Journal of Biological Engineering Research and Review, 2021; 8(1): 19-25
ISSN: 2349-3232 Online
Available online at www.biologicalengineering.in/Archive

Research Article

Larvicidal activity of Cymbopogon nardus (L) Rendle leaves and roots essential oil against Aedes larvae in Yangon Region, Myanmar

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ARTICLE INFO:

Article History:
Received: 23/04/2021
Revised: 20/05/2021
Accepted: 23/06/2021
Published: 30/06/2021

Keywords:
Larvicidal activity, Cymbopogon nardus, Myanmar, Mosquito-borne diseases, Aedes aegypti, Roots essential oil

Citation: Mya MM, New CT, Aung ZZ, Myint YY, Mu TN, Thaung S, Maung YNM, Htun MM. Larvicidal activity of Cymbopogon nardus (L) Rendle leaves and roots essential oil against Aedes larvae in Yangon Region, Myanmar Journal of Biological Engineering Research and Review. 2021, 8 (1): 19-25.

Abstract: - Mosquito-borne diseases are mostly harmful to children, and they are a public health problem in Myanmar. From April 2019 to March 2020, laboratory-reared Dagon Myothit North strain *Aedes aegypti* larvae were used to test the larvicidal properties of the Paung strain of *Cymbopogon nardus* (L) Rendle's leave and root essential oils. 100 grams of dried leaves and roots were separately extracted by the steam distillation method at 100°C for 3 hours and yielded 0.92 gm and 0.87 gm of essential oils. According to WHO, fifty third and fourth instar *Aedes aegypti* larvae were placed in 150 ml plastic cups with freshly prepared different concentrations of leaves and roots essential oil in 100 ml each of distilled water. Five replicates were done in the laboratory (26°C, 80%RH). The result revealed that 0.01g of leaves and root essential oil produced 100% knockdown within 60 minutes and 97.6% and 96.8% mortality within 24 hours, followed by 95.56%, 95.20% knockdown and 91.6 and 90.8% mortality at 0.005g, respectively. The effective lethal concentrations (LC_{50} and LC_{90} values) for leaf essential oils were determined to be 0.0015g and 0.00499g, respectively, and 0.0014g and 0.00524g for root essential oils. The LC_{50} and LC_{90} values of essential oils extracted from leaves and roots were not significantly different. Although leaves essential oil was found to be more effective than root essential oil against *Aedes* larvae within 24 hours. The essential oil of *Cymbopogon nardus* leaves and roots exhibited strong larvicidal activity against *Aedes* larvae and can be used as an alternative biological control of *Aedes* larvae.

INTRODUCTION

Mosquito-borne diseases such as Dengue fever (DF), Dengue Haemorrhagic Fever (DHF), malaria, filariasis, Japanese encephalitic (JE), and chikungunya are harmful to humans, and they are a public health problem in Myanmar [1]. Billions of people, primarily in tropical countries, are at risk from such diseases, which are transmitted by three different genera of mosquitoes: *Aedes, Culex,* and *Anopheles* species [2]. *Aedes aegypti* is one of the major vectors for dengue fever *(*DF), dengue hemorrhagic fever (DHF), chikungunya and yellow fever. *Culex tritaeniorhynchus* transmits Japanese encephalitis, and *Culex quinquefasciatus* is a major vector of filariasis. *Anopheles dirus* and *An. minimus* are major vectors of malaria in Myanmar. Mosquito vectors are generally controlled by insecticides, bio-control agents, and insect growth regulators. To control mosquitoes and mosquito-borne diseases, which have a worldwide health and economic impact, synthetic insecticide-based interventions are still necessary, particularly in situations of epidemic outbreaks and sudden increases of adult mosquitoes [3]. However, the indiscriminate use of conventional insecticides is fostering multifarious problems. 85 to 90% of applied pesticides never reach target organisms. The mosquito control approach has been almost completely based on synthetic organic insecticides. It can cause a chemical hazard. Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air. Therefore, there is a need for alternative methods for environmental safety, biodegradable, low-cost, and indigenous methods for vector control. In an attempt to overcome these problems, great emphasis has recently been placed on research and development of pest control using natural plant products. Many active insecticidal materials have been derived from plant sources, i.e. nicotine, pyrethrins, and rotenones. Orange, lemon, and kaffir lime leave extract have been used as pest control against rice weevil *Sitophilus oryzae* infestation in stored rice and as mosquito repellent as well as larvicide [4-6].

Among the plants investigated to date, one showing enormous potential is the *Cymbopogon nardus* (L) Rendle, whose plant was collected from Kalama Mountain, Paung Township, Mon State. *Cymbopogon nardus* (L) Rendle,

Ocimum basilicas L and *Eucalyptus globulus* are familiar through the use of their leaves for culinary purposes and traditional medicine [7,8]. Apart from being used as a food flavoring and appetizer, its leaves, barks, fruits, and roots have been used as traditional medicine in Asian countries such as China, India, Myanmar, Thailand, Malaysia, and Indonesia [7-9]. In Myanmar, essential oil extract of *Cymbopogon nardus* (L) Rendle leaves and roots powder and essential oil are very useful for making pain-relieving traditional medicines and cooking used as food flavoring. These plants are available in many parts of the country, although they are abundantly present in hilly areas of Myanmar, India, and Cylone, several Mediterranean countries, many parts of Asia, Africa, and the Pacific Islands [10, 11]. It chemically contains a low percentage of volatile and essential oils and is used in conventional medicine to alleviate pain, treat stress, mitigate vomiting and even as an insect repellent [7,10]. *Cymbopogon nardus* (L). Rendle leaves and roots can begin to be harvested any time after the plants have reached a height of 20–60 cm. The essential oil of *Cymbopogon nardus* (L) Rendle plant obtained by steam distillation with a yield of 1.3±0.11% was analyzed by chromatographic method (GC/MS) and the main oil compounds were citronellal (37.87%), nerol (19.88%), citronellol (9.11%), elemol (7.40%), and gamma murollene (4.65%) [12].

