Larvicidal and Ovicidal Effect of *Ocimum basilicum* L (ဝင်စိမ်း) Leaves Essential Oil on Dengue Vector *Aedes aegypti*

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Mosquito-borne diseases a public health problem in Myanmar. Dengue viruses are mostly harmful the children group. Larvicidal and ovicidal properties of *Ocimum basilicum* leaves essential oil was tested on the First filial generation (F1) of Laboratory reared *Aedes aegypti* larvae from Dagon Myothit North from June 2019 to May 2020 according to World Health Organization (WHO). The essential oil was extracted from 300 garms of fresh basil leaves using steam distillation at 100 °C for 3 hours, resulting in a yield % of 3.39 (w/w) essential oil. Different concentrations of *Ocimum* leaves essential oil was prepared in 100ml each of distilled water in 150 ml plastic cups. In the laboratory, 50 each of *Aedes* larvae were exposed for one hour to determine knockdown effect and 24 hrs for mortality in different concentrations. Five replicates were done. Results found that the maximum dose 0.1g *Ocimum* leaves essential oil produced 98.6% knockdown within 60minute and 98.4% mortality within 24hrs respectively and 100% ovicidal effect for 3 days as well as persistency was observed 100% mortality of larvae for 4 days. The effective lethal concentrations LC_{50} and LC_{90} values were observed 0.017 g and 0.0576 g of leaves essential oil. Therefore, the *Ocimum basilicum* leaves essential oil can be used in public sector as larvicide and ovicide of *Aedes* mosquitoes to control Dengue fever and Dengue hemorrhagic fever transmission via to control *Aedes* mosquito population.

Keywords: Essential oil, *Ocimum basilicum*, Dengue, *Aedes aegypti*, Knockdown, Mortality, Larvicidal, Ovicidal

INTRODUCTION

Mosquitoes are largest group of insects and they are act as intermediate hosts in the transmission of different principal human diseases which are malaria, dengue fever, yellow fever and filariasis. Three main groups of mosquitoes which are *Aedes*, *Culex* and *Anopheles*. *Aedes aegypti* is one of the faster allocated mosquitoes and mostly found in tropical and sub-tropical regions in the world and responsible vector for Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), Chikungunya and Yellow fever.[1] Dengue is an arbovirus disease it coursed DF and DHF and its subsequent Dengue Shock Syndrome (DSS) and have four serotypes of dengue virus. Transmission of the disease can be reduced by community participation in vector control.

Culex quinquefasciatus transmit filariasis. *Anopheles dirus* and *An. minimus* transmit malaria.[2] World Health Organization revealed that 3.9 billion people inhabiting 128 countries at risk of dengue infection in the World.[3] In India, dengue fever and Chikungunya are main Public Health Problem.[4] DHF, malaria, filariasis are harmful

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to human and they are a public health problem in Myanmar. Ministry of Health reported that considerably increase the number of dengue cases in Yangon, Mandalay, Mawlamyaing and Hpa-an in last few years.[5]

Vector of mosquitoes can be controlled by bio-control agent as larvivorous fish, and insect growth regulators as well as insecticides. However, unsystematic use of insecticides is creating various problems and they become resistance. Mostly synthetic organic insecticides have been used for mosquitoes control and these are toxic to environment by infecting the air, soil and water. Therefore, there is a need to control *Aedes* mosquito using alternative method which is cheap and effective, environmental safety, indigenous and biodegradable methods in community to avoid toxicity in air, soil and environment. Many active insect-cidal materials as nicotine, pyrethrins and rotenones have been extracted from plant sources.[6]

Among the plants sources *Ocimum basilicum* L is a potential plants species and their leaves was used for cooking and traditional medicine.[6, 7] In China, India, Myanmar, Thailand, Malaysia and Indonesia, have been used its leaves, fruits, barks and roots as traditional medicine and apart from use as food flavoring and appetizer. In Myanmar, *Ocimum basilicum* L leaves essential oil and leaves powder, fruits, seed, barks and roots are effective for formulation of pain release traditional medicines. It chemically contains a low percentage of volatile and essential oils and is used in the conventional medicine to alleviate pain, treat stress, mitigate vomiting and even as an insect repellent.[8] In Myanmar, India, Cylone, several Mediterranean countries, many parts of Asia, Africa and the Pacific Islands, *Ocimum basilicum* L plants are abundantly present in hilly areas.[9] When the plants height reached to 15-20 cm, the leaves can be picked any time. Chemical constituents of *Ocimum basilicum* L. leaves fresh essential oil containing little amount of estragol, eucalyptol, ocimene, linallol acetate, eugenol, 1-epibucyclose-squiphellandrene, menthol, menyhone, cyclo-hexanol, Cyclohexanone, myrcenol and nerol.[8] The essential oils in some article reported to possess other activities such as antibacterial, antifungal, mosquito larvicidal and repellent.[10] However, there is no reports of *Ocimum basilicum* L leaves essential oil on *Aedes* mosquitoes has been mentioned as an insecticidal agent in Myanmar. Therefore, attempt has been made to investigate the larvicidal and ovicidal action of *Ocimum basilicum* L leaves essential oil extract against *Aedes aegypti* larvae to support the future vectors control activity in environmental sound manner.

