Larvicidal activity of plant oil of *Rosmarinus officinalis* against the selected mosquitoes larvae

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Article History
Received: 17.05.2018
Revised and Accepted : 03.03.2020
Published: 16.03.2020

https://doi.org/10.56343/STET.116.013.003.008
http://stetjournals.com

Abstract

Being a low-risk insecticide, plant essential oils emerge as competent mosquitocidal and repellent candidates. However, essential oil may act differently in different mosquito species and different developmental stages of same mosquito species. In the current investigation, we have investigated the bio efficacy of different concentrations of plant oil, *Rosmarinus officinalis,* with special reference to its larvicidal activity against the fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The LC_{50} and its LCL and UCL were 513.57 ppm, 468.88 ppm, and 555.57 for *Ae. aegypti,* 500.32 ppm, 456.84ppm and 541.01 ppm for *An. stephensi* and 527.84 ppm, 483.40 ppm and 570.01 ppm for *C. quinquefasciatus.* The LC_{90} and its LCL and UCL values were 945.11 ppm, 877.917 ppm and 1033.968 ppm, 912.7 ppm, 849.48 ppm and 995.52 ppm for *An. stephensi,* and 962.92 ppm, 894.39 ppm and 1053.70ppm for *C. quinquefasciatus,* respectively. These findings *i.e.,*larvicidal activity and phytochemical compounds of *R. officinalis*eli suggest its possible role in combating the larvae of *Ae. aegypti, An. Stephensi* and *C. quinquefasciatus*. Further studies regarding the application of this oil in the field will pave the way for development of new green mosquitocide in the future.

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Keywords : *Ae.aegypti, An. stephensi, C. quinquefasciatus*, larvicidal activity, plant oil

INTRODUCTION

Mosquitoes are the vectors of the major infectious diseases and a cause of Public Health concern such as Malaria, Dengue, Lymphatic filariasis, Yellow fever, Chikungunya and Zika virus, which cause morbidity and mortality in tropical and sub-tropical zones (SenthamaraiSelvan *et al*., 2015; Senthamarai Selvan and Jebanesan, 2016). WHO has declared the mosquitoes as "public enemy number one". Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people per annum and four crores of Indian population. Mosquito control is considered as essential to prevent the spreading of mosquito borne diseases and to improve quality of sustainable environment and the health status of public (Gokulakrishnan *et al*., 2012; Krishnappa and Elumalai, 2012a; Thomson, 2014; Rafael *et al*., 2019; Baranitharan *et al*., 2020). Earlier, synthetic mosquitocides such as organochlorine and organophosphate compounds were used as the major tool in mosquito control operation but, this has not been completely successful due to human, technical, operational, economical and ecological factors. The present practice of using synthetic chemical insecticides to control mosquito vectors has resulted in the development of serious resistance, persistent pollution and damaging the ecosystem.In past few decades, the indiscriminate application of several synthetic insecticides in mosquito control programme has been banned or limited. It is due to lack of novelty, high cost, concern for environmental sustainability, harmful effect on human health, and other non-target creatures, prolonged persistence in nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and

P - ISSN 0973 - 9157
E - ISSN 2393 - 9249

target specific insecticides against mosquito species. Considering these, the application of eco-friendly alternatives such as biological control of vectors has become the central focus of the control programme in lieu of the chemical insecticides (Krishnappa *et al.,* 2012a; Govindarajan *et al.,* 2015a; Govindarajan *et al.,* 2016a; Benelli *et al.,* 2017b; Rekha *et al.,* 2019).

