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Original Research Article

Phytochemical Screening, Chemical Composition and Larvicidal Efficacy of *Syzygium aromaticum* Extracts and Essential Oil against *Culex pipiens*

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ABSTRACT

Culex mosquitoes, as nuisance insects and primary vectors of disease, present an important risk to animal and human health. The aim of the present study was to evaluate the larvicidal potential of various extracts (ethanolic, hydro-ethanolic, and essential oil) of *Syzygium aromaticum* (clove) against the larvae of *Culex pipiens*. Ethanolic and hydro-ethanolic extracts from *Syzygium aromaticum* were prepared by ultrasonication, and their composition was determined by phytochemical screening. Gas chromatography-mass spectroscopy (GC-MS) was performed to characterize the *Syzygium aromaticum* essential oil, which was produced through hydro-distillation. The extracts were tested for their larvicidal activity against *Culex pipiens* larvae and the LC_{50} and LC_{90} values were determined. The results of the phytochemical screening of the ethanolic and hydroethanolic extracts indicated the presence of flavonoids, sterols, terpenes, coumarins, glycosides, and gall tannins. The chemical composition analysis also revealed that eugenol (78.82%) and caryophyllene (16.51%) were the main compounds present in the essential oil. The LC_{50} and LC_{90} values obtained for the hydro-ethanolic extract were 557.90 and 939.71 ppm, respectively, while values of 400.14 and 743.32 ppm, respectively, were recorded for the ethanolic extract. The lethal LC_{50} and LC_{90} values of the *Syzygium aromaticum* essential oil demonstrated effectiveness at 1.220 and 5.260 ppm, respectively. Results of this study showed that extracts of *Syzygium aromaticum* have larvicidal activity against *Culex pipiens* and could be explored in the development of novel and sustainable mosquito control strategies.

Keywords: *Culex pipiens*, *Syzygium aromaticum*, Essential oil, Ethanolic and hydro-ethanolic extracts, Larvicidal activity, Fez, Morocco.

Introduction

International health organizations have been warning the public about the reemergence of vector-borne diseases such as malaria, dengue, and yellow fever for several years. These diseases, which spread between people via hematophagous vectors like mosquitoes, ticks, or other carriers, have caused numerous pandemics throughout history.[1] Mosquitoes and Culicidae can spread infectious diseases to humans, which has a significant impact on both human and animal health.

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Despite their reputation as harmful insects, their painful bites make them the primary group of vectors for transmitting parasitic diseases, including filariasis, yellow fever, dengue, Zika, chikungunya viruses, and West Nile virus, particularly those belonging to the *Culex* genus.[2-4] Widely distributed throughout the world, medically important mosquitoes belong to the Culicidae family. There are now 3,556 species in this large family.[5] In North Africa, Culicidian species have been historically associated with severe epidemics. Among these species, the female *Culex pipiens*, specifically, is recognized as a vector for numerous pathogens that can cause fatal infectious diseases in humans and animals.[6-11]
There are currently no effective preventive or curative methods for vector-borne diseases. The focus of strategies has been on controlling the vectors through the use of chemical insecticides. While this approach has proven to be highly effective against culicid mosquitoes, it also has several ecological disadvantages. The use of these products not only harms aquatic life but also contributes to a variety of environmental problems.[12-14] In addition to the aforementioned disadvantages, the emergence of insect resistance poses a serious problem with the use of chemical insecticides.[15-19] As a result, there has been a greater emphasis on investigating alternative insect pest management methods, such as the use of secondary plant compounds. This approach has generated a substantial amount of research.[20] Implementing comprehensive disease vector control methods at all phases is critical. Ethnobotanical and laboratory studies have

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discovered that various insecticidal plants from different families are present in different parts of the world.[21] Different levels of bioactivity against mosquitoes at different developmental stages have been found in crude solvent extracts of plant parts from different families, essential oils or their chromatographic fractions. Plant-derived chemical compounds have recently been promoted as a sustainable means of control.[22,23]
The efficacy of plant extracts against mosquito larvae has been reported in several studies conducted by researchers in Morocco.[24-27] Based on extensive bibliographic studies and its efficacy as a bio-insecticide, *Syzygium aromaticum* was selected for this study.[28] In this study, the larvicidal activity of essential oils and extracts of the aromatic plant against the larvae of mosquitoes was evaluated in Morocco. To our knowledge, the larvicidal activity of clove species against *Culex pipiens* larvae has not been previously documented in Morocco.
The purpose of the present study was the evaluation of the larvicidal activity of essential oils and extracts (hydro-ethanolic and ethanolic extracts) from *Syzygium aromaticum* (clove plant) against *Culex pipiens* larvae. A phytochemical screening of the hydro-ethanolic and ethanolic extracts of the clove plant was also conducted.