MATERIALS AND METHODS

Study area and study period

The study was conducted in the 31st Ward of in Dagon Myothit North Township and it focus on an area with a population of approximately 80,000 people residing in 13,000 houses. The key points about the lack of government piped water supply and the reliance on tube wells and storage containers for water remain relevant in this context. Most of the people are government staff, company staff, merchants, security persons, some are teachers, Doctors, and some are working as sellers and most of the residential are dependent. Study period for larval collection and larvicidal test was done June 2019 to May 2021.

Larval collection

Aedes mosquito larvae were collected from water storage containers in 31st Ward Dagon North Township Yangon Region. All collected *Aedes* larvae were carried by labeled plastic bags and supplying the battery oxygen pumps to Medical Entomology Research Division, Department of Medical Research. The larvae were fed on a larva food from DMR. The adult mosquitoes that emerged from larvae were provided with a 10% sucrose solution mixed with burplex. Larvae were fed on Entomology made larva food (1 Liver meal: 1 fish meal: 1 been powder: 1 rice powder). Adults that emerged from larvae were supplied with 10% sucrose

solution with burplux and eight weeks mice to feed blood meal for eggs maturation of colonization mosquitoes. Mosquitoes were reared at 26±2°C, 65-75% relative humidity with a photo period of 12 -hours light and 12-hours dark. Laboratory reared First filial generation (F1) of *Aedes aegypti* larvae were used for testing larvicidal efficacy of stream distillated essential oil of fresh *Ocimum basilicum* L. leaves from Mingaladon Township, Yangon Region.

Species identification of mosquitoes

For species of mosquito identification, adult mosquitoes that emerged from the larva survey were morphologically identified according to the keys of Rampa and Prachong.[11]

Collection and extraction procedure of *Ocimum basilicum* L leaves essential oil

Ocimum basilicum L. leave of (Myanmar Pinsein) was collected from agriculture farm in Htauk Kyant, Mingaladon Township, Yangon, in May because essential oil content in plant was high in the summer, for extraction of essential oil. A total of 10 Kilo grams of *Ocimum basilicum* L. leave was cleaned and 300 g each of *Ocimum basilicum* L. leave was extracted with 1000 ml distilled water by stream distillation method at 100 °C for 3 hours. Complete removal of the solvent from the extract was accomplished in glass rotary evaporator and 3.39 g of essential oil was obtained from 300 g of fresh *Ocimum basilicum* L. leave. The yield % of essential oil was 3.39% (w/w) and it was kept at 4°C in refrigerator until use. The extraction was done in Department of Pharmacology, University of Medical Technology (Yangon).

Larvicidal testing procedure

Larvicidal activity of the essential oil from *Ocimum basilicum* leaves was evaluated in a laboratory. According to the preliminary tests, different concentration of *Ocimum basilicum* L. leave essential oil as 0.1 g, 05 g, 025 g, 0.0125 g and 0.00625 g were prepared freshly in 100 ml each of distilled water in 150 ml plastic cups. Fifty (50) 3rd and 4th instars *Aedes aegypti* larvae were put into each concentration and simultaneously, negative control test was done. Detail testing was done according to WHO standard method.[12] Five replicates were done in laboratory at 27-29°C and 70-80% relative humidity. Knockdown was recorded after 60 minutes exposure period and mortality was recorded after 24 hours of exposure periods. Knockdown (failed to move upper surface and anywhere) and dead larvae were identified when the larvae failed to move after probing with a needle in the thorax region of the body.

