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Original Research Article

Toxicity and Histopathological Effects of *Lipia alba* Essential Oil on Late Instar Larvae of *Anopheles gambias SL* (Diptera: Culicidae)

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Abstract

In vector control, plant extracts are increasingly provided numerous sources of phytochemicals utilized on mosquito control. Essential oils such as *Lippia alba* have shown their effectiveness against insects. Our present study aims to show the toxicity of *L. alba* essential oil on *Anopheles gambiae* larvae and to demonstrate the histological damage. The larvae were exposed to serial concentrations from 200 ppm to 1000 ppm. Mortalities were recorded after 24 hours exposure to determine lethal doses LD_{50} and LD_{90}. Larvae treated with LD_{90} were fixed at 6h, 12h, and 24h to show the process of histological degradation. After 24 hours exposure, the results revealed that mortalities were 6.66%, 5%, 61.33%, 91.66%, and 91.66% for respectively 200, 400, 600, 800, and 1000 ppm doses. Fisher's test revealed that there was no significant difference in mortality between the control and low doses (200 ppm and 400 ppm), ($p = 1$). On the other hand, mortalities were significant between the control (0 ppm) and doses ≥ 600 ppm ($p = 0.0006$). The lethal doses LD_{50} and LD_{90} determined using the Muller and Tinter formula were 554.4 ppm and 788.2 ppm, respectively. The histological examnition revealed that, the product acts between 6h and 24h through with progressive destruction of the nervous system, muscle tissue, adipose tissue, and digestive tract. It appears that *L. alba* essential oil constitutes a product with a larvicidal effect and could be evaluated in a natural breeding sites against vector mosquitoes.

Keywords: Histopathology, *Lipia alba, Anopheles gambiae*.

INTRODUCTION

Mosquito vectors are of great medical importance worldwide due to the several diseases they cause that lead to serious health problems in humans, particularly in Africa. For malaria, the main vector species are *Anopheles arabiensis*, *An. gambiae* s.s., and *Anopheles coluzzii* of the Gambiae complex, and *Anopheles funestus* of the Funestus group (Sinka *et al*., 2010, 2012). Among these species, *Anopheles gambiae* s.l. is one of the primary vectors of malaria parasites in Africa. In Senegal, the transmission of Plasmodium by mosquito vectors contributes to the emergence of endemic zones. However, thanks to WHO programs, certain regions have shown pre-elimination trends for malaria, particularly in the department of Saint-Louis in Senegal (Furtado, 2018). Nevertheless, recent investigations in this area have revealed a resurgence of malaria, accompanied by a significant presence of Anopheles larvae breeding sites, which could increase the risk of transmission.

In vector control, conventional synthetic insecticides are widely used against adult and larval mosquitoes. However, the drawbacks of chemical products, such as resistance (Chen *et al*., 2013; Aikpon *et al*., 2020) and environmental pollution, have led to increased interest in natural products as an alternative. Among these products, fungi (Scholte *et al*., 2004; Seye *et al*., 2012; Bawin *et al*., 2016) and bacteria (Baumann *et al*., 1991; Dhang *et al*., 2009) have demonstrated effectiveness against mosquito vectors. Plant extracts have also shown promise, with various studies highlighting their efficacy against mosquito vectors (Seye *et al*., 2006; Amer and Mehlhorn, 2006; Bagavan and Rahuman, 2011; Seye *et al,* 2012, Rajasekar *et al*., 2015; Foko and Nyegue, 2016; Nkouandou *et al*., 2020). Botanical derivatives tend to pose fewer environmental

risks. Essential oils (EO), in particular, have proven effective against adult mosquitoes (Mahanta *et al*., 2019) and larvae (Seye *et al*., 2021). The essential oil of *L. alba*, known for its antiviral, antifungal, and antibacterial properties (Rahmatillah *et al*., 2011), has been shown to be effective against *Aedes aegypti* larvae (Santiago *et al*., 2006); *Anopheles gambiae* and *Aedes aegypti* adult (Coulibaly *et al*., 2024). Despite the diversity of these natural products, their mode of action varies depending on the developmental stage of the mosquitoes and the type of product used.

The objective of this study was to evaluate the efficacy of *Lippia alba* essential oil (EO) against *Anopheles gambiae* larvae collected from natural breeding sites in the department of Saint-Louis, and to assess the associated histological damage under laboratory conditions

MATERIALS AND METHODS

Mosquito larvae collection

Wild *Anopheles gambiae* larvae were collected from suitable breeding sites in the commune of Gandon, department of Saint-Louis, Senegal. The breeding site is a pond located at Diougop (Latitude: 16°2'54"N, Longitude: 16°25'12"W). Larvae were collected during the rainy season in September 2024 and transported to the laboratory of UFRS 2S at Gaston Berger University, sorted, and third- and fourth-stage larvae (3^{rd} and 4^{th}) were selected for testing. They were fed bread crumbs to sustain them during the experiment.