Recently, essential oils have emerged as potential renewable, cost-effective, and environmentally benign alternatives to synthetic pesticides for control of mosquitoes. Exploration of plants and plant based secondary metabolites are one of the positive approaches under the biological control programme in mosquito control (Krishnappa and Elumalai, 2013, 2014; Govindarajan *et al*., 2016b). Furthermore, unlike conventional insecticides which are based on a single active ingredient, insecticides of plant origin comprised of spectrum of chemical compounds which act concertedly in many processes by disturbing the insect's physiology or morphology. Hence, there is very little chance of developing resistance to such substances by the mosquitoes. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have broad spectrum of insecticidal properties and will obviously work as a new armament in the future that may act as suitable alternative product in combating the mosquitoes (Govindarajan and Benelli, 2016c; Krishnappa *et al*., 2019). Hence, in the present investigation, the larvicidal activity of *Rosmarinus officinalis* was investigated on the fourth instar larvae of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*.

MATERIALS AND METHODS

Preparation of the oil for the bioassay

Plant oil, *Rosmarinus officinalis,* was purchased from the distributor. 0.50mg, 0.100mg, 0.150mg, 0.200mg and 250mg oil was weighed and mixed with 250ml of

Table 1. Larvicidal activity of *R. officinalis*oil tested against the freshly moulted (0-6h old) fourth instar larvae of selected mosquito species.

Concentration (ppm)	Mortality* (%)	LC_{50} (LCL - UCL)	LC_{90} (LCL - UCL)
Aedes aegypti			
Control	1.8±0.8[a]	513.57 (468.88-555.57)	945.11 (877.917-1033.968)
200	17.8±1.2[b]		
400	36.2±1.2[c]		
600	63.2±1.8[d]		
800	75.4±2.2[e]		
1000	94.6±2.8[f]		
Anopheles stephensi			
Control	1.8±0.4 [a]	500.32 (456.84-541.01)	912.7 (849.48-995.52)
200	18.4±1.4 [b]		
400	36.4±1.6 [c]		
600	64.4±2.4 [d]		
800	78.6± 2.6 [e]		
1000	95.8±3.2 [f]		
Culex quinquefasciatus			
Control	1.8±0.8 [a]	527.84 (483.40-570.01)	962.92 (894.39-1053.70)
200	17.2±1.2 [b]		
400	33.4±2.2 [c]		
600	63.2±2.6 [d]		
800	73.4±3.4 [e]		
1000	93.8±3.6 [f]		

Values expressed mean ± SD of five replications. Values with different alphabets in the column differ statistically (DMRT, p<0.005)

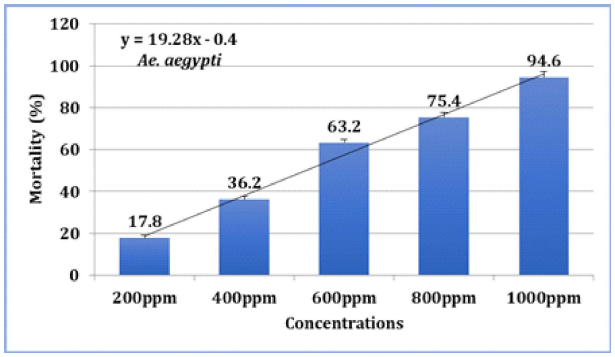

Fig. 1. Larvicidal activity of *R. officinalis*tested against the larvae of *Ae. aegypti.*

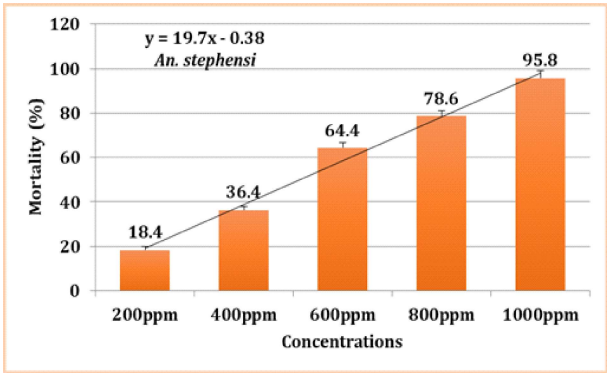

Fig. 2. Larvicidal activity of *R. officinalis*tested against larvae of *An. stephensi.*

