 mL of olive oil, respectively. The concentration calculations are summarized in Table 1 using $C_1V_1 = C_2V_2$. Formulations were applied to 1-mm-thick Whatman filter papers and allowed to stand for 1 h before use. The WHO tube-bioassay procedure was used as the experimental framework [24]. Twenty-five non-blood-fed females were introduced into each holding tube and acclimatized for 1 h before transfer to the corresponding treated-paper tube. Knockdown was recorded at 10, 15, 20, 30, 40, 50 and 60 min. Mosquitoes were then transferred to holding tubes, supplied with sugar solution and assessed for mortality after 24 h. Four exposure replicates were tested per nominal concentration, while mosquitoes exposed to olive-oil-impregnated papers served as controls. No control mortality was observed; therefore, Abbott correction was not required.

Table 1. Stock and test-formulation volumes for crude n-hexane *Ocimum suave* leaf extract in a final volume of 100 mL.

Formulation	Source concentration, C_1 (%)	Source volume, V_1 (mL)	Olive oil (mL)	$C_1V_1 = C_2V_2$
5% stock	100	5.0	95.0	(100 × 5.0)/100 = 5%
25% stock	100	25.0	75.0	(100 × 25.0)/100 = 25%
75% stock	100	75.0	25.0	(100 × 75.0)/100 = 75%
0.05% test	5	1.0	99.0	(5 × 1.0)/100 = 0.05%
0.25% test	25	1.0	99.0	(25 × 1.0)/100 = 0.25%
0.75% test	75	1.0	99.0	(75 × 1.0)/100 = 0.75%
1.00% test	100	1.0	99.0	(100 × 1.0)/100 = 1.00%
4.00% test	100	4.0	96.0	(100 × 4.0)/100 = 4.00%
7.50% test	100	7.5	92.5	(100 × 7.5)/100 = 7.50%

C_1 = source concentration; V_1 = source volume; C_2 = final nominal concentration; V_2 = final formulation volume (100 mL).

2.5 PBO synergist assay

For the synergist assay, mosquitoes were pre-exposed for 1 h to filter paper containing 4% piperonyl butoxide (PBO) and were then transferred to papers treated with the corresponding nominal *O. suave* extract concentration. Knockdown was recorded at the same intervals for 60 min, and mortality was assessed after 24 h. A separate PBO-only group was not included; consequently, the comparison evaluated the PBO-associated change in response to the botanical extract rather than an independent effect of PBO.

2.6 Post-exposure reproductive assessment

Two exposure replicates were designated for the post-exposure reproductive assessment after the 24-h mortality observation. Twenty males and twenty surviving females were transferred to holding cages, maintained on 10% sugar solution for two days and allowed to mate. Females were subsequently offered human blood on cotton wool on two consecutive nights. Each fully engorged female selected for the egg-count assessment was introduced separately into one prepared oviposition tube containing moist cotton wool. Each female–oviposition-tube combination constituted one independent experimental unit. The final dataset comprised 18 exposed females, three at each of the six nominal concentrations, and 18 independent control females. Eggs from each female were counted and monitored for hatching, and the same procedure was applied to the control group.

2.7 Data analysis

Data from the mortality, knockdown and reproductive assessments were entered into Microsoft Excel 2016. Results are presented as percentages, means and standard errors. Twenty-four-hour mortality comparisons were evaluated using the reported analysis of variance at a significance level of $p < 0.05$ in PAST Statistical Software version 4.06b. Knockdown was recorded repeatedly at pre-specified intervals in the same exposure tubes and is therefore presented descriptively as mean ± SE per tube for each treatment and time point; successive observations from the same tubes were not treated as independent biological replicates. Because 50% knockdown was not observed within 60 min in any group, KDT50 and KDT95 were treated as not estimable and were not extrapolated beyond the observation period. Egg counts from 18 independent exposed females and 18 independent control females were analysed using a negative-binomial generalized linear model with a log link because the count variance exceeded the mean. Exposure status was the explanatory variable, and results are reported as an incidence-rate ratio (IRR) with a 95% confidence interval. The negative-binomial model was fitted by maximum likelihood using Python 3.12 and SciPy 1.17. Hatching is reported as the observed outcome under the experimental conditions.