Essential oil (EO)

The *Lippia alba* essential oil (EO) used in this study was provided by the laboratory of UFR S2ATA at Gaston Berger University. The EO was extracted from the leaves via steam distillation at the Initiative Research Pole in Food Biotechnology and Support for Competitiveness (I_PREBAAC). The extracted EO was stored in a bottle at 4°C to preserve its properties for subsequent testing

Bioassay

The toxicity tests were conducted following a methodology inspired to the World Health Organization protocol (WHO, 2005). Six 250 mL jars were used under laboratory conditions (27.8 ± 2.2 °C and 75 ± 1.5% RH). Each jar contained 100 mL of tap water and 20 larvae. The larvae in the first five jars were exposed to *Lippia alba* essential oil concentrations of 200, 400, 600, 800, and 1000 ppm, with 0.1% Tween 80 added to each jar. The control group received only 0.1% Tween 80. Mortality was recorded after 24 hours. Five replicates were performed for each concentration and the control. Larval mortality was corrected using Abbott's formula (Abbott, 1925). The LD_{90} was determined and applied to advanced-stage larvae under the same conditions. For histological analysis, untreated and larvae treated with LD_{90} were randomly collected after 6, 12, and 24 hours of exposure, fixed in Carnoy's solution, and stored in 50 mL jars

Statistical analysis

Data were collected and analyzed using Excel 2016 and R software (version 4.4.3). The package was used for data structuring, and basic functions were employed for statistical tests. Fisher's exact test was applied to contingency tables in pairs to compare mortality proportions between different dose levels. To control the risk of type I error due to multiple testing, a Bonferroni correction was applied to the p-values obtained. The significance threshold was set at $\alpha = 0.05$ for all analyses. The lethal doses (LD_{50} and LD_{90}) were calculated using the Miller and Tainter formula

Histology

After 6, 12, and 24 hours of exposure to the LD_{90} of *Lippia alba* essential oil, the larvae were fixed in Carnoy's solution for at least 48 hours. Dehydration was performed using a Leica ASP300S automated processor with successive baths of formalin, 90%, 95%, and 100% alcohol, followed by clearing with xylene and impregnation with paraffin. The samples were then embedded in paraffin blocks using a Leica Tissue-Tek TEC embedding station. Sections of 3 µm thickness were cut using a Leica RM2245 semi-automatic microtome. The sections were stained with Hematoxylin-Eosin (HE) using a Tissue-Tek DRS automated stainer. Observations were made with a BioBlue optical microscope.

RESULTS AND DISCUSSION

Biocidal effects of *Lippia alba* essential oil on advanced-stage (3^{rd} and 4^{th}) larvae of *Anopheles gambiae* s.l.

The results demonstrated the toxicity of *Lippia alba* essential oil against Anopheles larvae within 24 hours (Figure 1). The mortality rate was dose-dependent, with 0% mortality in the control group and 91.66% at the highest dose of 1000 ppm. Low concentrations (200 and 400 ppm) resulted in low mortality rates (6.66% and 5%, respectively), while a significant increase in mortality (63.33%) was observed onl at 600 ppm.

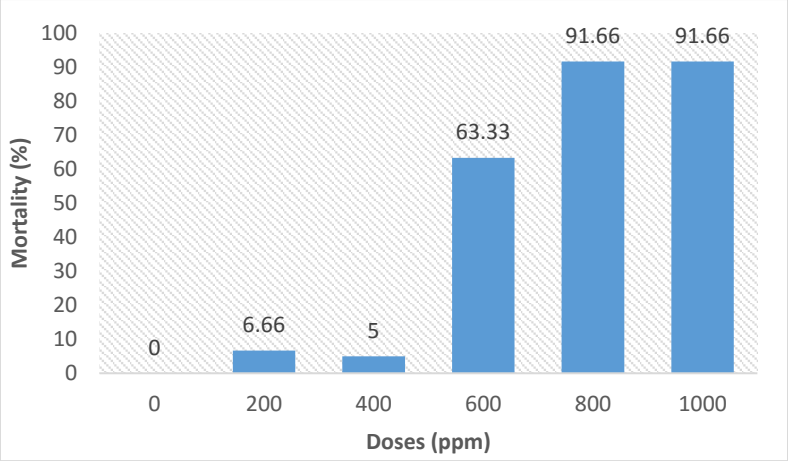

Figure 1: Variation in cumulative mortality rate of late-stage *Anopheles gambiae* larvae treated with *Lippia alba* essential oil after 24 hours of exposure