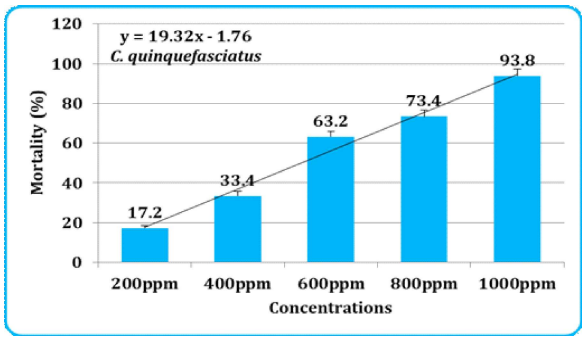

Fig. 3. Larvicidal activity of *R. officinalis*tested against the larvae of *C. quinquefasciatus*

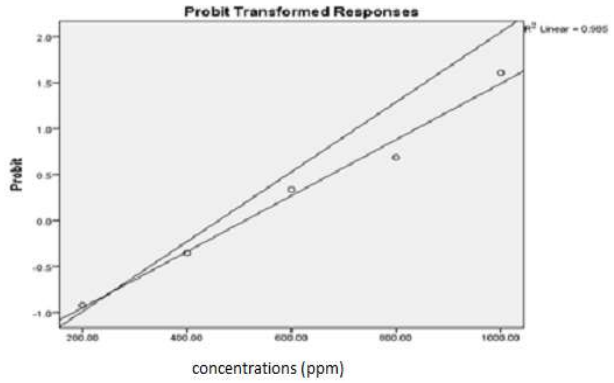

Fig. 4. Probit transformed responses observed on *R. officinalis* tested against the *Ae. aegypti*larvae.

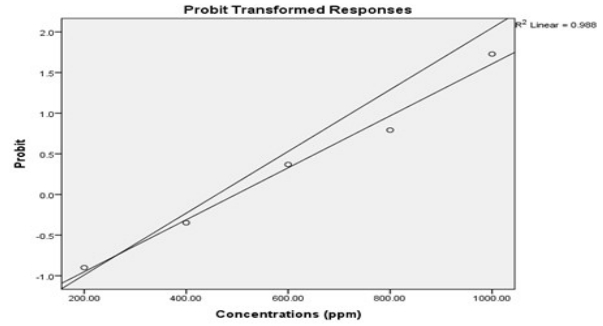

Fig. 5. Probit transformed responses observed on *R. officinalis* tested against the *An. stephensi* larvae.

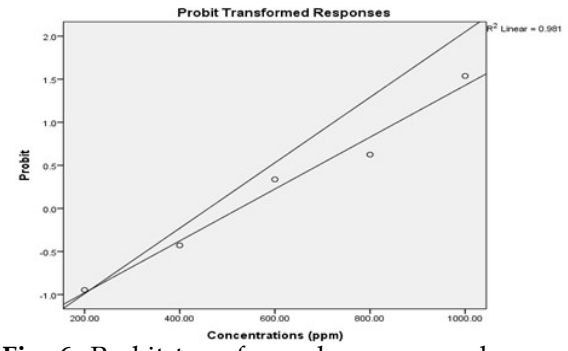

Fig. 6. Probit transformed responses observed on *R. officinalis*tested against the *C. quinquefasciatus* larvae.

double distilled water to obtain 200ppm, 400ppm, 600ppm, 800ppm and 1000ppm concentrations, respectively, with this 0.05ml of dimethyl sulphoxide (DMSO) was used as an emulsifier to blend the oil with water.