Ocimum basilicum L and *Eucalyptus globulus* leaves yield essential oils containing a small amount of estragol, ocimene, linallol acetate, eugenol, 1-epibicyclosesquiphellandrene, menthol, menthone, cyclohexanol, cyclohexanone, myrcenol and nerol [8]. Essential oils have also been reported to possess antibacterial, antifungal, mosquito larvicidal, insecticidal, and repellent activities [13–15]. The study of biologically active materials derived from plant sources can act as larvicides, insect growth regulators, repellents, and ovipositional attractants and have deterrent activities as observed by many researchers [16–18]. Essential oils have received much attention as potentially useful bioactive compounds against insects [19]. Larvicide, adulticide, and repellency are known to play an important role in preventing vector-borne diseases by reducing mosquito density and man-vector-contact. Natural plant products are safe for human beings when compared to those of synthetic or chemical insecticides and repellents. Therefore, Novak emphasized the urgent need for the investigation of phytochemicals as larvicide, ovicide, and repellents for mosquito control [20].

Cymbopogon nardus (L.) Rendle shows some propensity for the latter option. *Cymbopogon nardus* is a monocotyledonous plant in the Poaceae family whose essential oil has anti-fungal activity against *Aspergillus* and *Penicillium* fungus strains [21]. In addition, this essential oil, which contains mainly citronellal, geraniol, and elemol [22], has insecticidal effects on *Aedes aegypti*, a species of mosquito vector of dengue [23]. The genus *Cymbopogon* of the Poaceae family has been investigated for its pharmacological potential. *Cymbopogon nardus* (L.) Rendle, popularly known as citronella, is a grass cultivated in subtropical and tropical regions of Asia, Africa, and America, including Brazil [24]. The Essential oil of the leaves of *Cymbopogon nardus* is commonly used in perfumes, the production of cosmetics, and as an insect repellent. The major chemical constituents are geraniol, citral, citronellal, and citronellol [25]. Other studies revealed the antiviral [26], antibacterial [27], and antifungal [28,29] activities of this oil. In the study by Silva et al. [30] involving compounds isolated from the essential oil of *Aristolochia trilobata*, the monoterpenes p-cimene and limonene stood out, revealing a lot of toxicity for the larvae of *Aedes aegypti,* although α- and β-pinene monoterpenes also have larvicidal activity.
However, there is no derivative of *Cymbopogon nardus (L).* Rendle leaves and roots: essential oil on *Aedes* mosquito larvae has not been mentioned as an insecticidal agent in Myanmar. As a result, research into the larvicidal action of *Cymbopogon nardus* (L) Rendle leaves and roots essential oil extract on 3rd and 4th instar *Aedes* larvae will aid in future vector control efforts in an environmentally responsible manner.

MATERIALS AND METHODS

Mosquito larvae raining

Aedes aegypti mosquito larvae and adults *Aedes* mosquitoes emerged from pupae were reared in the laboratory of the Medical Entomology Research Division, Department of Medical Research. Larvae were fed on DMR larva food. Adults were given a 10% sucrose solution and a blood meal from an 8-week-old mouse. Mosquitoes were held at 26±2°C, 65–75% relative humidity, with a photoperiod of 12 h light and 12 h dark. The insecticidal properties of steam distilled essential oil of *Cymbopogon nardus* (L) Rendle's leaves and roots, which were collected from Kalama Mountain, Paung Township, Mon State from April 2019 to March 2020, were tested using laboratory-reared larvae and mosquitoes.

Mosquito species identification

Larvae and adult mosquitoes emerging from larval surveys were identified by morphological methods according to the key of Rampa and Prachong [31].

Collection and preparation of essential oil from the leaves and roots of Cymbopogon nardus (L) Rendle

Cymbopogon nardus (L) Rendle, Myanmar name Sabalin leaves and roots were collected from Paung Township, Mon State. A total of 10 kilos of *Cymbopogon nardus* (L) Rendle leaves and roots were cleaned and dried in a shed at room temperature for 7 days. Dried leaves and roots were separately ground by a grinding machine to make powder and finely ground. 100 g each of dried leaves and root powder were separately extracted with 800 ml of distilled water by the steam distillation method at 100°C for 3 hours. Complete removal of the solvent from the extract was accomplished in a glass rotary evaporator. The resulting 0.92 gm of essential oil was obtained from 100 gm of dried leave powder and 0.87 gm of essential oil was obtained from 100 gm of dried root powder. The essential oils were stored at 4°C until use. The extraction was done in the Department of CRDC, Yangon.