Fisher's exact test revealed significant differences in mortality between the control (0 ppm) and doses ≥ 600 ppm ($p = 0.0006$). Similarly, comparisons between low doses (200 ppm and 400 ppm) and doses ≥ 600 ppm showed significant differences in mortality ($p = 0.003$ and $p = 0.0006$, respectively). In contrast, no significant differences were observed between the control and low doses (200 ppm and 400 ppm; $p = 1$) or between high doses (600 ppm vs. 800 ppm, 600 ppm vs. 1000 ppm, and 800 ppm vs. 1000 ppm; $p = 1$). The LD_{50} and LD_{90}, calculated using the Miller and Tainter formula, were 554.4 ppm and 788.2 ppm, respectively.

Histological effects:

Histological analysis of *Anopheles gambiae* larvae exposed to the LD_{90} (788.2 ppm) of *Lippia alba* essential oil for 6, 12, and 24 hours revealed progressive degradation of various larval structures.

Anterior part (Head and thorax):

Microscopic observations revealed a near-total disorganization of *Anopheles gambiae* larvae's internal structures 24 hours after exposure to *Lippia alba* essential oil, compared to the control (Figure 2). Longitudinal sections showed that the essential oil caused extensive damage to the digestive tube, nervous system, muscle tissue, and adipose tissue. Specifically, the digestive tube's anterior components (esophagus and gastric caeca) were severely degraded (Figure 2). The nervous system and muscle tissue were also destroyed, highlighting the oil's potent larvicidal effects.

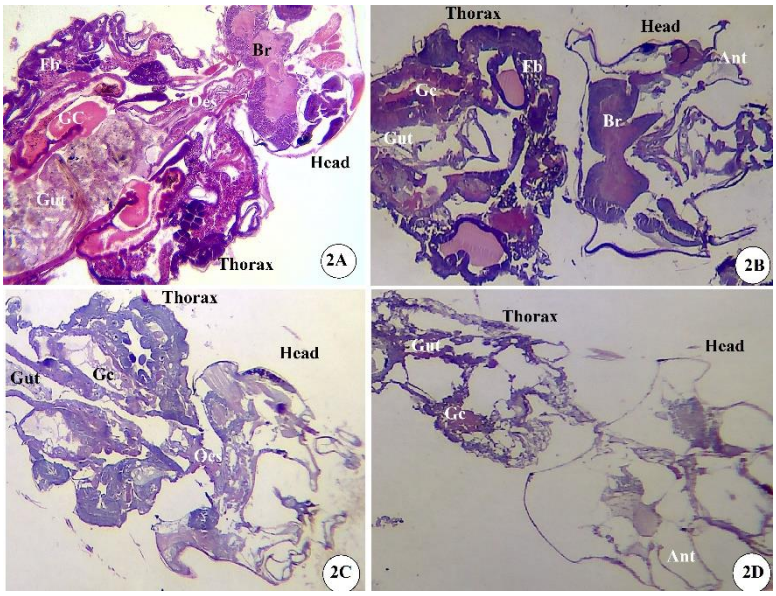

Figure 2: Longitudinal sections showing evolution of the anterior structures of *Anopheles gambiae* larvae untreated (Figure 2A) and treated with essential oil (EO) at LD_{90} (788.2 ppm) after 6h (Figure 2B), 12h (Figure 2C), and 24h (Figure 2D) exposure. stained with hematoxyline and eosin (HE) X100. Abreviation: Brain (Br), Oesophagus (Oes), gastric caeca (Gc), Fat body (fb), Antena (Ant)

Compared to the control larva (Figure 2A), longitudinal sections of treated larvae showed progressive disorganization of internal structures. After 6 hours of exposure (Figure 2B), initial damage was observed, although it was less pronounced. However, with time, the damage intensified: the nervous system (brain and cerebral ganglion) was partially disorganized after 12 hours (Figure 2C) and completely degraded after 24 hours (Figure 2D). Similarly, the gastric caeca and intestinal cells became disorganized after 12 hours and were completely degraded after 24 hours. The adipose tissue, visible 6 hours after exposure, began to disappear after 12 hours and was completely desorganized after 24 hours.

At the abdominal level

Following exposure to LD_{90} (788.2 ppm) of *Lipia alba* EO, longitudinal section of the *An. gambia* larvae showed progressive disruption of the midgut through destruction of cells between 6h and 24h (Figure 3).