Ovicidal test

The Ovicidal study was followed by the method of WHO.[12] Laboratory reared Dagon Myo Thit North strain of five to seven days old fully gravid female *Aedes aegypti* mosquitoes 100 were kept in with the dimensions of (59x59x59cm) steel net cage. The *Ocimum basilicum* L leaves essential oil was diluted in distilled water to achieve various concentrations ranging from 0.00625 g, 0.0125 g, 0.025 g, 0.05 g and 0.1 g. These diluents were carefully put into a fully gravid exposed steel cage and simultaneously distilled water in a plastic cup was exposed as control for one day. After one day of oviposition the diluents and control cups were kept for 4 days for fully eggs hatching and amount of egg and hatched larvae were recorded. The test was carried out in a (12x15x15 ft) room at 24-28°C and relative humidity of 70-85%. The three replicates were done in each experiment.

Gas chromatography-mass spectrometry analysis (GC-MS)

The qualitative and quantitative analysis of the sample (*Ocimum basilicum* L. leaves essential oil) was carried out by using GM/MS technique (model GC/MSQP2010-Ultra, Japans' Simadzu Company), GCMS model number Trace 1300 ISQ QD and capillary column (Rtx-5ms-30m×0.25mm ×0.25 μm). The sample was injected by using split mode, instrument operating in EI mode at 70eV. The carrier gas Helium passed with flow rate 1.69 ml/min, the temperature program was started from 50°C

with rate 7°C/min to 180°C then the rate was changed to 10°C/min reaching 280°C as final temperature. The injection port temperature was 300°C. The ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of 40-500 m/z charges to ratio and the total run time was 28 minutes (British Pharmacopoeia 2007). Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents were available in the Library of West Yangon University and results were recorded.

Data analysis

The larval mortality data from larvicidal and ovicidal tests were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence of upper and lower confidence limit, and Chi-square values were calculated using Finney.[13] Results with $p<0.05$ were considered to be statistically significant. The hatching rate was measured after 48 hours of treatment according to following formula.[14]

$$\% \text{ of egg mortality} = \frac{\text{Number of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

RESULTS

In Table 1, highest mortality of *Aedes aegypti* larvae was found 98.40% at 0.1g essential oil dilution and followed by 81.20% at 0.05g dilution of *Ocimum basilicum* leaves essential oil. Lowest mortality was found 16.40% of larvae at 0.00625 g dilution of essential oil.