Materials and Methods

Source of plant materials
Syzygium aromaticum (cloves) (Figure 1) was selected for this study after extensive research on the efficacy of several plants as bio-insecticides. Cloves used in this study were purchased in the historic city of Fez in May 2022 and essential oils prepared in the Biology Department, Faculty of Science, Dhar El Mehraz, Fez, Morocco.

Collecting and morphological identification of Culex pipiens larvae
Culex pipiens (Diptera: Culicidae) larvae were collected in May 2022 from the Oued El-Mehraz breeding area (altitude: 423 m; 34°02′13, 74″N and 4°59′59.279″W). This is an area that is rich in organic matter, providing a suitable environment for Culicidae species, particularly *Culex pipiens* larvae, to thrive. Rectangular plastic plates were used to collect the larvae, then kept at the same location under constant conditions of 22.6°C±2°C water temperature and 70%±5% relative humidity. Following a two-day rearing period, only mosquito larvae in their third and fourth stars were selected for experimental testing, in accordance with the World Health Organisation (WHO) protocol. To identify the larvae, a morphological characterization was conducted using the Moroccan identification key, [29] as well as the Culicidae and the Mediterranean African mosquito identification software.[30]

Preparing hydroethanolic extract of Syzygium aromaticum
About 50 g of *Syzygium aromaticum* powder was subjected to sonication for 45 minutes at a temperature of 25°C in a mixture of ethanol and water (350/150 ml). A Whatman No.1 paper filter was used to filter the resulting hydroethanolic extract. The ethanol/water mixture was then evaporated from the filtrate at 40°C, producing a dark brown residue with a strong aroma, which was stored at 4°C for future use. [31]


Figure 1: Cloves (*Syzygium aromaticum*) of Morocco.

Preparation of ethanol extract of Syzygium aromaticum
The extraction process required sonicating 50 g of *Syzygium aromaticum* powder for 45 minutes at 25°C with 500 ml of ethanolic solvent. Filter paper used was Whatman No.1 to filter the resultant ethanolic fractions. After that, the filtrate was evaporated at 40°C and kept at 4°C until needed.[31]

Extraction and chemical characterisation of the essential oil of Syzygium aromaticum
The essential oils were prepared from *Syzygium aromaticum* using modified Clevenger-type equipment by hydro-distillation for 3 hours. The chemical analysis was conducted using gas chromatography coupled with mass spectrometry (GC-MS), which allows for the chromatographic characterization and qualitative determination of the majority of compounds in each oil. A Hewlett-Packard instrument was used, equipped with an HP1 fused silica column (30 m × 0.25 mm, thickness: 0.25 μm). The instrument was connected to a quadrupole detector (GC-quadrupole MS system, model 5970). The column temperature has been programmed for a 10°C/min rise of 70-200°C and the injection temperature has been programmed for 200°C. The mass detector was operated at 70 eV using helium as the carrier gas at a flow rate of 0.6 ml/min.

Phytochemical screening of Syzygium aromaticum extracts
To characterise the different chemical constituents, a quantitative phytochemical screening was carried out of the ethanolic and hydro-ethanolic extracts prepared from the plant material of *Syzygium aromaticum*.

Determination of extracts' larvicidal activity
The standard WHO protocol was followed to conduct the larvicidal tests. Preliminary experiments with the hydro-ethanolic extract, the ethanolic extract and the essential oil were carried out to determine a suitable range of concentrations. The test concentrations for hydro-ethanolic extract were 100, 200, 300, 400, 500, 600, 700, and 800 ppm, while for the ethanolic extract and essential oil, the concentrations were 500, 1250, 2000, 5000, and 10000 ppm using ethanol as the solvent. Each prepared suspension, consisting of 1 ml of extract or oil, twenty larvae in their fourth or final third instar were placed in a beaker containing 99 ml of distilled water. Twenty larvae were added to 99 ml of distilled water with 1 ml of ethanol as a control. The larvicidal bioassay, as well as the control assay, were performed in triplicate. The mortality rate was determined after 24 hours of treatment for all of the concentrations tested. Mortality of larvae exposed to hydro-ethanolic extract, ethanolic extract and essential oils was corrected using Abbott's formula below when mortality in the control exceeded 5%.[32] If the control assay mortality exceeded 20%, the test was invalid and was repeated.