Larvicidal testing procedure

Based on preliminary tests, further dilutions were prepared with distilled water. Different concentrations of essential oil of leaves and roots of *Cymbopogon nardus* (L) Rendle as 0.01g, 0.005g, 0.0025g, 0.00125g, and 0.000625g of essential oil of leaves and roots of *Cymbopogon nardus* were prepared freshly in 100 ml each of distilled water in 150 ml plastic cups. Fifty (50) 3rd and 4th instar *Aedes aegypti* larvae were put into different concentrations and also a negative

control test was done simultaneously. Detail testing was done according to the standard method [32]. Larvae were exposed for 24 hours in different concentrations in the laboratory at 27-29°C and 70-80% relative humidity for each replication. Five replicates were carried out, and knockdown was checked and recorded after a 60minute exposure period, and mortality was checked and recorded after a 24 hour exposure period. Knockdown and dead larvae were identified when the larvae failed to move after probing with a needle in the thorax region of the body. Lethal concentration LC_{50} and LC_{90} values for 95% confidential limits were calculated by the following formula [33].

$$X^2 = (O-E)^2 / E \times (100-E)$$

(X^2 stands for Chi squire, O stands for observed value, E stands for expected value, and O-E stands for observed minus expected)

Data analysis

Data entry and processing were done using Microsoft Excel software. Average larval mortality data were subjected to probit analysis to calculate LC_{50} and LC_{90} values, as well as other statistics at 95% confidence limits of upper and lower confidence limits [33], and chi-squared values were calculated using dose-effect probit analysis. Results with p 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

According to Bowers et al. [34], the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. Plant parts contain a complex of chemicals with distinct biological activity [35, 36], which is thought to be due to toxins and secondary metabolites that act as mosquitocidal agents [37]. Natural products are safe for humans when compared to those of synthetic compounds and chemical insecticides. Mosquitoes alone infect over 700 million people each year with diseases such as malaria, filaria, dengue hemorrhagic fever (DHF), dengue fever (DF), Japanese encephalitis, Zika, and yellow fever [38]. Therefore, the control of mosquitoes is an important public health problem around the world. *Aedes aegypti* (Culicidae) occurs in tropical countries like Asia, Africa, and Central and South America and transmits four different types of flavivirus viruses (as DNV1 to DNV4), etiologic agents of human diseases like DF and DHF dengue, Zika, and yellow fever. Chemicals and synthetic insecticides used for control of vectors are causing irreversible damage to the eco-system and human beings and animals. The indiscriminate use of insecticides to control dengue has aided the emergence of resistant populations of *Aedes aegypti*, as well as having negative consequences such as long-term persistence in the environment [39-41]. An alternative would be the use of natural insecticides to control this vector.

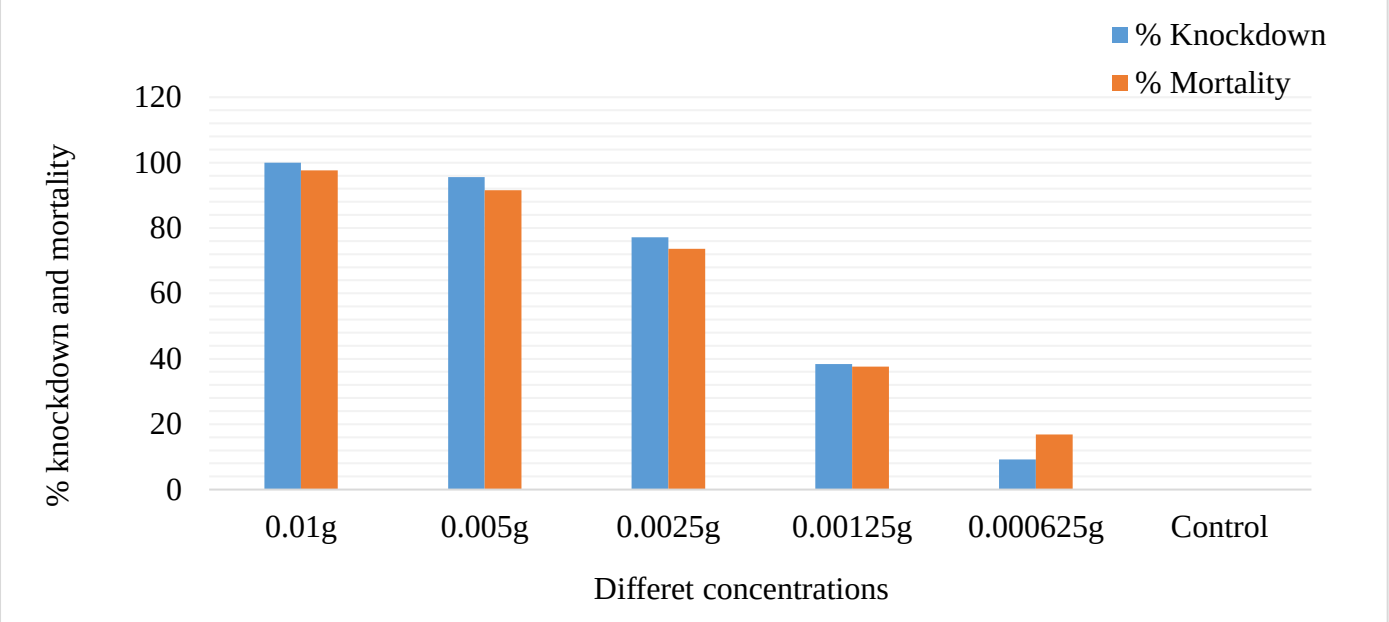

Fig.1. Knockdown and mortality effect of different dilutions of essential oil of *Cymbopogon nardus* (L) Rendle leaves against 3rd and 4th instars of *Aedes aegypti* larvae.