Larvicidal bioassay

The larvicidal activity of the selected oil at various concentrations were studied following the the method described by the World Health Organization (WHO, 2005). Fourth instar larvae of *Ae. aegypti, An. stephensi and C. quinquefasciatus* were used in the present study. Twenty larvae were placed in a paper cup with 250 ml of aqueous suspension of test material at various concentrations, and an emulsifier dimethyl sulfoxide (DMSO) was added in the final test solution (0.05%). Five replicates per concentration were maintained simultaneously and with each experiment, a set of control using 0.05 % DMSO and acetone and untreated sets of larvae in distilled water, were also run for comparison. The assay was carried out in the laboratory and the larval mortality was recorded after 24 h of exposure.

Statistical analysis

Per cent mortality was corrected for control mortality using Abbott's formula (Abbotts, 1925). Results from

all replicates for the oil were subjected to probit analysis using SPSS (v20.0) to determine $LC_{50}$and LC_{90}values and their 95 % confidence intervals (Sakuma, 1998). Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

RESULTS

The larvicidal activity of *R.officinalis* was tested against the fourth instar larvae of *Ae. Aegypti, An. stephensi and C. quinquefasciatus* with 200, 400, 600, 800 and 1000 ppm concentrations. The data pertaining to the experiment are shown in Table 1 and Figures 1 to 3. It was observed that 17.8±1.2, 36.2±1.2. 63.2±1.8, 75.4±2.2 and 94.6±2.8% larval mortality at 200, 400, 600, 800 and 1000 ppm concentrations respectively, against the larvae of *Ae.aegypti*. Similarly, 18.4±1.4, 36.4±1.6, 64.4±2.4, 78.6± 2.6 and 95.8±3.2% larval mortality at 200, 400, 600, 800 and 1000 ppm concentrations respectively, against the larvae of *An. stephensi*. Likewise, 17.2±1.2, 33.4±2.2, 63.2±2.6, 73.4±3.4 and 93.8±3.6% larval mortality at 200, 400, 600, 800 and 1000 ppm concentrations, respectively, against the larvae of *C. quinquefasciatus*. The LC_{50} value of 513.57ppm was recorded with the LCL and UCL values of 468.88 and 555.57ppm, respectively ,which was noted against the larvae of *Ae. aegypti.* In the same way, the LC_{90} value of 945.11ppm was recorded with the LCL and UCL values of 877.92 and 1033.97ppm, respectively, against the larvae of *Ae. aegypti*. The LC_{50} value of 500.32ppm was recorded with the LCL and UCL values of 456.84 and 541.01ppm, respectively, was noted against the larvae of *An. stephensi*. In the same way, the LC_{90} value of 912.70ppm was recorded with the LCL and UCL values of 849.48 and 995.52ppm, respectively, against the larvae of *An. stephensi*. The LC_{50} value of 527.84pm was recorded with the LCL and UCL values of 483.40 and 570.01ppm, respectively, was noted against the larvae of *C. quinquefasciatus*. In the same way, the LC_{90} value of 962.92ppm was recorded with the LCL and UCL values of 894.39 and 1053.70ppm, respectively, against the larvae of *C. quinquefasciatus*. At the same time, the probit responses of the plant oil of *R. officinalis* oil tested on the fourth instar larvae of *Ae. aegypti*, *An. Stephensi* and *C. quinquefasciatus* showed incremental responses to the concentrations tested in the present experiment and the respective figures are shown in Figures 4-6.