$$\%\ \text{mortality corrected} = \frac{(\%\ \text{mortality observed} - \%\ \text{mortality control})}{(100 - \%\ \text{mortality control})} \times 100$$

Statistical analysis of results
The CIRAD-CA/MABIS Loge-probit analysis software (WinDL version 2.0) was used to statistically analyse the data.[33] Toxicity was assessed by calculating the LC_{50} and LC_{90}. These are the lethal concentrations that cause 50% and 90% mortality, respectively, in the treated larval population. These values were obtained by plotting an experimental curve that shows mortality at 24 hours according to increasing dose of essential oil.

Results and Discussion

The present study aimed at phytochemical screening of hydro-ethanolic, ethanolic and essential oil extracts of *Syzygium aromaticum* and their larvicidal activity against the West Nile mosquito vector *Culex pipiens* for the first time in Morocco. The results obtained for the yield of the extracts from the *Syzygium aromaticum* plant showed that the hydro-ethanolic extract had a higher yield than the ethanolic extract (Table 1). This difference in yield is likely attributable to the

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different solvents used for extraction.[34] Phytochemical screening involves qualitative characterization reactions to detect all secondary metabolite families present in the plant part under study. These reactions rely on precipitation or coloration phenomena using specific reagents for each compound family.[35]The results of the phytochemical tests performed on the ethanolic and hydro-ethanolic extracts are presented in Table 2. Jimoh *et al*. (2017) conducted a similar study in a related context.[36] Based on the initial screening, the methanolic extract of *Syzygium aromaticum* did not contain phlobatannins, reducing sugar, and steroids, whereas they were found in the distilled water extract. The average yield of two extracts (ethanolic and hydro-ethanolic), calculated based on dry matter, was found to be around 14.37 and 26.15%, respectively. In another study, oils from *Syzygium aromaticum* were obtained by hydrodistillation with a yield of 6.3±0.23% by Aimad *et al*.[7]

It was observed that the ethanolic extract contained a significant amount of catechin, tannin, sterol, and glucoside, whereas the hydroethanolic extract contained a high value of glucosides. Additionally, a higher quantity of sterols was also observed in the hydroethanolic extract. The GC and GC/MS analysis of the essential oil revealed that the primary components of *Syzygium aromaticum* are eugenol (78.82%) and caryophyllene (16.51%). The presence of tannins and phenolics was indicated by a dark green color change, while a reddish-brown interface suggested the presence of terpenoids and glycosides. A greenish color indicated the presence of steroids, while flavonoids were indicated by a yellow to colorless color change. The analysis of the chemical composition of the essential oils from *Syzygium aromaticum* indicated the presence of major compounds including 78.82% eugenol and 16.51% caryophyllene. Previous studies have shown that clove oil contains caryophyllene (11.34%), heptadecane (10.29%), hexadecane (7.93%), docosane (7.92%) and a small amount of eugenol (3.98%).[38] In another study conducted in Fez, Morocco, The oil of *Syzygium aromaticum* was characterised by a high content of eugenol (80.26%), eugenyl acetate (9.62%), beta-caryophyllene (6.74%) and c-humulene (1.14%).[37] It has been reported that the eugenol content in clove oil can vary greatly depending on the country of origin, ranging from 72-90%,[39] to as high as 99.10%.[40] However, in the present study, the eugenol content in the sample was found to be very significant, which could be attributed to several factors such as the plant's growing location, extraction method, plant parts used for extraction, and time of harvest.[41]