Fig.1. shows that the knockdown effect of *Aedes aegypti* larvae was found to be 100% knockdown at 0.01g in essential oil of *Cymbopogon nardus* (L) Rendle, followed by 95.56% knockdown at 0.005g dilution of essential oil. The lowest knockdown effect of 9.2% was found at a 0.000625g dilution of essential oil of leaves. The highest mortality rate of *Aedes aegypti* larvae was found to be 97.6% at 0.01g, while the effective mortality rate was found to be 90.6% at 0.005g dilution of the leaves essential oil, mortality effect was found at 16.8% of larvae at a 0.000625g dilution of leave essential oil. The same result has been found in the essential oil of the Kisumu strain *Cymbopogon nardus* in Kenya, which revealed 100% mortality at 80ppm on sensitive larvae from Kisumu and 200ppm on wild larvae [12].

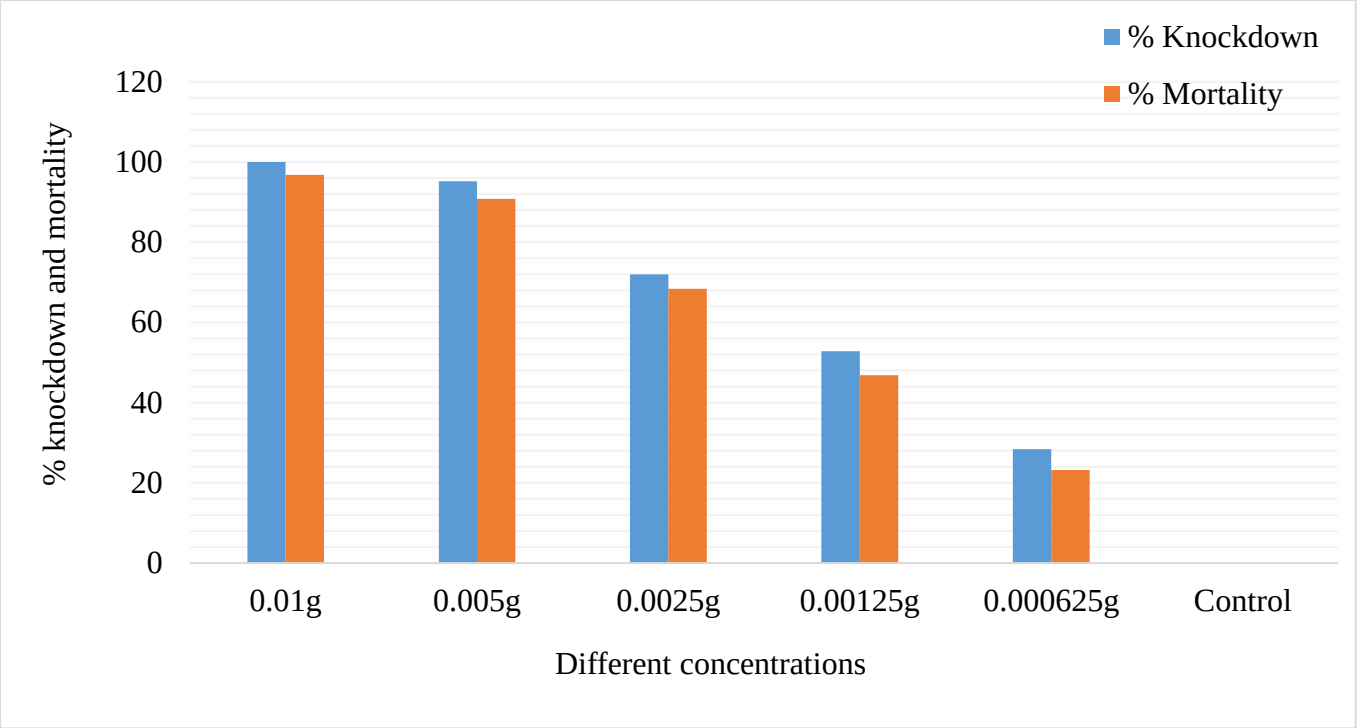

Fig.2. shows the knockdown and mortality effects of different dilutions of essential oil of *Cymbopogon nardus* (L) Rendle roots on *Aedes aegypti* larvae in the third and fourth instars.

Fig. 2. shows that the effective knockdown effect of *Aedes aegypti* larvae was found at 100% at 0.01g in the roots of *Cymbopogon nardus,* followed by 95.20% knockdown at 0.005g dilution of essential oil. The lowest knockdown effect of 28.40% was found at a 0.000625g dilution of essential oil of root. The highest mortality rate of *Aedes aegypti* larvae was found at 96.8% at 0.01g and effective mortality was found at 90.8% at 0.005g of roots of *Cymbopogon nardus,* followed by 68.4% at 0.0025g dilution of root essential oil. The lowest mortality effect was found to be 23.20% of larvae were dead at a 0.000625g dilution of root oil. Other researcher observed that the larvicidal properties of leaves and root essential oil from *Cymbopogon nardus* were found to be very low amounts that were needed to destroy 3rd and 4th instar larvae. Brito and his party observed that at the concentration of 2.5L, 63.33% of the larvae died after 24 hours, 83.33% with 7.5L and 100% mortality with 10L against *Aedes aegypti* mosquito larvae [42]. It may be due to the fact that *Cymbopogon nardus* essential oil contains phytochemicals such as oxygenated monoterpenes (Citronellal, Geraniols, Cetronellol) and hydrocarbon sesquiterpenes (Elemol), which are very effective larvicidal, pupaecidal, and ovicidal in nature [22, 42]. Table 1. shows that LC_{50} and LC_{90} values of *Cymbopogon nardus* leaves and roots essential oil against 3rd and 4th instar *Aedes aegypti* larvae were observed at 0.0015g and 0.0014g for 50% mortality and 0.00499 and 0.00529g for 90% mortality, respectively. *Aedes* larvae were killed by a small amount of essential oil extracted from the leaves and roots. For 50% mortality, the lethal concentration (LC_{50}) of leaves and root essentials was 0.0015g and 0.0014g, and for 90% mortality (LC_{90}), lethal doses were 0.0049g and 0.0052g. Another sensitivity of *Cymbopogon nardus* essential oil tests in Kenya performed on 3rd instar *Anopheles gambiae* larvae revealed that the larvicidal properties of the lethal concentrations (LC_{50}) figures obtained were 36.83 ppm and 97.33 ppm for the Kisumu strain and the local wild population, respectively. The LC_{90} adopted the same trend with 55.72 ppm and 147.9 ppm, respectively, for sensitive and wild larvae. The essential oil of *Cymbopogon nardus* was very effective for controlling *Aedes* larvae. It may be due to the high content of main oil compounds such as citronellal (37.87%), nerol (19.88%), citronellol (9.11%), elemol (7.40%), and gamma murollene (4.65%) [12].