DISCUSSION

In the present investigation, plant oil of *R.officinalis* oil showed statistically significant activity against the fourth instar larvae of *Ae. aegypti, An. Stephensi* and *C. quinquefasciatus*. Among the three kinds of larvae, *C. quinquefasciatus*.was more susceptible than *Ae. aegypti* and *An. stephensi*. Furthermore, while conducting the experiment the application of oil with the respective concentration formed a thin film over the surface of the experimental cups, suggesting that the oil film could prevent the further exchange of gases in to the medium of experimental cups thereby causing death of the larvae by asphyxia. In more recent publications, it was also noted that the plant oils have promising insecticidal activity. Compared to the results of these few studies the larvicidal activity was more higher in this study for mosquitoes than that findings reported earlier. The high level of larvicidal activity of the plant oil of *R. officinalis*is could possibly be due to the higher concentration of the major insecticidal compounds present in it. *The* present findings are hand in hand with the earlier findings of several authors. Earlier, Sarita Kumar *et al*. (2012) studied peel hexane extract of *C. limetta* and reported higher larvicidal potential against *An. stephensi* and the phytochemical study of the said extract showed the presence of terpenoids and flavonoids . Correspondingly, the results of the present studies are on par with previous reports that the 15 per cent *C. limetta* oil exhibited highest percentage of larval mortality and knockdown effects as elucidated by Prakash Rao *et al*. (2016). Rosalinda (2016) reported that hexane extract of *C. grandis* peel tested for 3rd and 4th instar larvae of *Ae. aegypti* and showed maximum activity. *C. limetta* oils are also had prospective larval killing activity and have knockdown effects (Manimaran *et al*., 2012; Mallik *et al*., 2016). Manh *et al*. (2020) showed the larvicidal activity of essential oils extracted from *Cymbopogon citratus, Cymbopogon winterianus, Eucalyptus citriodora,* and *Eucalyptus camaldulensis,* and the aromatic plants grown in Vietnam when tested on *Ae. aegypti* larvae and found to be the most efficient against *Ae. aegypti.*The larvicide based on food grade orange oil encapsulated in yeast was shown to be highly active against all larval stages of *Ae. aegypti.* These findings demonstrated its potential for incorporation in an integrated approach to larval source management of *Ae. aegypti.* This novel approach can enable development of affordable control strategies that may have significant impact on global health. (Workman *et al*., 2020). Aksorn Chantawee and Mayura Soonwera (2020), evaluated larvicidal, pupicidal and oviposition deterrent activities of four plant essential oils from *Alpinia galanga* (L.) Willd rhizome, *Anethum graveolens* L. fruit, *Foeniculum vulgare* Mill.fruit, and *Pimpinella anisum* L. fruit against *Ae. aegypti*. and showed that *A. graveolens* oil had a good potential as a larvicidal, pupicidal and oviposition deterrent agent for controlling *Ae. aegypti.* Zhang *et al*. (2020) in a study of bioassay on essential oil which is extracted from the leaf of *Cinnamomum camphora*(L.) against *A. stephensi* showed that the essential oil of *C. camphora*leaf had an excellent larvicidal potential for the control of *A. stephensi*. Rijusarma *et al*.,(2019)

prepared twenty-eight combinations of plant essential oil-based terpene compounds were and tested against larval and adult stages of *A. aegypti* and the result revealed that the combination of Temephos and Diallyldisulfde and combination of Malathion and Eudesmol were the most effective combination. These effective combinations bear potential prospect to be used against *A. aegypti.*

CONCLUSION

Indiscriminate application of chemical pesticides in general,mosquitocides in particular cause an imbalance in the environment as well as to the human health . Thus, scientific community world wide started to search for new and a novel alternative chemicals of plant origin. In this context, application of plant oil to control the mosquito larvae proved to be the best strategy in integrated vector control program. As, a part of it, the present investigation of larvicidal activity of plant oil of *R. officinalis* elicited its possible role in combat with the larvae of *Ae. aegypti, An. Stephensi* and *C. quinquefasciatus*. Thus, in a nut shell, the essential oil of *R. officinalis* could be promoted as an efficient larvicidal agent, which is more effective against *C. quinquefasciatus* than *A. aegypti* and *An. stephensi*. As natural products are favoured in vector control measures due to their less deleterious effect, such kind of studies could encourage a researcher to explore new alternatives to synthetic repellents and insecticides.

ACKNOWLEDGEMENT

Authors are thankful to the Head, Department of Zoology and The Principal, Govt. Arts College for Men (Autonomous), Nandanam, Chennai for providing the laboratory facilities.

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