Results of this research indicate that *Syzygium aromaticum* essential oils have a significant larvicidal effect on 3rd and 4th-stage larvae, with the most effective essential oils having LC_{50} and LC_{90} values of 1,220 and 5,260 ppm, respectively. Figure 2 illustrates the percentage mortality rate of *Culex pipiens* larvae after 24 hours of exposure to different concentrations (100, 200, 300, 400, 500, 600, 700, and 800 ppm) of the ethanolic extracts of *Syzygium aromaticum*. Figure 3 displays the percentage mortality rate of *Culex pipiens* larvae after 24 hours of exposure to five different concentrations (100; 200; 300; 400; 500; 600; 700 and 800 ppm) of hydro-ethanolic extract of *Syzygium aromaticum*. The percentage mortality rate of *Culex pipiens* larvae at 24 hours of exposure to the essential oil of *Syzygium aromaticum* at five different concentrations (500, 1250, 2000, 5000, and 10000 ppm) is shown in Figure 4. Table 3, as well as Figures 2, 3, and 4, provide evidence that the ethanolic extract, the hydro-ethanolic extract, and essential oils of *Syzygium aromaticum* have larvicidal activity. The LC_{50} and LC_{90} values for the essential oils of *Syzygium aromaticum* were approximately 1.220 ppm (0.414 ± 3.07) and 5.260 ppm (1.62 ± 10.9), respectively (Table 3). The hydro-ethanolic extract had LC_{50} and LC_{90} values of about 557.90 ppm (646±761) and 939.71 ppm (712±1332), while the ethanolic extract had LC_{50} and LC_{90} values of around 400.14 ppm (286 ± 477) and 743.32 ppm (620±1023) (Table 3). Similar results were reported in a recent study conducted in Nigeria, which showed that clove essential oil had larvicidal activity against larvae of Aedes aegypti collected in the field, with LC_{50} values of 92.56 and 62.3 ppm, respectively.[42] Clove essential oil and eugenol were also evaluated against *Anopheles stephensi* for their larvicidal activity. LC_{50} and LC_{90} of essential oil of cloves (57.49 and 93.14 ppm, respectively)[43] were significantly lower than eugenol (86.96 and 128.18 ppm).[44] The results of this study indicate that the use of essential oils derived from cloves can be an effective way to control the population of *Culex pipiens* larvae. Compared to the ethanolic or hydro-ethanolic extracts, the essential oils showed a higher level of effectiveness in eliminating larvae. This suggests that the active compounds responsible for larvicidal activity are more concentrated in essential oils. These observations are in agreement with the findings from a study that reported that the essential oil of cloves was found to be more effective than ethanolic or hydro-ethanolic extracts in controlling the population of *Culex pipiens* larvae.[43,44] Another study also reported that the essential oil of clove could be considered a promising natural larvicide with potent and persistent larvicidal activity against *Culex pipiens* and used as a potential source of natural insecticide for mosquito control.[42,45] Furthermore, essential oils have high larvicidal activity, demonstrating their potential as a natural and eco-friendly alternative to synthetic pesticides. These discoveries could have significant consequences for the development of innovative and sustainable mosquito control strategies.[46]

Table 1: Yield of clove plant extract

Extract	Weight	Yield
Sa EE	7.1851g	14.37 %
Sa HEE	13.075g	26.15%

Sa EE: *Syzygium aromaticum* ethanolic extract; Sa HEE: *Syzygium aromaticum* hydro-ethanolic extract.

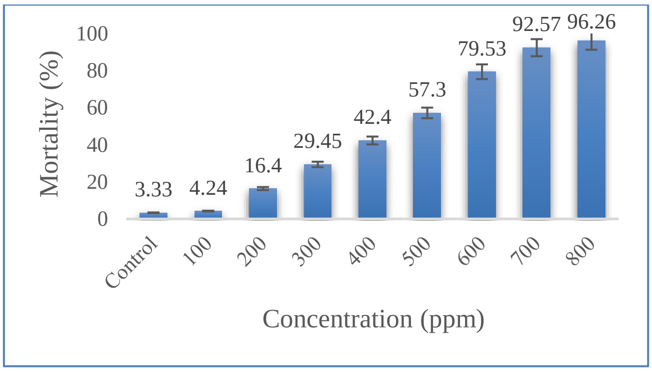

Figure 2: The percentage of mortality of *Culex pipiens* larvae based on the concentrations of ethanolic extract of *Syzygium aromaticum* after 24 hours of exposure.

Table 2: Qualitative phytochemical screening of *Syzygium aromaticum* extracts

Extract	Flavonoid	Alkaloid (Mayer)	Alkaloid (Wagner)	Terpenes Heterosides	Gallic tannins	Catechic, Tannins	Saponins	Sterols	Glycosides
Sa EE	+	_	+	_	+	+++	_	+++	+++
Sa HEE	+	+	_	++	+	_	_	+++	+++

Sa EE: *Syzygium aromaticum* ethanolic extract; Sa HEE: *Syzygium aromaticum* hydro-ethanolic extract
(+) = present; (−) = absent

Table 3: Lethal concentrations (LC_{50} and LC_{90}) of *Syzygium aromaticum* extracts against *Culex pipiens* larvae after 24 hours