Table (1.) LC_{50} and LC_{90} values of essential oil of *Cymbopogon nardus* leaves and roots on Aedes aegypti larvae in the third and fourth instars.

Treatment	Hours	Essential oil	X^2	df	LC_{50} corrected limits and 95 % confidence interval (upper and lower limit (gm)	LC_{90} corrected limits and 95 % confidence interval (upper and lower limit) (gm)
Leaves of *Cymbopogon nardus*	24	Oil of Leaves	3.0216	4	0.0015g	0.00499
Roots of *Cymbopogon nardus*	24	Oil of roots	2.1131	4	0.0014g	0.00529

Although other studies of leaf essential oil extracts of *Ocium basilicum* revealed a moderate LC_{50} and LC_{90} value of 141.95 ppm and 445.12 ppm, respectively, against early fourth instars of *Ae aegypti* for 24 h. The investigations also showed that oil did not have any hormono-mimetic effects as it did not result in the formation of any larval-pupal intermediates. A recent study of *Ocium basilicum* leaves essential oil found very small amount of essential oil need to destroyed *Aedes* larvae, $LC_{50\ and}$ LC_{90} found to be 0.017g and 0.0576g for 50 and 90% killing of 3rd and 4th instar larvae of *Ae. aegypti* [43]. However, the basil essential oil exhibited significant effective repellency against adult *Ae. aegypti* [14,43]. For *Eucalyptus globulus,* 0.1 mg/mL of essential oil has been tested against *Ae. aegypti* larvae and showed >80% mortality [44]. Additionally, another study of *Eucalyptus globulus* essential oil against 3rd instar *A. aegypti* larvae found 20% mortality at 1000 mg/L [45]. For 50% and 90% mortality, the current study of *Cymbopogon nardus* leaves and roots essential oil requires a very small amount of oil (LC_{50}= 0.0015g, 0.0014g and LC_{90}= 0.00499g, 0.00521g). The essential oils of *Cymbopogon nardus* collected from Paung Township, Mon State, may contain a high level of larvicidal compounds such as citronellal, geraniol, and elemol.

These results contrast with those previously obtained by Koumaglo in 2004, who reported that citronellal and geraniol are the majority compounds, with respective levels of 47.80% and 25.00% in samples of *Cymbopogon nardus* in Benin [46]. Similarly, Silou analyzed the essential oil of *Cymbopogon nardus* from Congo-Brazzaville and found citronellal (44.87%) and geraniol as major compounds (22.99%) [47]. Abena obtained citronellal (41.3%) and geraniol (23.4%), which are mainly present in this essential oil in Benin [48]. Veloso et al., [49] describe the larvicidal activity of the essential oil of the species *Cymbopogon nardus* (Citronella) collected in a northern Brazilian state against the *Aedes aegypti*, with aliquots of 5, 7.5, and 10L with greater larvicidal action, and found 100% of dead larvae of the exposed vector in the first 6 hours, showing the potential of the essential oil to counter the development and proliferation of this vector. The yield of essential oil obtained for *Cymbopogon nardus* is significantly higher than that obtained in Brazil (0.4%) by Rios [50]. These results can be explained by the influence of certain particular factors such as the growing season, harvest condition, origin, climatic and edaphic conditions of each region on the plant [51]. And associates described the effects of sublethal concentrations and constituent compounds (citral and geranyl acetate) of *Cymbopogon flexuosus* essential oil on the development *of Aedes aegypti* and found that all geranyl acetate concentrations decreased egg hatching and increased the larval length and head width compared with the control group. *Cymbopogon flexuosus* essential oil caused a similar effect to diflubenzuron, namely decreased hormone concentrations, an extended larval period, and death [52].

Another study of *Citrus hystrix* fruit extract found higher larval mortality. The dry fruit and peel extracts resulted in significantly higher 100% mortality ($P < 0.05$) when compared to the mortality (86.8%) caused by internal material of *Citrus hystrix* fruit at a concentration of 0.1gm/100ml against *Aedes* larvae. The dose of 0.0125g/100 ml of *Citrus hystrix* fruit extract was found to be 100% protection from oviposition of gravid *Aedes aegypti* mosquitoes in the laboratory [53]. The toxicity study of the essential oil of *Cymbopogon nardus* has been confirmed on eggs and larvae of *Aedes aegypti* [54]. *Cymbopogon nardus* shows very interesting activity compared with that obtained on larvae of *Anopheles stephensi* and *Ajuga remota* for 330 ppm in 24 hours and 290 ppm in 48 hours [55]. In addition, important pharmacological properties of the essential oil of *Cymbopogon nardus* (L.) Rendle include anti-tumor, anti-nociceptive, and antibacterial activities, and so an investigation of this compound against pathogenic fungi is interesting [29]. The essential oils of *Cymbopogon nardus* leaves and roots collected from Paung Township, Mon State, very effective and toxic to control third and fourth instar *Aedes* larvae in water storage containers in DHF prone areas.