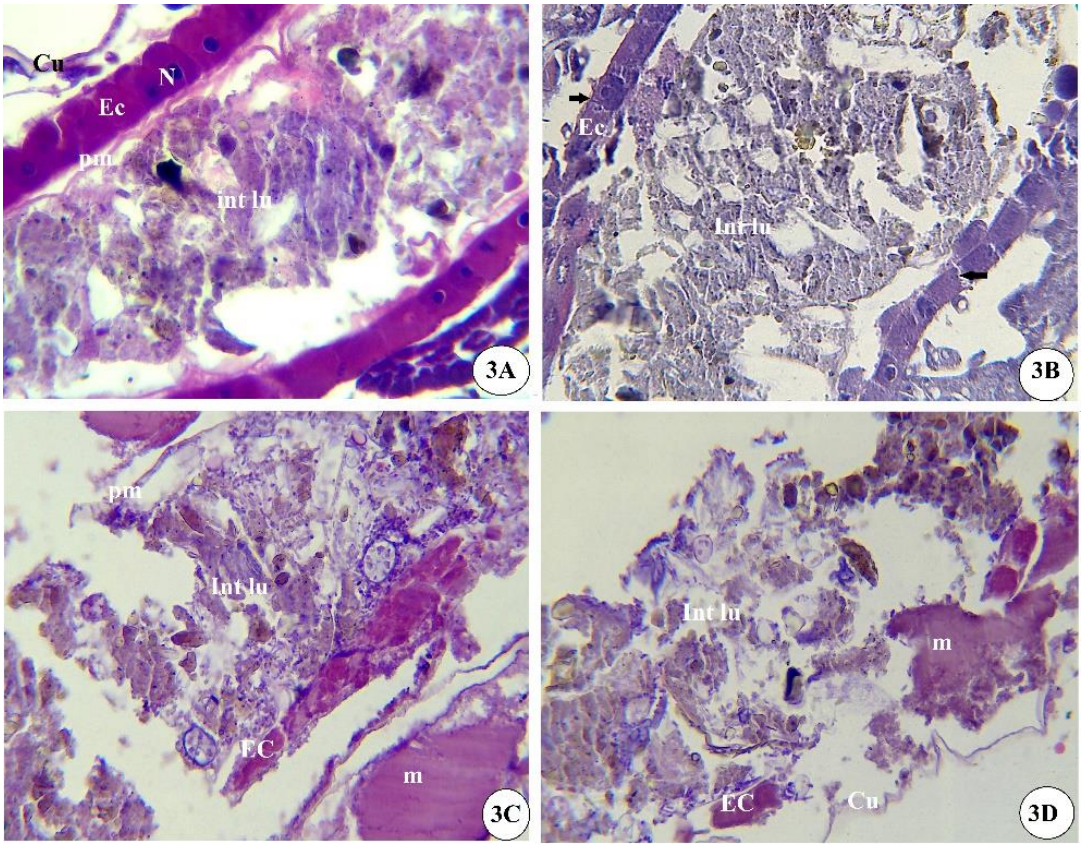

Figure 3: Longitudinal section of the abdomen of *Anopheles gambiae larvae*, untreated (figure 3A) and treated with *Lippia alba* Essential Oil at LD_{90} (788.2 ppm) after 6h (Figure 3B), 12h (Figure 3C), and 24h (Figure 3D) of exposure, stained with hematoxyline and eosin (HE) X400. Abbreviation: nucleus (N), Epithelial cell (EC), Intestinal lumen (Int lu) peritrophic membrane (pm), Cuticle (Cu), muscle (m).

In the control larva (Figure 3A), the intestinal epithelium was intact, with nucleated cells joined together, a visible peritrophic membrane surrounding the food column, and an intact cuticle. In contrast, larvae treated with *Lippia alba* essential oil showed progressive disorganization. As early as 6 hours post-exposure (Figure 3B) intestinal cells began to lose adhesion (arrow). By 12-24 hours (Figures 3C-3D), the food column became disorganized, and the peritrophic membrane disappeared. After 24 hours, intestinal cells were completely destroyed. Additionally, muscle tissue lost its normal structure and invaded the internal cavity of the larva

At the muscle tissue

High-magnification observations showed that during 24h exposure of larvae to the LD_{90} of *L. alba* essential oil, muscle tissue was found in different degenerative stages (Figure 4).