3. Results

3.1 Adult mortality response of *Anopheles gambiae* s.l.

Twenty-four-hour mortality increased with nominal extract concentration (Table 2). Extract-only mortality was 24%, 29%, 45%, 56%, 61% and 70% at 0.05%, 0.25%, 0.75%, 1.00%, 4.00% and 7.50%, respectively. PBO pre-exposure enhanced mortality at every corresponding concentration, producing values of 56%, 67%, 80%, 82%, 85% and 91%. These changes represent absolute mortality increases of 32, 38, 35, 26, 24 and 21 percentage points, respectively. The highest mortality was therefore observed in the PBO + 7.50% extract group. The reported analysis indicated a difference between the extract-only and PBO-pre-exposed mortality responses ($p = 0.009$).

Table 2: Twenty-four-hour mortality of *Anopheles gambiae* s.l. exposed to nominal concentrations of crude n-hexane *Ocimum suave* leaf extract, with and without 4% PBO pre-exposure.

Nominal extract concentration (%)	Extract-only mortality (%)	PBO + extract mortality (%)	Mortality increase (percentage points)
0.05	24	56	32
0.25	29	67	38
0.75	45	80	35
1.00	56	82	26
4.00	61	85	24
7.50	70	91	21

Four exposure replicates were tested per nominal concentration. Control mortality was zero. PBO = piperonyl butoxide.

3.2 Knockdown response over 60 min

Knockdown generally increased with observation time, but the pattern varied among concentrations and between extract-only and PBO-pre-exposed groups (Table 3). At 0.05%, extract-only knockdown increased from 0 at 10 min to 1.33 ± 0.67 mosquitoes per tube at 60 min, while the corresponding PBO group increased to 4.67 ± 1.20. The 0.25% extract-only group remained at 0.67 ± 0.33 from 10 to 60 min, whereas the PBO + 0.25% group recorded no knockdown throughout the observation period. At 0.75%, knockdown became evident after 30 min and reached 4.00 ± 1.00 for extract alone and 3.33 ± 0.88 after PBO pre-exposure. The 1.00% extract-only treatment produced the highest early knockdown, rising from 2.67 ± 0.88 at 10 min to 6.00 ± 1.53 at 60 min.

At 4.00% and 7.50%, the strongest 60-min response was 6.00 ± 1.53 in the PBO-pre-exposed groups. These treatment-specific trajectories describe the development of knockdown during the 60-min exposure.Figures 1 and 2 present the mean percentage knockdown trajectories with standard-error bars. All groups remained below 25% knockdown at 60 min. Extract-only knockdown was highest at 1.00% (24% at 60 min), while the lowest 60-min response occurred at 0.25% (approximately 3%). Following PBO pre-exposure, the highest 60-min knockdown values were recorded at 4.00% and 7.50% (24%). The concentration-specific variation in the PBO series shows that rapid knockdown and 24-h mortality were distinct components of the observed adult response.

Table 3: Knockdown over time of *Anopheles gambiae* s.l. exposed to nominal concentrations of crude n-hexane *Ocimum suave* leaf extract, with and without 4% PBO pre-exposure