CONCLUSION

Therefore, the study concluded that *Cymbopogon nardus* (L.) Rendle leaves and root essential oil extracts were very effective to control larvae and they can be used as a larvicidal agent against *Aedes aegypti* mosquitoes. Further studies on the identification of active compounds and field trials are needed to recommend the active fraction of *Cymbopogon nardus* (L.) Rendle leaves and roots essential oil extracts for the development of eco-friendly chemicals and indigenous plant base materials were developed for the protection of *Aedes* larvae density. To avoid mosquito resistance to chemical/synthetic insecticides and to protect the environment and public health, native larvicidal and insecticidal plant base extracts or essential oils have been suggested as an alternative source of material for mosquito larval control in the public environment. An insect repellent of plant origin ought to be well-defined and harmless to humans and other non-target organisms. Therefore, use of these botanical derivatives in mosquito control could reduce the cost and environmental effects. The study documented the promising larvicidal, ovicidal, and repellent potential of essential oil extracts of *Cymbopogon nardus* (L.) Rendle leaves and roots, which could be considered as a potentially alternative source for developing novel larvicides to be used in controlling vectors of mosquito borne diseases.

REFERENCES:

1. Ministry of Health. Communicable diseases, Health in Myanmar, Ministry of Health 2019.
2. Service M.W. Mosquitoes (*Culicidae*). In: Lane R.P., Crosskey R.W. eds. Medical insects and arachnids. London, Chapman and Hall, 1993; 723.
3. Nathan S.S., Kalaivani K., and Sehoon K. Effects of *Dysoxylum malabaricum Bedd*. (Meliaceae) extract on the malarial vector *Anopheles stephensi* Liston (Diptera: *Culicidae*). Bioresource Technol, 2006; 97:2077-2083.
4. Tawtsin A., Wratten S.D., Scott R., Thavara U, Techadamrongsin Y., Repellency of volatile oils from plants against three mosquito vectors. Engl. J. Med. 2001; 26:76-82
5. Moses M., Johnson E.S., Anger W.K., Buse V.W., Horstman S.W., Jackson R.J., Lewis R.G., Maddy K.T., McConnell R., Meggs W.T., Zahm S.H. Environmental equity and pesticide exposure. Toxicol. Ind. Health, 1993; 9(5):913-959.
6. Maung Maung Mya, Sein Min, May Aye Than, Sein Thaung, Chit Thet New, Zar Zar Aung and Yan Naung Maung Maung. Effects of indigenous plant Garlic bulb