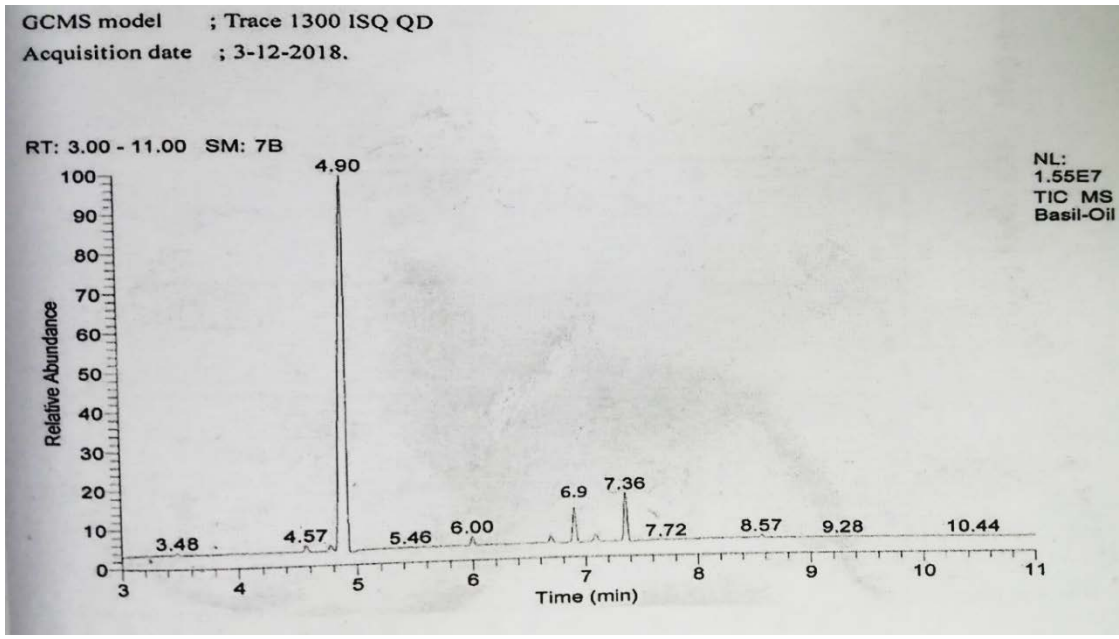

Fig. 1. Gas Chromatography profile of *Ocimum basilicum* leaves essential oil

Table 2 shows that dose effect analysis of LC_{50} and LC_{90} values of *Ocimum basilicum* leaves essential oil against 3rd and 4th instars *Aedes aegypti* larvae were found 0.0178g for 50% mortality and 0.0576g for 90% mortality, respectively, $p<0.05$, $X^2=0.0620$.
Table 3 shows that 0.1 g of *Ocimum basilicum* leaves essential oil can ovicide the eggs for 3 days, at the fourth day, 42% of eggs were destroyed and 58% of the eggs were hatched to 1st instar larvae. 0.05% concentration of *Ocimum basilicum* leaves essential oil can ovicide for one day. Other concentrations were found no ovicidal effect on *Aedes* eggs.

Table 1. Knockdown and mortality effect of different concentrations of *Ocimum basilicum* leaves essential oil against 3rd and 4th instar *Aedes aegypti* larvae

Concentration (gm)	Total larvae	Knock-down	% knock-down	Mortality	% mortality
0.1	250	247	98.80	246	98.40
0.05	250	178	71.20	203	81.20
0.025	250	89	35.6	148	59.20
0.0125	250	48	19.2	84	33.60
0.00625	250	25	10.0	41	16.40
Control	250	0.00	0.00	0.00	0.00

Table 3. Ovicidal effect of *Ocimum basilicum* leaves essential oil against *Aedes* eggs in laboratory

No.	Concentration	Duration and eggs mortality (%)			
		Day 1	Day 2	Day 3	Day 4
1	0.1	100	100	100	42
2	0.05	100	78.6	34.3	11.2
3	0.025	75	38.8	12.6	0
4	0.0125	32.24	17.5	0	0
5	0.00625	9.6	0	0	0
6	Control	0	0	0	0

Table 2. LC_{50} and LC_{90} values of *Ocimum basilicum* leaves essential oil on 3rd and 4th instar *Aedes aegypti* larvae

Treatment	Hours	Essential oil	X^2, p<0.05	df	LC_{50} confidence upper, lower and corrected limits (95% confidence interval)	LC_{90} confidence upper, lower and corrected limits (95% confidence interval)
Ocimum basilicum leaves essential oil	24	Oil of *Ocimum basilicum* leaves	0.1248 P<0.05	4	Upper 0.0177g Lower 0.0173g Corrected 0.0175g	Upper 0.0717g Lower 0.0502g Corrected 0.061g

X^2=Chi squire, P=probability, df= degree of freedom

Fig. 1. shows that according to the gas chromatography-mass GC-MS spectrometry analysis, total four peaks were detected as active major constituents such as Linalool at 4.90RT, Geranyl isobutyrate at 6.00RT, Citronellene at 6.90RT and Geranial or Citral A at 7.36 RT in essential oil of *Ocimum basilicum* L leaves.

DISCUSSION

In the tropic region, malaria, yellow and dengue fevers, are a major public health problem and transmitted to over 2 billion people.[1] Over 1005 plant species are found to possess insecticidal properties, of these 384 contained anti-feed ants, 297 contained repellents and 27 contained attractants and growth inhibitors. All these indicate that the plant kingdom is a vast store-house of potentially useful plant chemicals for pest control. The leaf and seed extract of plant *Agave Americana*[15] had mosquito larvicidal properties, and the extract of *Tagetes minuta* flowers had mosquito larvicidal activity against *Aedes aegypti*.[16]

In the present study, *Ocimum basilicum* leaves essential oil concentration 0.1g/100 ml water found 96.80% knockdown and 98.40% mortality and LC_{50} and LC_{90} values were found 0.0178g and 0.0576g. Same larvicidal effect has been found the essential oil of Orange, Lemon and Orange plus Lemon mixture at concentration of 0.01g/100ml water caused 98.33%, 100% and 96.67% mortality rate of 3rd and 4th instar *Ae. aegypti* larvae.[17]

This finding revealed that Lemon essential oil (0.01g/100ml) was found 100% mortality and had a higher potency than the present *Ocimum basilicum* leaves essential oil 0.1 g/100 ml. Maung Maung Mya and his associates revealed that *Citrus hystrix* DC leaves, fruit and fruit peel extracts were found to very effective and high mortality against 3rd and 4th instar larvae and LC_{50} and LC_{90} values were found 1.73% and 2.08% concentrations for leaves extract. 0.0138 g and 0.0515 g for fruit extract, 0.0142g and 0.0522 g for peel extract.[18, 19]

