



# Ovicidal Potential of *Jasminum Officinale*, *Mentha Piperita* and *Prunus Dulcis* Oils Against the Eggs of Selected Vector Mosquitoes, *Aedes Aegypti* (L.) and *Anopheles Stephensi* (Liston)

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## ABSTRACT

In the present investigation, the three volatile oils such as *Jasminum officinale*, *Mentha piperita* and *Prunus dulcis* oils were tested for their ovicidal activity against the eggs of selected vector mosquitoes, *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston). Among the three oils, the *J. officinale* and *P. dulcis* showed a notable effect on the eggs of the selected vector mosquitoes. Since the plant, volatile oils are a mixture of various phytochemical compounds, and thus the various degrees of the activity was noted in the present investigation. As an important component in the intensive vector control program, the application of environmentally safer plant oils will play an imminent role soon.

**Keywords:** Volatiles, Ovicidal, Mosquitoes, plant oils, phytochemical.

## INTRODUCTION

Mosquitoes have challenged human progress for many centuries. Mosquitoes are among around 3,600 species of small flies belonging to the family Culicidae (Maria del Carmen Marquetti, 2019). More diseases are transmitted by mosquitoes than any other group of arthropods, and millions of people around the world are affected by mosquito-borne diseases every year (Anupam Ghosh *et al.*, 2012). Mosquito can act as vectors for many diseases causing viruses and parasites. Infected mosquitoes carry these organism from person to person without exhibiting symptoms themselves (Warikoo *et al.*, 2011). Many of the world's most dangerous diseases are transmitted through mosquitoes, such as malaria, yellow fever, dengue fever,

chikungunya fever, filariasis, encephalitis, etc., and this occurs in virtually all tropical and subtropical countries and elsewhere in the world.

Mosquito control is crucial to prevent the proliferation of mosquito-borne diseases and to ensure that the quality of the environment and public health is improved. Applying synthetic insecticides is a major tool in the mosquito control program. But this has not been very successful due to human, technical, operational, ecological and economic factors. Lack of novel insecticides, concern for environmental sustainability, harmful effect on human health and other non-target population, their non-biodegradable nature, higher rate of biological magnification through the ecosystem and increasing insecticide resistance on a global scale limited the use of synthetic insecticides in the mosquito control program. Considering the above factors, there is an urge to look for environment-friendly, cost-effective, biodegradable and target-specific insecticides against mosquito species. The application of eco-friendly alternatives such as biological control of vectors has become the central focus of the control program.

A simple and sustainable method of reducing mosquito populations can be achieved by utilizing safer insecticides of botanical origin as an alternative within the biological control program. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and may act as a suitable alternative product to fight against mosquito-borne diseases. Studies proved that the plant products have antifeedant, ovicidal, larvicidal, pupicidal, repellents and adulticidal activities. In addition, plant essential oils have been evaluated for insecticidal activities. Secondary metabolites of plants and their synthetic derivatives provide an alternative source of vector control. In the present investigation, the essential oil of three commonly available Indian medicinal plants and their major chemical constituents were tested for mosquitocidal activities against *Aedes aegypti* and *Anopheles stephensi*.

## MATERIALS AND METHODS

### Rearing of vector mosquito

Eggs, larvae and pupae of *Aedes aegypti* and *Anopheles stephensi* were collected within the college campus (Govt. Arts college Coimbatore) by placing water-filled plastic trays (23X15X6.5 cm) with a lining of partially immersed filter paper. In the plastic trays (30X24X10 cm), each containing 2 l of tap water, the eggs have been placed to ensure that larval incubation will take place. The trays were kept at room temperature ( $27 \pm 2^\circ\text{C}$ ) with a photoperiod of 12:12 h (L:D) to ensure larval hatching. The larvae and pupae were maintained in separate trays under the same laboratory conditions and larvae were fed with yeast powder. The pupal trays have kept in separate cages at  $27 \pm 2^\circ\text{C}$  and relative humidity of  $75 \pm 5\%$  for the emergence of adult mosquitoes. Cotton soaked in 10% aqueous sucrose solution has been placed in a Petridish to feed adult mosquitoes to induce the eclosion and provided with blood meal from an immobilized young chick by placing inside the cage. A plastic tray (30 × 15 × 6 cm) with partially immersed blotting paper has provided for the female mosquitoes to lay eggs. The eggs obtained were immediately used to maintain the stock culture in the laboratory.

## Preparation of essential oils

The fully matured, uninfected and diseases free plant leaves of *Jasminum officinale*, *Mentha piperita*, *Prunus dulcis* were collected from North-western Nilgiri Hills (Blue Mountains), Nilgiri District, Tamil Nadu, India (11.56230 N, 76.53450 E). The leaves were collected between January and March 2018. In addition to air and shade drying, the collected plants were ground to a fine powder and sieved through a kitchen strainer for a period of 15 days. 1000 grams of the fine powder of plants were sequentially extracted through hydro-distilled in a Clevenger apparatus for 6 hrs. The distilled oil has been stored in a refrigerator under 4°C and packed in an aseptic amber bottle until the bioassay test.