(*Allium sativum*) extract against larvae of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. Department of Medical Research Bulletin, 2015a; 27(11):1-2.
7. Gimletle J. D. &Thomson H.W. A Dictionary of Malayan Medicine. Oxford University Press, Kuala Lumpur. 1983.
8. Yeung H.C. Handbook of Chinese Herbs and Formulas. Institute of Chinese Medicine, Los Angeles 1985.
9. Kiran R.S., Bhavani K., Devi S.P., Rao R.B.R. and Reddy J.K.,' Composition and larvicidal activity of leaves and stem essential oils of *Chloroxylon swietenia* D.C. against *Aedes aegypti* and *Anopheles stephensi*. Bioresource Technol.2006; 97:2481-2484.
10. Maung Maung. Mya, Yin Yin Aye, Aye Win Oo and R.K. Saxena. Effect of *Citrus hystrix* DC Leaves Ethanol Extract on Larvae of *Aedes aegypti*. *Journal of Biological Engineering Research and Review*; 2015b; 2(2): 01-06
11. Ibrahim J. Abu SA., Abdul RA., nor AMA., Norsiha A. Chemical composition some Citrus oils from Malaysia. J. Essent. Oil Res.,1996; 8:627-632
12. Ayidé C. Ahouansou, Sèdami R. Medegan Fagla, Jean M. Tokoudagba, Habib Toukourou, Yvette K. Badou and Fernand A. Gbaguidi. Chemical composition and larvicidal activity of the essential oil of *Cymbopogon nardus* (L.) Rendle on *Anopheles gambiae.* Int. J. Biol. Chem. Sci.2019; 13(3): 1861-1869.
13. Gazali GE, Khalid H, El Tohami M, Abdalla W, Yagi S. Medicinal plants commonly used in Khartoum State. National Centre for Research, Khartoum.1988.
14. Kumar S, Radhika Warikoo, Monika Mishra, Roopa R Samal, Shrankhla, Kungreiliu Panmei, Vinay S Dagar and Aarti Sharma. Impact of *Ocimum basilicum* Leaf Essential Oil on The Survival and Behaviour of An Indian Strain of Dengue Vector, *Aedes aegypti* (L.).Vector Biol J. 2017; 2(2): 1-6
15. Dan B, Steven C, Erich S, Andrew G. Chinese herbal medicine. Materia Medica. Eastland Press, USA.2004.
16. Babu, R. & Murugan, K. Interactive effect of neem seed kernel and neem gum extracts on the control of *Culex quinquefasciatus* Say. Neem Newsletter. 1998; 15: 9-11.
17. Venkatachalam, M.R. & Jebanesan, A. Repellent activity of *Ferronia elephantum* Corr. (Rutaceae) leaf extracts against *Aedes aegypti*. Bioresource Technology. 2001a; 76: 287-288.
18. Venkatachalam, M.R. & Jebanesan, A. Larvicidal activity of *Hydrocotyle javanica* Thunb. (Apiaceae) extract against *Culex quinquefasciatus*. Journal of Experimental Zoology. 2001b; 4: 99-101.
19. Kim, S.I., Shin, O.K., Song, C., Cho, K.Y. & Ahn, Y.J. Insecticidal activities of aromatic 22.c plant extracts against four agricultural insects. Agricultural Chemistry and Biotechnology. 2001; 44: 23-26.
20. Novak D. Nonchemical approaches to mosquit`o control in Czechoslovakia. In : Control methodologies, v 2. San Diego, CA : Academic Press 1985; p. 185–96.
21. Kpatinvoh B, Adjou ES, Dahouenon-Ahoussi E, Konfo TRC, Atrevi B, Soumanou MM, Sohounhloue DCK. Efficacité des huiles essentielles de trois plantes aromatiques contre la mycoflore d'altération du niébé (*Vigna unguiculata* L., Walp) collecté dans les magasins de vente du Sud-Bénin. J. Appl. Biosci. 2017; 109: 10680-10687. http://dx.doi.org/10.4314/jab.v109i1.12.
22. Ouedraogo I, Sawadogo A, Nebie RCH, Dakouo D. Evaluation de la toxicité des huiles essentielles de *Cymbopogon nardus* (L) et *Ocimum gratissimum* (L) contre *Sitophilus zeamais* Motsch et *Rhyzopertha dominica* F, les principaux insectes nuisibles au maïs enstockage au Burkina Faso. Int J Biol Chem Sci. 2016;10: 695-705.
23. Warikoo R, Wahab N, Kumar S. Oviposition-altering and ovicidal potentials of five essential oils against female adults of the dengue vector, *Aedes aegypti* L. Parasitol Res, 2011; 109: 1125-31. DOI: 10.1007/s00436-011-2355-y.
24. Chen, Q.; Xu, S.; Wu, T.; Guo, J.; Sha, S.; Zheng, X.; Yu, T. Effect of citronella essential oil on the inhibition of postharvest Alternaria alternata in cherry tomato. J. Sci. Food Agric. 2014; 94: 2441–2447.
25. 25 Chanthai, S.; Prachakoll, S.; Ruangviriyachai, C.; Luthria, D.L. Influence of extraction methodologies on the analysis of five major volatile aromatic compounds of citronella grass *Cymbopogon nardus* and lemongrass (*Cymbopogon citratus*) grown in Thailand. J. AOAC Int. 2012; 95: 763–772.
26. Aini, M.N.N.; Said, M.I.; Nazlina, I.; Hanina, M.N.; Ahmad, I.B. Screening for Antiviral activity of sweet lemon grass (*Cymbopogon nardus* (L.) rendle) fractions. J. Biol. Sci. 2006; 6: 507–510.
27. Innsan, M.F.M.F.; Shahril, M.H.; Samihah, M.S.; Asma, O.S.; Radzi, S.M.; Jalil, A.K.A.; Hanina, M.N. Pharmacodynamic properties of essential oils from *Cymbopogon* species. Afr. J. Pharm. Pharmacol. 2011, 5.
28. Nakahara, K.; Alzoreky, N.S.; Yoshihashi, T.; Nguyen, H.T.T.; Trakoontivakorn, G. Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (Citronella Grass). Jpn. Agric. Res. Q. 2013; 37: 249–252.
29. Toledo De LG, Matheus ADSR, Larissa S., Elza MC, Fernando RP, Érica De OL,et al., Essential Oil of *Cymbopogon nardus* (L.) Rendle: A Strategy to Combat Fungal Infections Caused by Candida Species. International Journal of Molecular Sciences. 2016; 17: 1252-1268; doi:10.3390/ijms17081252.
30. Silva, I. M., Martins, G. F., Melo, C. R., Santana, A. S., Faro, R. R., Blank, A. F., Alves, P. B., Picanço, M. C., Cristaldo, P. F., & Araújo, A. P. A. (2018). Alternative control of *Aedes aegypti* resistant to pyrethroids: Lethal and sublethal effects of monoterpene bioinsecticides. Pest Management Science. 2018; 74(4): 1001–1012.
31. Rampa R., and Prachong P. Illustrated keys to the medically important mosquitos of Thailand, Southeast Asian Journal of tropical Medicine and public Health 1994; 25(1): 1-66.
32. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. WHO Geneva 2005, *WHO/CDS/WHOPES/GCDPP/1.3*
33. Finney. Probit Analysis. Third ed. Cambridge University press, Cambridge,1971; 9968-72
34. Bowers WS, Sener B, Evans PH, Bingol F, Erdogan I. Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti* and *Anopheles gambiae*. Insect Sci Appl. 1995; 16(3/4):339–342.
35. Govindarajan M, Jebanesan A., Pushpanathan T, Samidurai K. Studies on effect of *Acalyoha indica* L. (Euphorbiaceae) leaf extract on the malaria vector *Anopheles stephensi* Liston (Diptera:Culicidae). *Parasitology Research* 2008a; 103(3): 691-695.