Singhi *et al.*,[20] have reported that the latex of *Citrus procera* has shown larvicidal efficacy against all three important vector species as

Ae. aegypti, An. stephensi, and *Cx .quinque-fasciatus* in India. The insecticidal activity of *Zingiber officinale* against *Anopheles pharoensis* 3rd stage showed 100% larval mortality rate.[21] Other study of bio-larvicide activity testing of Orange peel, Lemon peel and Orange and Lemon peel essential oil mixture were analyzed and found that LC_{50} and LC_{90} values of lemon essential oil 0.001 g and 0.004 g found highest bio-larvicide property against 3rd and 4th instar *Aedes* larvae was more toxic and effective as bio-larvicide.[17]

Mosquito insecticide and repellent properties were found in many plant extracts on vector mosquitoes although very little amount of plant extract have been found practically useful to repeal and kill the vector mosquitoes. In India, many species of plants such as *Cleome viscosa, Ocimum basilicum, Vitex negundo, Delonixregia, Oligo chaetaramosa, Azadirachta indica, Ouassia amara, Anacardium occidentale, Thevetianerii folia* etc. were tested on different species of mosquito larvae. Naturally extracted plants materials are favorable due to high degree of ecofriendly, easily degradability and without or fewer toxic than the synthetic chemicals.[22]

In the present study, ovicidal properties of *Ocimum basilicum* leaves essential oil 0.1 g in 100ml water could be ovicided 100% of *Aedes* eggs for 3 days. Although a researcher revealed that ovicidal activity of castor seed oil against *An. stephensi*, *Cx. fatigans* and *Ae. aegypti* mosquitoes successfully in laboratory.[23] The ovicidal activity of neem products *Azadirachtin* against mosquitoes *Cx. tarsalis* and *Cx. quinquefasciatus* was found potential effect of ovicidal on both mosquito eggs.[24] These ovicidal effects of the different plants extracts were agreed with the present *Ocimum basilicum* leaves essential oil against *Aedes* eggs. It may be due to the fact that the chemical constituents of *Ocimum basilicum* leaves essential oil like Linalol, Gerantyl isobutyrate, Citronellene and Geranial may be more effective phyto-chemical to destroyed eggs and larvae of *Aedes* mosquitoes.

According to the gas chromatography-mass GC-MS spectrometry analysis, four active major constitutes were observed in *Ocimum basilicum* L leaves essential oil which were Linalool at 4.90RT, Geranyi isobutyrate at 6.00RT, Citronellene at 6.90RT and Geranial or Citral A at 7.36 RT in *Ocimum basilicum* L. leaves essential oil. Other researcher observed that there is different morphological structure, texture, and chemical contents of *Ocimum basilicum* L. plants species were available.[4] In *Ocimum basilicum* L. plants consist of small percentage of essential oils, volatile oils, linalool, lineol, geraniol and poly-phenolic acids. These are very useful to make conventional medicine as sooth pain, treat vomiting and stress and insect repellent. Fifty-one compounds were characterized and identified by GC–MS, comprising 100% of the total oil.[25]

Conclusion

The study was conducted in laboratory for Larvicidal and Ovicidal properties of the indigenous plants *Ocimum basilicum* L leaves essential oil against *Aedes* larvae and eggs. 0.1g of *Ocimum basilicum* L leaves essential oil can destroy 98.40% of 3rd and 4th instar *Aedes* larvae within 24 hours and 100% of ovicidal properties for 3 days. LC_{50} and LC_{90} values of *Ocimum basilicum* L leaves essential oil was found to be 0.0178 g and 0.0576 g, respectively. The chemical constituents of *Ocimum basilicum* leaves essential oil were observed Linalol, Gerantyl iso-butyrate, Citronellene and Geranial by GC-MS technique and these were more effective phytochemical to destroyed eggs and larvae of *Aedes* mosquitoes. Larvicidal and Ovicidal properties of the indigenous plants *Ocimum basilicum* L leaves essential oil *against Aedes* mosquito larvae was found very effective to control *Aedes* larvae and eggs.

Therefore, the essential oil can be reduced the dengue infection via to reduce the *Aedes* mosquito population and it could be suitable for the use in mosquito control programme for a Public Health purpose.

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