Journal of Academia and Industrial Research (JAIR)
Volume 6, Issue 3, August 2017

ISSN: 2278-5213

Research Article

A comparative Study of the Larvicidal Activity of Lemongrass (*Cymbopogan citratus*) from Different Methods of Extraction

O.N. Goselle*, D.A. Gyang, O.F. Adara, K.T. Effiong, N. Nanvyat, I.A. Adulugba, D. Kumbak, Y.M. Ahmadu[1] and H.B. Mafuyai

Applied Entomology and Parasitology unit, Department of Zoology, University of Jos-Nigeria
obeto247@yahoo.com*

Abstract

Pure compounds or standardized extracts obtained as natural products from medicinal plants have for long been used as toxicants to mosquito larvae and they have provided limitless opportunities for novel medicine due to their unequaled ease of access of chemical diversity. Owing to a growing demand for chemical diversity in programmed screening selections and the quest for therapeutic drugs from natural products, concern principally in edible plants has increased all over the world. Despite achievement of various strides in the formulations and usage of various bioactive compounds from herbal preparations and botanicals for medicinal usage against mosquitoes, the resurgence of mosquitoes and the scourge of diseases transmitted by these vectors are still felt worldwide. Here, we report an amazing case where we conducted a comparative study on the efficacy of the various extraction methods on lemongrass in the mortality of the larvae of *Culex* mosquitoes. To achieve our set objectives, we used three common methods of extraction of plants extract i.e. Decoction, Infusion and Maceration on lemongrass and then compared their potency on the mortality rate of *Culex* larvae. To our chagrin, although all the methods of extraction were efficient but the highest larval mortality rate was observed in ethanol extract of dried lemongrass, followed by boiled extract and wet extract with the Chi-square value significant at $P<0.05$. In conclusion, although we were unable to ascertain the specific compound in lemongrass that is responsible for the mortality of the larvae, we recommend that lemongrass is a safe and natural insect repellant that is just as effective as the commercial chemical product and should be planted around homes, as they could have potentials of repelling mosquitoes and reducing mosquito borne diseases.

Keywords: Bioactive compounds, lemongrass, *Culex* mosquitoes, larvicidal activity, mosquito borne diseases.

Introduction

Mosquitoes as holometabolous insects have a complex life cycle interspersed between the aquatic immature and terrestrial adult stages found in dispersed ecologically distinct environment that could best support their life pattern (Muturi *et al.,* 2012). The biotic and abiotic conditions which larvae experience during development is usually carried over into the adult stage and significantly in both adult life history traits and vector competence for arboviruses (Klowden *et al.,* 1988; Grimstad and Walker, 1991; Nasci 1991; Alto *et al.,* 2011; Muturi *et al.,* 2012). Moreover, interspecies interactions which occur almost exclusively among larvae can influence arboviral susceptibility, vectorial capacity and the geographical distribution of mosquito species (Alto *et al.,* 2005; Bevins, 2007; Reiskind and Lounibos, 2009). Importantly, adult body size has a genetic basis in mosquito, but is a very plastic trait that to a large extent is influenced by environmental or physiological conditions (Clements 1992).

It is also important to note that the resources these larvae are able to accumulate as they develop determine its adult body size and life span, fecundity, immune function, sperm capacity and susceptibility to arboviruses (Clements 1992; Briegel *et al.,* 2001; Ponlawat and Harringhon 2007; Alto *et al.,* 2008; Telang *et al.,* 2012). To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is necessary. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been that successful due to man, technical, operational, ecological, and economical factors (Schmidt *et al.,* 2017). In recent years, the use of many of the formal synthetic insecticides in the control of mosquitoes has been limited due to lack of novel insecticides, high cost of synthetic insecticides and concern for environmental sustainability.

***Corresponding author**

They cause harmful effects on human health and other non-target populations, their non-biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale (Schmidt *et al.,* 2017). The Environmental Protection Act in 1969 had rules governing the application of chemical control agents in nature and that prompt the research to look for alternative methods of approaching transparent mosquito control measures that focuses on public education, monitoring and surveillance, source reduction and environment friendly least toxic larval control. These factors have resulted in urgent search for environmental friendly, cost-effective, biodegradable and 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 program in lieu of chemical insecticides (Centers for Disease Control and Prevention, 1993).