36. Govindarajan M, Jebanesan A., Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. *Parasitology Research* 2008b; 102(2): 289-292.
37. Venkatachalam, M.R. & Jebanesan, A. Larvicidal activity of *Hydrocotyle javanica* Thunb. (Apiaceae) extract against *Culex quinquefasciatus*. Journal of Experimental Zoology.2001b; 4: 99-101.
38. Taubes, G. Vaccines. Searching for a parasites weak spot. Science. 2000; 290 (5491), 434–437.
39. Ryan, S. J., Mundis, S. J., Aguirre, A., Lippi, C. A., Beltrán, E., Heras, F., Sanchez, V., Borbor-Cordova, M. J., Sippy, R., & Stewart-Ibarra, A. M. Seasonal and geographic variation in insecticide resistance in *Aedes aegypti* in southern Ecuador. PLoS Neglected Tropical Diseases. 2019; 13(6), e0007448.
40. Satoto, T. B. T., Satrisno, H., Lazuardi, L., & Diptyanusa, A. Insecticide resistance in *Aedes aegypti*: An impact from human urbanization? Plos One. 2019; 14(6): e0218079.
41. Demok, S., Endersby-Harshman, N., Vinit, R., Timinao, L., Robinson, L. J., Susapu, M., Makita, L., Laman, M., Hoffmann, A., & Karl, S. Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* mosquitoes in Papua New Guinea. Parasites & Vectors. 2019; 12(1): 1–8.
42. Brito LCdF., Dias LMF., Pereira GSS., Alves NB., Rocha MdS., Junior JFdS., Barros VC and Maria Christina Sanches Murator MCS. Analysis of the chemical composition, antifungal activity and larvicidal action against *Aedes aegypti* larvae of the Essential Oil *Cymbopogon nardus.* Research, Society and Development, v. 10, n. 13, e543101321452, 2021, ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i13.21452. 2021
43. Maung Maung Mya, Aung Chan Pyae Maung, Aye Win Oo, Than Myat Htay, Chit Thet New, Thu Zar Nyein Mu, Zar Zar Aung, Yee Yee Myint and Sein Thaung 2020, Larvicidal, Ovicidal and repellency properties of the indigenous plants *Ocimum basilicum* L leaves essential oil against *Aedes aegypti* mosquitoes. 50th Myanmar Health Research Congress Programme and Abstracts 2022; p 95. (Best Poster Award 2022).
44. Park H, Kim J, Chang K, Kim B, Yang Y, Kim G, et al. Larvicidal activity of Myrtaceae essential oils and their components against *Aedes aegypti,* acute toxicity on *Daphnia magna*, and aqueous residue. J Med Entomol. 2011; 48(2): 405-10.
45. Tennyson S, Samraj DA, Jeyasundar D, Chalieu K. Larvicidal efficacy of plant oils against the dengue vector *Aedes aegypti* (L.) (Diptera: *Culicidae*). Middle East J Sci Res 2013; 13(1): 64-8.
46. Koumaglo HK. Production de l'essence de citronnelle par les coopératives villageoises en Afrique occidentale. Québec, Canada : Université de Chicoutimi.2004; 81-97.
47. Silou T, Bikanga R, Nsikabaka S, Nombault J, Mavoungou C, Figuérédo G, Chalchat JC. Plantes aromatiques du Plateau des Cataractes (Bassin du Congo). Caractérisation du chémotype de l'huile essentielle de *Cymbopogon nardus* (L.) Rendle. Biotechnol Agron Soc Environ. 2017; 21: 105-116. DOI: 10.25518/1780-4507.13727.
48. Abena AA, Gbenou JD, Yayi E, Moudachirou M, Ongoka RP, Ouamba JM, Silou T. Comparative chemical and analgesic properties of essential oils of *Cymbopogon nardus* (L) Rendle of Benin and Congo. Afr J Tradit Complement Altern Med. 2007; 4: 267-72.
49. Veloso, R. A., de Castro, H. G., Cardoso, D. P., Chagas, L. F. B., & Júnior, A. F. C. Óleos essenciais de manjericão e capim citronela no controle de larvas de *Aedes aegypti*. Revista Verde de Agroecologia e Desenvolvimento Sustentável. 2015; 10(2): 16.
50. Ríos N, Stashenko EE, Duque JE. Evaluation of the insecticidal activity of essential oils and their mixtures against *Aedes aegypti* (Diptera: *Culicidae*). A Journal on Insect Diversity and Evolution Medical and Veterinary Entomology. 2017; 61: 307-311.
51. Anton R, Lobstein A. Plantes aromatiques. Épices, aromates, condiments et huiles essentielles. Tec & Doc. Paris, 2005; p522.
52. Morales RMC., Serrano SO., Villamizar ALR., Sanchez SCM., & Duque JE. Impact of *Cymbopogon flexuosus* (Poaceae) essential oil and primary components on the eclosion and larval development of *Aedes aegypti* (Poaceae) essential oil. Nature Portfolio 2021, www.nature.com/scientificreports2021, https://doi.org/10.1038/s41598-021-03819-2.
53. Maung Maung Mya, Zar Zar Aung, Khine Khine Lwin,Chit That Nwe, Aye Win Oo, Than Myat Htay, Sein Thaung, Khin Phyu Phyu, and Yan Naung Maung Maung. Larvicidal properties of *Citrus hystrix* DC fruit (Taw-Shaut-Khar-thee) extracts against *Aedes aegypti* larvae collected from different Townships of Yangon Myanmar Health Sciences Research Journal 2019; 31(1):23-29.
54. Warikoo R, Wahab N, Kumar S. Oviposition-altering and ovicidal potentials of five essential oils against female adults of the dengue vector, *Aedes aegypti* L. Parasitol Res. 2011; 109: 1125-31. DOI: 10.1007/s00436-011-2355-y.
55. Preeti S, Lalit M, Cn S. 2004. Larval susceptibility of *Ajuga remota* against *Anopheline* and *Culicine* mosquitos. Southeast Asian J Trop Med Public Health. 2044; 35: 608 - 610.

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