## Ovicidal bioassay:

To conduct the ovicidal assay, batches of 100 mosquito eggs were placed in 100 ml of test medium containing concentrations of the different essential oils (*Jasminum officinale*, *Mentha piperita*, and *Prunus dulcis* oils) as previously described. All containers have been maintained at room temperature ( $27\pm 2^\circ\text{C}$ ) with naturally general photoperiod (12:12 hrs L:D). The experimental medium changed after 24 hrs with a fresh medium containing the same oil and test concentration. Every 24, 48, and 72 hours, the test media were carefully examined for the presence of intact (unhatched) eggs as well as the number of larvae of the first instar. The latter indicated that the eggs had successfully hatched. This maximum time point for egg hatchability was fixed since the embryogenesis in mosquitoes under reasonable conditions has been reported to be completed within 72hrs. Besides, the unhatched eggs remaining in the test media after 72hrs of exposure were transferred to tap water and maintained up to 24 hrs to ascertain the mortality of these eggs. The eggs that failed to hatch out even under this ideal condition were dead due to their previous exposure to a particular test medium. Percentage mortality of the eggs, representing the ovicidal effect of the test material, was calculated from the total number of eggs introduced into the medium and the number of unhatched or dead eggs (Deepa *et al.*, 2014).

## RESULT AND DISCUSSION

In the present study different concentrations used were 50, 100, 150 and 200 ppm. In the case of *Jasminum officinale* treated against *Aedes aegypti* at 200 ppm caused 42.55% mortality in 24 hrs, 64.63% mortality in 48 hrs and 70.32% after 72 hrs. *Mentha piperita* against *Aedes aegypti* showed 52.44%, 43.26% and 60.55% after 24, 48 and 72 hrs under 200 ppm. Plant oil *Prunus dulcis* against *Aedes aegypti* at 200 ppm caused 52.14%, 54.99% and 70.89% after 24, 48 and 72 hrs. In the case of *Anopheles stephensi* at 200 ppm *Jasminum officinale* showed 40.77%, 51.22% and 50.33% after 24, 48 and 72 hrs. *Mentha piperita* against *Anopheles stephensi* caused 30.55%, 43.21% and 50.64% after 24, 48 and 72 hrs under 200 ppm. *Prunus dulcis* plant oil against *Anopheles stephensi* at 200 ppm after 24, 48 and 72 hrs caused 42.55%, 57.73% and 70.33% mortality. In the case of *Aedes aegypti*, *Prunus dulcis* showed moderate mortality at 72 hrs, followed by *Jasminum officinale* and *Mentha piperita* under 200 ppm. *Prunus dulcis* plant oil showed moderate efficacy against *Anopheles stephensi* at 72 hrs under 200 ppm, followed by *Mentha piperita* and *Jasminum officinale* (Table 1-6).

Table1. Ovicidal activity of *J. officinale* tested against the eggs of *Aedesaegypti*

Concentration(ppm)	Egg Mortality (%)		
	24hrs	48hrs	72hrs
50	10.55	17.44	35.24
100	19.77	40.22	49.22
150	35.64	51.26	55.66
200	42.55	64.63	70.32

Values represent the mean of five replications.

Table2. Ovicidal activity of *Mentha piperita* tested against the eggs of *Aedesaegypti*

Concentration(ppm)	Egg Mortality (%)		
	24hrs	48hrs	72hrs
50	17.22	25.64	33.87
100	21.65	27.22	47.29
150	33.93	36.41	51.34
200	52.44	43.26	60.55

Values represent the mean of five replications.

Table3. Ovicidal activity of *Prunus dulcis* tested against the eggs of *Aedesaegypti*

Concentration(ppm)	Egg Mortality (%)		
	24hrs	48hrs	72hrs
50	12.66	23.44	33.55
100	24.51	39.24	47.62
150	40.32	45.88	55.22
200	52.14	54.99	70.89

Values represent the mean of five replications.

Table4. Ovicidal activity of *Jasminum officinale* tested against the eggs of *Anopheles stephensi*

Concentration(ppm)	Egg Mortality (%)		
	24hrs	48hrs	72hrs
50	15.47	20.54	27.44
100	24.66	34.28	38.12
150	33.18	43.55	40.55
200	40.77	51.22	50.33

Values represent the mean of five replications.

Table5. Ovicidal activity of *Mentha piperita* tested against the eggs of *Anopheles stephensi*

Concentration(ppm)	EggMortality (%)		
	24hrs	48hrs	72hrs
50	7.66	18.44	27.64
100	16.23	27.33	36.41
150	24.22	36.44	44.58
200	30.55	43.21	50.64

Values represent the mean of five replications.