Extracts from plants may be alternative sources of mosquito egg and larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for use in control of mosquito larvae (Pushpanathan *et al.,* 2008). In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Pushpanathan *et al.,* 2008), such as *Cymbopogan citratus* (lemon grass). According to an article of New Tech Bio., Lemongrass is very popular and used for medicinal, food and insect repellent products. The lemongrass oils are used in cosmetics, soaps, perfume, dyes, and odorizes along with thousands of other products. Lemongrass are extremely safe and is listed on the EPA'S GRAS list (Generally Regarded As Safe), unlike other insecticides containing chemical compound like DEET (N,N-Diethlymetatoluamide) used as active chemical ingredient in insect repellent. People all over the world have come to rely on the potent insect repellent properties of lemongrass and utilized it as a personal and areas spray (Helmerdinger *et al.,* 2006). As noted by Goddard (2002), insect repellents are important public health tools for the prevention of vector-borne infectious diseases The actions to reduce vector-borne diseases can result in major health gains and relieve an important constraint on development in poor regions (Amer and Mehlhorn, 2006). Technically, an insect repellent is any chemical and natural insecticide that causes insects or other arthropods to make directed, oriented movements away from the source of repellent, and lemon grass can be used as natural insect repellent (Helmenstine, 2011). The aim of the study is to therefore evaluate the preferred extraction techniques on lemongrass extract that is most potent against the larval stages of *Culex*, with the specific objectives of determining the most efficacious extraction techniques against *Culex* larval stages; determining the lethal concentration (LC_{50}) of lemongrass against larvae; determining the lethal time (LT_{50}) of lemon grass against larvae; and to infer where possible the effectiveness of lemongrass extract as natural insect repellent at the larval stage.

Materials and methods

Study area: The study was conducted in the Department of Zoology of the University of Jos, Plateau State, Nigeria.

Sample collection

Grass species: Samples of lemongrass were obtained from a residence, then compared with the preserved species in the Department of Plant Sciences and Technology of the University of Jos, Plateau state and then subjected to the various extraction techniques.

Collection of mosquito and identification of larvae: To obtain *Culex* larvae, tires filled with water were placed where adults of mosquitoes were found to be common in the Department of Zoology, University of Jos. The tires were then left for 4 d, during which time it was anticipated the adults would have bred. As a confirmation after the expected 96 h period, more than 200 mosquito larvae were collected in a white container containing yeast (for feeding) and covered with a muslin cloth using rubber band. Identification of the *Culex* species was carried out with the aid of a microscope and further comparison with the preserved specimen in the Department of Zoology laboratory of the University. After which breeding was continued in the undergraduate laboratory of Department of Zoology, University of Jos.

Extraction method

Maceration: In this process, whole or coarsely powdered crude drug is placed in a stoppered container with the solvent (ethanol) and allowed to stand at room temperature for at least 3 d with frequent agitation until the soluble matter has dissolved. The mixture is then strained, the marc (the damp solid material) is pressed and the combined liquids are clarified by filtration or decantation after standing (Woisky *et al.,* 1998; Cunha *et al.,* 2004; Phrompittayarat *et al.,* 2007; Sasidharan *et al.,* 2008).

Infusion: Fresh infusions were prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs (Sukhdev *et al.,* 2008).

Decoction: In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water soluble, heat stable constituents.

*Corresponding author

Table 1. Mortality rate of mosquito larvae in dry lemongrass extract (Maceration).

	Time (h)											
	2 h			6 h			24 h			48 h		
Treatment (mL)	R1	R2	C	R1	R2	C	R1	R2	C	R1	R2	C
30	15	15	0	-	-	0	-	-	0	-	-	0
50	15	15	0	-	-	0	-	-	0	-	-	0
100	15	15	0	-	-	0	-	-	0	-	-	0

Table 2. Mean mortality rate of mosquito larvae in dry lemongrass (Maceration).

Concentration (mL)	Log Conc.	No. of larvae in each replicate	Mortality in hours/time (mean) 2	6	24	48	Total mortality	Mean mortality (%)	Probit mortality
0.00	-	15	0	0	0	0	0	0	2.5
30	1.477	15	15	-	-	-	15	100.00	97.5
50	1.699	15	15	-	-	-	15	100.00	97.5
100	2.000	15	15	-	-	-	15	100.00	97.5

The starting ration of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentration extract is filtered and used as such or processed further (Sukhdev *et al.*, 2008).

Larvicidal bioassay: The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (WHO/CDS/WHOPES/GCDPP/2005). From the stock solution, three different test concentrations (viz: 30, 50, and 100 mL) for crude extracts were tested against the freshly moulted (0-6 h) 4th instar larvae of *Culex* mosquitoes. The larvae of these mosquito species (15 larvae) were introduced into 200 mL plastic cups containing 20 mL of water and the required amount of plant extract was added. The larval mortality was observed and recorded after 2, 6, 24 and 48 h of post-treatment. For each experiment, two replicates were maintained at a time.

Statistical analysis: The LC_{50}, LC_{90}, LT_{50}, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL) and regression were calculated. The chi-square values and the degrees of freedom were also calculated by using probit analysis with Statistical Package for Social Sciences (SPSS) 16.0 Version in MS-Excel, 2007.