		Knockdown time (minutes)						
Treatment	**Conc. (%)**	**10**	**15**	**20**	**30**	**40**	**50**	**60**
O. suave	0.05	0.00 ± 0.00	0.67 ± 0.33	0.67 ± 0.33	0.67 ± 0.33	1.33 ± 0.67	1.33 ± 0.67	1.33 ± 0.67
PBO+ *O. suave*	4+0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 0.33	2.67 ± 0.88	4.00 ± 1.00	4.67 ± 1.20
O. suave	0.25	0.67 ± 0.33	0.67 ± 0.33	0.67 ± 0.33	0.67 ± 0.33	0.67 ± 0.33	0.67 ± 0.33	0.67 ± 0.33
PBO+ *O. suave*	4+0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
O. suave	0.75	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 1.00	4.00 ± 1.00	4.00 ± 1.00
PBO+ *O. suave*	4+0.75	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.33 ± 0.67	1.33 ± 0.67	3.33 ± 0.88
O. suave	1.00	2.67 ± 0.88	2.67 ± 0.88	3.33 ± 1.20	4.67 ± 1.20	5.33 ± 1.33	6.00 ± 1.53	6.00 ± 1.53
PBO+ *O. suave*	4+1.00	0.67 ± 0.33	1.67 ± 0.67	1.67 ± 0.67	1.67 ± 0.67	2.00 ± 1.00	2.33 ± 1.33	2.33 ± 1.33
O. suave	4.00	2.00 ± 0.58	2.00 ± 0.58	3.33 ± 0.88	4.00 ± 1.00	4.67 ± 1.20	4.67 ± 1.20	4.67 ± 1.20
PBO+ *O. suave*	4+4.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.58	3.33 ± 0.88	3.33 ± 0.88	6.00 ± 1.53
O. suave	7.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.75 ± 0.25	1.75 ± 0.25	2.25 ± 0.48	2.75 ± 0.75
PBO+ *O. suave*	4+7.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.67 ± 1.20	3.33 ± 0.88	2.25 ± 0.48	6.00 ± 1.53

Values are descriptive mean ± SE per 25-mosquito tube at each observation time. PBO = piperonyl butoxide.

3.3 Post-exposure reproductive response

Adult exposure to the *O. suave* extract was followed by lower egg production (Table 4). Exposed females produced a mean of 1.33 ± 0.44 eggs, compared with 4.39 ± 0.74 eggs among the independent control females. The negative-binomial model estimated an IRR of 0.304 (95% CI: 0.147–0.627; Wald z = −3.22; p = 0.0013), corresponding to an estimated 69.6% lower egg-production rate following adult exposure. Egg production declined across the displayed concentration groups, and no hatching was recorded among eggs produced by exposed females. In contrast, hatching was observed in the control group under the same rearing conditions. These findings demonstrate a marked post-exposure reproductive response among females surviving the adult contact assay.

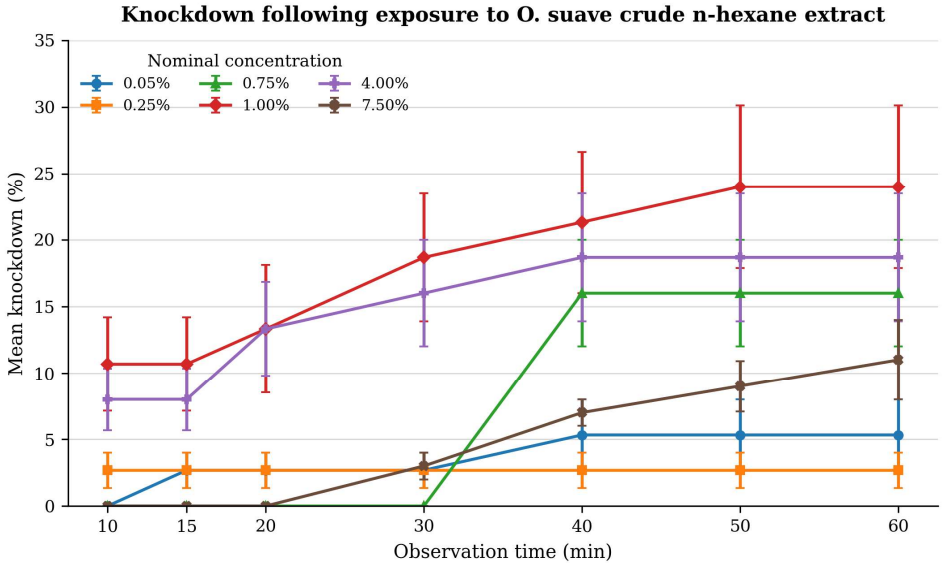

Figure 1. *Descriptive mean percentage knockdown (± SE) of adult Anopheles gambiae s.l. exposed to nominal concentrations of crude n-hexane Ocimum suave leaf extract.*