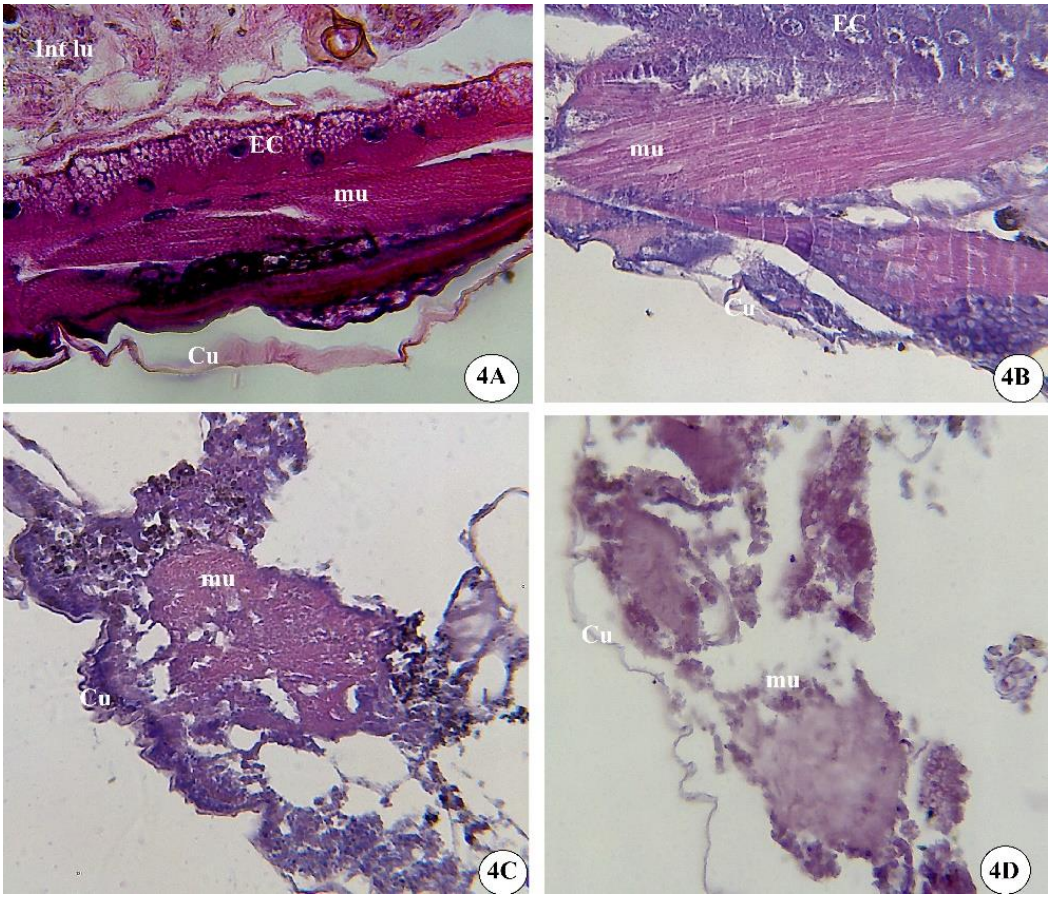

Figure 4: Longitudinal section showing muscle tissue of *Anopheles gambiae* larvae, untreated (Figure 4A) and treated with LD_{90} (788.2ppm) of *Lippia alba* essential oil after 6h (Figure 4B), 12h (Figure 4C), and 24h (Figure 4D) of exposure, stained with hematoxyline and eosin (HE) X400. Abbreviation; Cuticle (Cu), mu (muscle), Epithelial cell (Ec), Intestinal lumen (Int. lu.)

High-magnification sections of *Anopheles gambiae* larvae treated with *Lippia alba* essential oil revealed progressive degradation of muscle tissue compared to untreated larvae (Figure 4). This degradation began was early at 6 hours post-exposure (Figure 4B), intensified after 12 hours (Figure 4C), and culminated in complete disaggregation after 24 hours (Figure 4D).

DISCUSSION

Vector control aimed at reducing larval populations of mosquito vectors is a real challenge in endemic areas. Natural products seems increasingly indicated as an alternative to chemical products for msquito control. Plant extracts are not only effective mosquito control agents but also promising as environmentally safe alternatives (Choochote *et al.,* 1999). *Lippia alba* essential oil revealed in our study potent larvicidal activity against the 3rd and 4th instar larvae of *Anopheles gambiae* within 24 hours. The mortalities were 6.6%, 5%, 63.33%, 91.66%, and 91.66% for respectively 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm doses. These mortalities are almost similar to those obtained by Seye *et al*. (2021) with *Cymbopogon citratus* essential oil against *Aedes aegypti* larvae in 24 hours, but only for high doses (800 ppm and 1000 ppm). In contrast, at low doses (200 ppm and 400 ppm), *L. alba* essential oil appears to be less toxic to *An. gambiae* larvae (6.6% and 5% mortality) compared to *C. citratus* (19.4% and 43.2% mortality) against *A. aegypti* for the same respective doses. However, our study showed that, in addition to its repellent and adulticidal effects (Coulibaly *et al*., 2024), *L. alba* essential oil also exhibits larvicidal effects on vector mosquitoes, such as *An. gambiae*.