Plant extract	LC_{50} (ppm) (Ll-Ul)*	LC_{90} (ppm) (Ll-Ul)*	Equation of the regression line	Calculated *Chi*-square ($\chi 2$)
Hydro-Ethanolic	557.90(646 ± 761)	939.71(712 ± 1332)	Y = -15.54677 + 5.66045* X	35.710
Ethanolic	400.14 (286 ± 477)	743.32(620 ± 1023)	Y = -12.40052 + 4.76537* X	13.642
Essential oil	1220(0.414 ± 3.07)	5260(1.62 ± 10.9)	Y = -0.17824 + 2.02316* X	8.770

*Ll-Ul: Lower limit-Upper limit; LC_{50} and LC_{90}: Lethal concentrations.

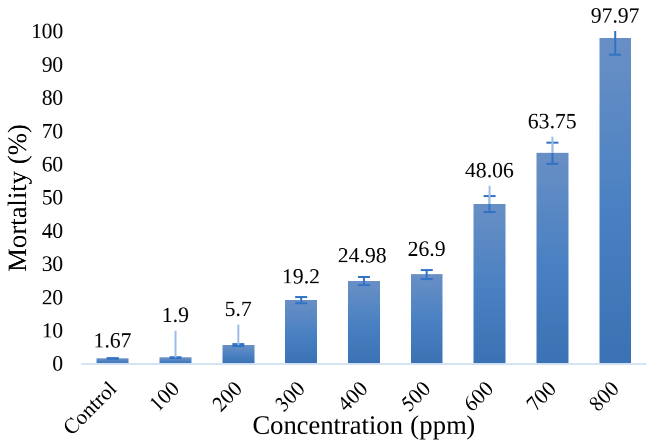

Figure 3: Mortality rate (%) of *Culex pipiens* larvae based on the concentrations of hydro-ethanolic extract of *Syzygium aromaticum* after 24 hours of exposure.

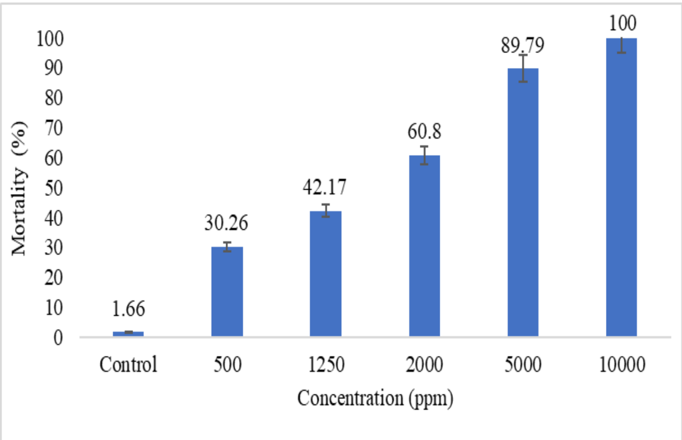

Figure 4: Mortality rate (%) of *Culex pipiens* larvae based on the concentrations of essential oil of *Syzygium aromaticum* after 24 hours of exposure.

The findings of the present study support the use of essential oils as a viable bioinsecticide option in Morocco, This is especially true given its efforts to reduce the use of Persistent Organic Pollutants through the Stockholm Convention, which Morocco ratified between 2015 and 2019. However, more research is needed to investigate the exact mechanisms by which essential oils act on larvae and to identify the specific compounds responsible for their larvicidal activity. Furthermore, the potential effects of essential oils on non-target organisms and the environment should be thoroughly evaluated before their widespread use in mosquito control programs.

Conclusion

The findings of the present study indicate that the oils and extracts of *Syzygium aromaticum* had varying effects on the larvae, with mortality rates ranging from low to high. Additionally, the concentration of the extracts was found to influence the evolution of larval mortality. The hydro-ethanolic and ethanolic extracts of clove extracts exhibit a larvicidal effect against *Culex pipiens* larvae, with LC_{50} and LC_{90} values of 557.90 and 939.71 ppm for the hydro-ethanolic extract respectively, while 400.14 and 743.32 ppm, respectively for the ethanolic extract. Also, the oils are more potent than the plant extracts due to their higher activity. The essential oil, with LC_{50} and LC_{90} values of 1,220 and 5,260 ppm, showed larvicidal activity against *Culex pipiens* larvae of stages 3 and 4. These findings could have significant consequences for the development of novel and sustainable mosquito control strategies.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby 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 them.

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