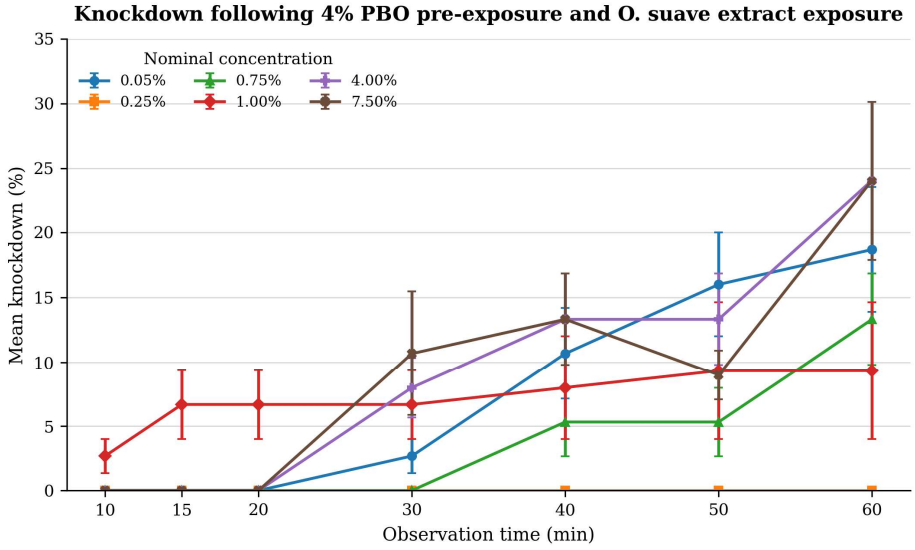

Figure 2. Descriptive mean percentage knockdown (± SE) of adult Anopheles gambiae s.l. following 4% PBO pre-exposure and exposure to nominal concentrations of crude n-hexane Ocimum suave leaf extract.

Table 4: Post-exposure egg production of *Anopheles gambiae* s.l. following adult exposure to nominal concentrations of crude n-hexane *Ocimum suave* leaf extract.

Independent female/tube no.	Pooled control egg count	Nominal treatment concentration (%)	Exposed-group egg count
1	6	0.05	6
2	9	0.05	4
3	10	0.05	3
4	3	0.25	3
5	3	0.25	3
6	2	0.25	3
7	4	0.75	0
8	6	0.75	0
9	5	0.75	2
10	10	1.00	0
11	7	1.00	0
12	3	1.00	0
13	4	4.00	0
14	4	4.00	0
15	3	4.00	0
16	0	7.50	0
17	0	7.50	0
18	0	7.50	0
Mean ± SE	*4.39 ±0.74*		*1.33 ± 0.44*

Each row represents one independently housed female–oviposition-tube unit. Exposed observations comprised three females per nominal concentration (n = 18); the independent control females (n = 18) were pooled for the overall comparison and are displayed alongside the concentration rows for presentation only, not as paired observations.

4. Discussion

This study demonstrates that crude n-hexane *O. suave* leaf extract exerts measurable adulticidal and post-exposure reproductive effects on *An. gambiae* s.l. The experimental design extends botanical-vector research beyond acute mortality by integrating 60-min knockdown, 24-h mortality, PBO pre-exposure and subsequent egg production and hatching. Such an integrated approach is valuable because botanical products may influence mosquito populations through both lethal and post-exposure pathways. The combined endpoints are a particular strength of the work: mortality establishes adult

contact activity, the knockdown series describes the development of the response during exposure, and the egg data show that the biological effect continued among surviving females. This broader experimental perspective complements the substantial literature on botanical larvicides and supports the continuing evaluation of plant-derived products within integrated vector-management strategies [10-12,25]. It also provides a practical basis for selecting concentrations and endpoints for subsequent laboratory and semi-field evaluation of *O. suave*.