On the other hand, in our study, the LD_{50} of *L. alba* essential oil against *An. gambiae* larvae within 24 hours (554.4 ppm) is higher than that reported by Mahanta *et al*. (2019) for *Culex quinquefasciatus* larvae (275.66 ppm). In contrast, it is lower than the LD_{50} against *Aedes aegypti* (631.29 ppm) Mahanta *et al*., 2019). This highlights the difference in species sensitivity to the same plant extract, potentially due to variations in extraction processes (Kébé *et al*. 2024) or inherent species-specific responses. Moreover, this variability in sensitivity is also observed within the same mosquito species (*Aedes aegypti*) when exposed to different types of essential oils (Christina *et al*., 2018).

Lippia alba essential oil exhibits toxicity against *Anopheles gambiae* larvae within 24 hours, as

confirmed by histological analysis. In afct, the histological sections revealed that *L. alba* essential oil, applied at LD_{90} (788.2 ppm), targets various tissues in *An. gambiae* larvae. Microscopic observations showed degradation of nervous tissue, muscle tissue, adipose tissue, and the digestive tube. These findings are consistent with those of Seye *et al*. (2021), who observed similar changes in *Aedes aegypti* larvae treated with *Cymbopogon citratus* essential oil. The damage from digestive tube cells suggests that the oil acted after ingestion, disrupting tissues in the anterior part of the larvae (nervous tissue, esophagus, and gastric caeca) before affecting the food column and midgut cells. Given its composition of diverse molecules (Mahanta *et al*., 2019), *L. alba* essential oil may diffuse through internal structures such as muscle and adipose tissue. These types of destruction are consistent with previous studies showing the mode of action of larvicidal products after ingestion (Alves *et al.,* 2004; Al-Mehmadi and Al-Khalaf, 2010; Costa et al, 2014 ; Bawin *et al*., 2016; Dhayalan *et al*., 2019; Seye *et al*., 2021; Moola *et al*., 2023). The damage to tissues responsible for movement (nervous and muscle tissue) likely leads to immobilization of moribund larvae, preventing them from surfacing to breathe and ultimately resulting in death within 24 hours of exposure. The larvicidal effect of *L. alba* essential oil at LD_{90} (788.2 ppm) and the associated histological damage suggest its potential use as an insecticide against mosquito vectors.

However, the effectiveness of a larvicide depends on its persistence in relation to various environmental factors. It's essential to evaluate the efficacy of essential oils in semi-natural and natural environments. Although short exposure times (24-48 hours) can induce high larval mortality in laboratory settings, the efficacy and safety of these doses need to be assessed in natural breeding sites to determine their impact on non-target organisms.

CONCLUSION

The present study revealed the larvicidal potential of *Lippia alba* essential oil against the 3rd and 4th instar larvae of *Anopheles gambiae* under laboratory conditions. The essential oil, with an LD_{90} value of 788.2 ppm, caused histological damage within 24 hours. Further studies are needed at natural breeding sites to evaluate the potential larvicide against wild populations of mosquito vectors in environmental conditions.