Table6. Ovicidal activity of *Prunus dulcis* tested against the eggs of *Anopheles stephensi*

Concentration(ppm)	Egg Mortality (%)		
	24hrs	48hrs	72hrs
50	11.59	24.89	33.88
100	22.34	34.66	49.93
150	39.44	43.87	54.66
200	42.55	57.73	70.33

Values represent the mean of five replications.

There are some authors who suggest that essential oils possess their general effect due to their lipophilic nature (Tolozaet *al.*, 2006). The oil components interact with cellular membranes, causing them to lose their integrity, as well as that of other structures that contain lipopolysaccharide molecules. There has also been a proposal that spontaneous molecular complexes create an increase in cellular permeability, encouraging the circulation of ions and macromolecules and causing death by causing a functional failure in the organism (Ralph 2008; Govindarajan *et al.*, 2008). An outer membrane called chorion surrounds mosquito eggs to provide protection to the embryos and to allow the exchange of gases and water with the outside through the aeropils. It has been described that ovicidal compounds can cross this barrier, preventing the hatching of the egg or interrupting the development of the embryo and thus the survival of the larva inside. A majority of the studies with essential oils evaluating their ovicidal activity have omitted not only the bioactivity found but also the mechanism by which this effect is induced. Nevertheless, what was previously mentioned by other writers about the lipidic properties of oils and their interaction with lipid membranes might have been related to the mechanism of action. In this work, we found that the doses used caused a moderate percentage of hatching of the exposed eggs, and this caused the larvae to die shortly after hatching. As a result of our study, it is inferred that the studied essential oil solutions were able to cross the blocks of the chorion, causing the larvae to emerge as a survival mechanism. The primary conditions that led to their emergence were also the conditions for which they emerged, which led to the death of first-stage individuals as a secondary consequence (Kuppusamy and Murugan, 2008).

Studies carried out by Jarial *et al.*,(2001) showed that the non- detachment of the exocorion in the eggs after being exposed to the extracts of *Allium sativum* caused the non-hatching of the embryos (Ismanet *al.*, 2008). Some authors stated that the eggs become waterproof once they harden, resulting in an inversely proportional relationship between concentration and hatching in young eggs (Giovanni Benelli, 2015; Jarial, 2001). The study by De Lima Santos *et al.* (2013b) suggested that the interaction between the lectin from *M. oleifera* results in the dissolution of embryos in eggs of different life stages subjected to the ovicide solution for 72 hours using chitin in their oocytes and chorion.

Warikoo *et al.*(2011) observed that the efficacy to act on the embryo inside the eggshell depends on an efficient penetration of the insecticide, which in turn is influenced by the exposure period which they have observed it against the *Sitophilusoryzae*; the same trend was observed in the present study also against the selected species of vector mosquitoes. The results disclosed that the treatment of 24 hrs was less effective in inducing a higher rate of mortality than 48 and 72hrs treatment. Ovicidal action of *Jasminum officinale* against *Aedes aegypti* at 200ppm after 48 hrs was 64.63%, but in 72 hrs, the mortality was 70.32%.

In the case of *Mentha piperita* at 200ppm after 48 hrs was 43.26% but in 72 hrs, the mortality was 60.55%. *Prunus dulcis* against *Aedes aegypti* showed 54.99% in 48 hrs and 70.89% in 72hrs under 200ppm. In the case of *Anopheles stephensi*, at 200ppm, *Jasminum officinale* showed 51.22% in 48 hrs and 50.33% in 72hrs. Ovicidal action of *Mentha piperita* against *Anopheles stephensi* at 200ppm after 48hrs was 43.21%, but in 72hrs, the mortality was 50.64%. In the case of *Prunus dulcis* against *Anopheles stephensi* at 200ppm after 48hrs, the mortality was 57.73% and in 72hrs, it was 70.33%.

In this study, the concentration of essential oils also played a crucial role in the effectiveness of the ovicidal activity. The highest mortality was observed in the highest concentration of plant oil. Broadbent and Prec (1984) reported more entry of the chemical inside the eggshell when eggs were directly exposed to higher concentrations of the compounds, which affected the embryogenesis. Similarly, longer exposure periods also facilitated the increased penetration of the compounds into the shell, thus increasing their effectiveness. Therefore the current study indicated that the ovicidal activity of the plant oils against *Aedes aegypti* and *Anopheles stephensi* depends upon factors such as concentrations of the plant oils and period of exposure.

## CONCLUSION

Today environmental safety is considered to be of paramount importance. For an insecticide to be considered acceptable, it does not need to cause high mortality rates in target organisms, but it should be an environmentally friendly insecticide. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts. In conclusion, we investigated the toxicity of three plant oils such as *Jasminum officinale*, *Mentha piperita* and *Prunus dulcis*. All three essential oils showed different ovicidal properties against *Aedes aegypti* and *Anopheles stephensi*. The public, in general, should be made aware of the benefit of using plant essential oils. We should spread awareness about the ill effects of chemical pesticides and the benefits of biopesticides to laypeople.

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