The increase in extract-only mortality from 24% to 70% across the nominal concentration range demonstrates a clear concentration-related adult response. The graded increase across six concentrations, together with zero mortality in the olive-oil controls, supports the contact activity of the crude extract under the study conditions. The use of extract-impregnated filter papers within the WHO tube-bioassay framework also provided a consistent surface for comparing the nominal formulations. The progressive increase at higher concentrations provides a rational basis for formulation optimization and isolation of the fractions contributing to activity. Related botanical studies have similarly shown that adulticidal efficacy depends on concentration, mosquito population, extraction method and chemical composition [26-28]. The present findings therefore add evidence for the adulticidal potential of *O. suave*, a plant more commonly investigated for effects on immature mosquito stages, and extend previous work on the biological activity of *Ocimum*-derived preparations.

PBO pre-exposure increased 24-h mortality at every corresponding concentration, with absolute enhancements of 21–38 percentage points and a maximum mortality of 91% in the PBO + 7.50% group. The consistency of the 24-h mortality enhancement across the full concentration series is biologically informative and suggests that PBO-sensitive detoxification pathways may influence mosquito tolerance to constituents of the crude extract. PBO has been widely used to examine oxidase-associated detoxification in mosquito populations exposed to conventional insecticides [29-30]. In the present study, the PBO-associated increase provides direction for targeted biochemical and molecular confirmation while the observed mortality values remain the primary evidence. Importantly, PBO did not uniformly increase rapid knockdown. This concentration-specific pattern shows that the 60-min knockdown and 24-h mortality endpoints captured different components of the adult response and reinforces the value of measuring both endpoints. Because a separate PBO-only group was not included, the comparison is interpreted specifically as PBO-associated enhancement of the extract response rather than as an independent effect of PBO.

Knockdown remained below 25% during the 60-min exposure, whereas mortality continued to develop over the subsequent 24 h. This pattern suggests that the crude extract acted more strongly through delayed toxicity than through immediate incapacitation. Because 50% knockdown was not reached in any group, KDT50 and KDT95 were not estimable within the observation period and were not extrapolated. Time-dependent response is important in vector-control research because rapid incapacitation and delayed mortality need not arise at the same rate or contribute equally to overall efficacy. The comparatively strong 24-h mortality in the presence of modest 60-min knockdown identifies delayed toxicity as a prominent feature of the preparation. The distinction between rapid knockdown and delayed mortality therefore adds useful biological information and provides clear targets for formulation development, particularly where improved delivery, chemical standardization or enrichment of active fractions may accelerate contact toxicity while retaining the observed delayed effect.

The reproductive observations provide an additional and notable outcome. Females that survived adult exposure produced fewer eggs than independent control females, and none of the eggs recorded from exposed groups hatched. The negative-binomial analysis strengthened this observation by estimating a 69.6% lower egg-production rate after exposure (IRR = 0.304, 95% CI: 0.147–0.627). Because the extract was applied to adults rather than directly to the oviposition substrate or eggs, these findings are appropriately interpreted as post-exposure effects on reproductive performance and egg viability, rather than as oviposition deterrence or direct ovicidal activity. This distinction identifies a continuing biological effect following adult contact with the extract. A botanical preparation that affects both adult survival and the reproductive output of survivors may have greater population-level relevance than would be indicated by mortality alone. The model-supported egg-count difference, together with the absence of recorded hatching in the exposed group, makes this endpoint a strong priority for confirmatory study. The combined mortality and reproductive responses strengthen the biological relevance of the extract and justify focused studies using chemically characterized preparations and standardized laboratory and semi-field designs.

5. Conclusion

Crude n-hexane *O. suave* leaf extract produced concentration-related adult mortality in *An. gambiae* s.l., and PBO pre-exposure consistently enhanced the 24-h mortality response. Adult exposure was also followed by reduced egg production and an absence of recorded hatching among eggs from exposed females. Together, these findings identify *O. suave* as a promising botanical source for adulticidal and post-exposure reproductive effects. Chemical standardization, optimized formulation and confirmatory laboratory and semi-field studies are warranted to advance its potential contribution to integrated mosquito management.

Declarations

Consent for publication: The author, declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by him.

Consent to Participate: Not applicable.

Conflict of Interest: The authors declare no complicit of interest

Data availability statement: All data generated from this study is present in this report, any further information required are available from the corresponding author on reasonable request.

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Ethics: The Faculty of Science Ethical Review Committee, Delta State University, approved the study (REL/FOS/2023/12).

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