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REFERENCES

- Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Okara, R. M., Van Boeckel, T., Godfray, H. C. J., Harbach, R. E., & Hay, S. I. (2010). The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East : Occurrence data, distribution maps and bionomic précis. *Parasites & Vectors*, 3(1), 117-150. https://doi.org/10.1186/1756-3305-3-117
- Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Burkot, T. R., Harbach, R. E., & Hay, S. I. (2012). A global map of dominant malaria vectors. *Parasites & Vectors*, 5(1), 69-79. https://doi.org/10.1186/1756-3305-5-69
- Furtado, A., « La surveillance du paludisme à Saint-Louis (Sénégal). (2018). comprendre les écarts entre morbidité réelle et "morbidité compilée" », *Espace populations sociétés* [on line], /1-2 | 2018: DOI : https://doi.org/10.4000/eps.7607
- Chen, C. D, WA Nazni, HL Lee, Norma-Rashid, Y, Lardizabal, ML and Sofian-Azirun, M. (2013). Temephos resistance in field *Aedes (Stegomyia) albopictus* (Skuse) from Selangor, Malaysia. *Tropical Biomedicine*, 30: 220-230.
- Aikpon R., Missihoun A., Lokossou A, Aikpon G, Salifou S, Dansi A et Agbangla C. (2020). Hétérogénéité génétique et résistance des vecteurs du paludisme (*Anopheles gambiae* s.l) aux insecticides en zone cotonnière au Benin. *International Journal of Biological and Chemical Sciences* 14(8) : 2724-2736. DOI : https://dx.doi.org/10.4314/ijbcs.v14i8.6
- Scholte E.-J., Knols, B. G. J., Samson, R. A., & Takken, W. (2004). Entomopathogenic fungi for mosquito control : A review, *Journal of Insect Science*, 4(19), 1-24
- Seye, F., Ndiaye, M., Faye, O., & Afoutou, J. M. (2012). Evaluation of entomopathogenic fungus *Metarhizium anisopliae* formulated with Suneem (Neem Oil) against *Anopheles gambiae s.l.* and *Culex quinquefasciatus* adults. *Malaria Chemotherapy, Control and Elimination*, (1), 1-6. https://doi.org/10.4303/mcce/235494
- Bawin T, Seye F, Boukraa S, Zimmer J-Y, Raharimalala FN, Ndiaye M, COMPERE P., Delvigne F., Francis F. (2016). Histopathological effects of *Aspergillus clavatus* (Ascomycota: Trichocomaceae) on larvae of the southern house mosquito, *Culex quinquefasciatus* (Diptera: Culicidae). *Fungal Biology*. 120: 489-99. doi: 10.1016/j.funbio.2016.01.002
- Baumann, P., Clark, M. A., Baumann, L., & Broadwell, A. H. (1991). *Bacillus sphaericus* as a mosquito pathogen : Properties of the organism and its toxins. *Microbiological Reviews*, 55(3), 425-436. https://doi.org/10.1128/mr.55.3.425-436.1991

- Dhang, C. C., Lim, L. H., Ahmad, N. W., Benjamin, S., Weng, L. K., Rahim, D. A., Safian, E. S., & Sofian-Azirun, M. (2009). Field effectiveness of *Bacillus thuringiensis israelensis* (Bti) against *Aedes* (Stegomyia) *aegypti* (Linnaeus) in ornamental ceramic containers with common aquatic plants. *Tropical Biomedicine*, 26(1), 100-105.
- Seye, F., Ndione, R., & Ndiaye, M. (2006). Etude comparative de deux produits de neem (huile et poudre) sur les stades préimaginaux du moustique *Culex quinquefasciatus* (Diptera : Culicidae). *Afrique Science*, 2(2), 212-225. https://doi.org/10.4314/afsci.v2i2.61165
- Amer A, Mehlhorn H. (2006). Larvicidal effects of various essential oils against Aedes, Anopheles, and Culex larvae (Diptera: Culicidae). *Parasitology Research* (99): 466-472. DOI : 10.1007/s00436-006-0182-3
- Bagavan A, Abdul Rahuman A. (2011). Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. *Asian Pacific Journal of Tropical Medicine.* 29-34
- Rajasekar P., Jebanesan A., Shamsudin M, Jerold I.,. Meeramaideen J M and Kasim M. (2015). Larvicidal activity of three chosen plants' essential oil against the mosquito vector, *Culex quinquefasciatus* (say). *Life Science Archives* (LSA) 1 (4) : Page : 272 – 276
- Foko, G. A. D., & Nyegue, M. A. (2016). Effet insecticide de l'huile essentielle des graines de *Aframomum sulcatum* (Oliv. & D. Hanb. Ex Baker) K. Schum. Sur les stades aquatiques de *Anopheles gambiae* Giles 1902, vecteur du paludisme. Syllabus Review, *Science Series*. (6), 69-80.
- Nkouandou, P. M., Ntonga, P. A., Djeukam, C. A., Dongmo, P. M. J., & Menut, C. (2020). Evaluation des propriétés insecticides des huiles essentielles de quelques Zingiberaceae contre les larves d'*Anopheles gambiae*. L. Collectées à Ayos (sud-Cameroun). *Journal of Animal & Plant Sciences*, 43(3), 7469-7482. https://doi.org/10.35759/JAnmPlSci.v43-3.3
- Mahanta S, Sarma R and Khanikor B. (2019) The essential oil of *Lippia alba* Mill (Lamiales:Verbenaceae) as mosquitocidal and repellent agent against Culex quinquefasciatus Say (Diptera: Culicidae) and *Aedes aegypti* Linn (Diptera: Culicidae). *The Journal of Basic and Applied Zoology*. 80:64. https://doi.org/10.1186/s41936-019-0132-0
- Seye F.**,** Fall A., Toure M., Ndione R D and Ndiaye M. (2021). Histopathological effects of *Cymbopogon citratus* (lemongrass) essentiel oil on late third instar larvae of *Aedes aegypti* L (Diptera: Culicidae). *Biolgy and Medicine* (Aligarh), 13 (1): 1-5
- Rahmatillah, M., Jahan, R., Azam, F. M. S., Hossan, S., Mollik, M. A. H., & Rahman, T. (2011). Folk medicinal uses of verbenaceae family plants in Bangladesh. *African Journal of Traditional Complementary and Alternative Medicines*, 8(S), 53–65.
- Santiago G M. P. , Lemos T L. G., Pessoa O. D. L., Arriaga Â. M. C., Matos F. J. A., Lima M A S., Santos H S., Lima M. da C. L., Barbosa F. G., Luciano J. H. S., Silveira E. R. and De Menezes G. H. A. (2006). Larvicidal Activity Against *Aedes aegypti* L. (Diptera: Culicidae) of Essential Oils of *Lippia* Species from Brazil. *Natural Product* Communications. 1 (7) 573-576.
- Coulibaly F.H., Rossignol M., Haddad, Carrasco D., Azokou A., Valente A, Ginibre C., Koné M W, & Chandre F. (2024). Biological effects of *Lippia alba* essential oil against *Anopheles gambiae* and *Aedes aegypti*. Scientific *Reports*, (14**)**, 3508. https://doi.org/10.1038/s41598-024-52801-1
- WHO. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; (2005). Available from: https://apps.who.int/iris/handle/10665/69101.
- Abbott WS. A method of computing effectiveness of insecticides. (1925). *Journal of Economic Entomology*.18(2):265-7. doi: 10.1093/jee/18.2.265a
- Choochote W, Kanjanapothi D, Panthong A, Taesotikul T, Jitpakdi A, Chaithong U, Pitasawat B. (1999). Larvicidal, adulticidal and repellent effects of Kaempferia galangal. South East Asian, *Journal of Tropical Medicine Health*; 30(3):470-6.
- Kébé, M., Diouf, E. H. G., Ndour, M. L., Faye, A., Sarr, A., Ndior, T., Camara, N. B., Ngom, M., Sarr, B., & Sène, A. (2024). Phytochemical screening and evaluation of the larvicidal activities of three organic extracts derived from the leaves of *Cassia sieberiana* to control *Anopheles gambiae*, the vector of malaria. *Chemical Science International Journal*, 33(1), 47-53. https://doi.org/10.9734/CSJI/2024/v33i1884
- Christina P M, Fabiola M, Johnson A. J, JMV Kalaiarasi. (2018). Larvicidal activity of selected essential oils against *Aedes aegypti* (Insecta: Diptera: Culicidae). *International Journal of Entomology Research*. 3 (2) ; pp : 139-142
- Alves S N, J E Serrão, Mocelin G. and de Melo A. L. (2004). Effect of Ivermectin on the Life Cycle and Larval Fat Body of *Culex quinquefasciatus*. *Brazilian Archives of Biology and Technology*. Vol.47, n. 3 : pp. 433-439,
- Al-Mehmadi R.M, Al-Khalaf AA. (2010). Larvicidal and histological effects of *Melia azedarach* extract on *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *Journal of King Saud University* (Science) 22, 77–85. https://doi.org/10.1016/j.jksus.2010.02.00
- Costa M. S., Cossolin J F. S, Pereira M J. B., Sant'Ana A. E. G., Lima M D., Zanuncio J C. and Serrão J E. (2014). Larvicidal and Cytotoxic

Potential of Squamocin on the Midgut of *Aedes aegypti* (Diptera: Culicidae). *Toxins*, (6), 1169-1176 ; doi:10.3390/toxins6041169

- Dhayalan A., Kannupaiyan J, Govindasamy B., Pachiappan P. (2019). Extraction and Characterization of Secondary Metabolites from the Soil Bacterium, *Acidovorax* sp. SA5 and Evaluation of Their Larvicidal Activity Against *Aedes aegypti.* International Journal of Environmental Research. *International Journal of Environmental Research* 13, 47–58. https://doi.org/10.1007/s41742-018-0152-5
- Moola A K, Ayyadurai T, Balasubramani S., Vignesh R., Mohan P. K, Sathish S., Diana R K B. (2023). *Journal of Natural Pesticide* Research, (3) 100018. https://doi.org/10.1016/j.napere.2022.100018