Results

Effect of the different extract of lemongrass on mosquito larvae: The potency of the different extract was tested on the mosquito larvae. The mortality rate was noted at various times i.e. 2, 6, 24 and 48 h in different concentration of the various samples (Dried lemongrass, wet lemongrass and boiled lemongrass extracts).

Mortality rate, mean mortality rate and regression line of mosquito larvae in dry lemongrass (Maceration): Table 1 indicates that with all the varying concentrations of lemongrass used, the mortality rate was still the same i.e. all larvae died within 2 h of application.

Fig. 1. Regression line obtained when Probit values from maceration techniques were plotted against Log Concentration.

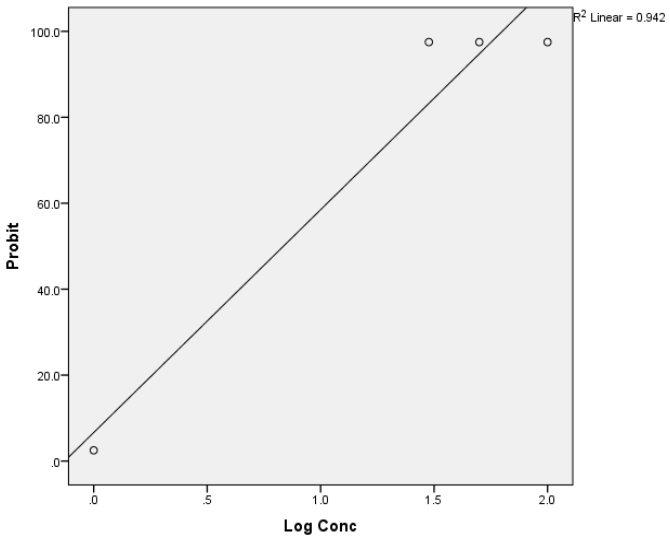


Table 2 showed the Log concentration, Percentage mean mortality and Probit mean mortality for both the control and the various volumes of macerated products applied. The results from Table 2 indicated that all larvae under the controlled environment survived the specified time period, but the same recorded number of deaths were recorded in all the volumes. This prompted the plotting of a scatter plot and a straight line was drawn to obtain the confidence interval as shown in Fig. 1. The results indicated that the confidence intervals are not wide apart therefore the line is obviously highly reliable. There is a clear relationship that the regression line slope is significantly different from zero and it explains 94.2% of the variation in the uptake observations. The 95% confidence intervals are quite close to the best-fit line confirming that the relationship is robust. This hampered the calculations of the Lethal Time (LT_{50}).

*Corresponding author

Table 3. Mortality rate of mosquito larvae in wet lemongrass extract (Maceration).

	Time (h)											
	2 h			6 h			24 h			48 h		
Treatment (mL)	R1	R2	C	R1	R2	C	R1	R2	C	R1	R2	C
30	0	0	0	2	1	0	3	3	0	3	1	0
50	0	2	0	4	4	0	3	1	0	6	0	0
100	1	7	0	5	6	0	3	2	0	0	-	0

Table 4. Mean mortality rate of mosquito larvae in wet lemongrass (Maceration).

Concentration (mL)	Log Conc.	No. of larvae in each replicate	Mortality in hours/time (mean)				Total mortality	Mean mortality (%)	Probit mortality
			2	6	24	48			
0.00	-	15	0	0	0	0	0	0	2.5
30	1.477	15	0	1.5	2.5	2.0	6	20.00	4.158
50	1.699	15	1.0	4.0	2.0	3.0	10	33.33	4.568
100	2.000	15	4.0	5.5	2.5	0.0	12	40.00	4.747

Mortality rate, mean mortality rate and regression line of mosquito larvae in wet lemongrass extract (Infusion): The result from Table 3 indicates that from the varying concentrations by Infusion techniques of lemongrass used, although mortality rate was recorded in all instances, a marked variation was recorded with those treated with 100ml volumes as more larvae died within the first 24 h as compared to other volumes. Table 4 showed the log concentration, percentage mean mortality and probit mean mortality for both the control and the various volumes of Infusion products applied. The results from Table 4 indicated that all larvae under the controlled environment survived the specified time period, but varying survival rate were recorded in all the volumes. Those treated with 100 mL volumes were observed to die within 12 h as compared to the other volumes applied. This prompted the plotting of a scatter plot and a straight line was drawn to obtain the confidence interval as shown in Fig. 2. The results indicated that the confidence intervals are not wide apart; therefore the line is obviously highly reliable. There is a clear relationship that the regression line slope is significantly different from zero and it explains 99.4% of the variation in the uptake observations. The 95% confidence intervals are quite close to the best-fit line confirming that the relationship is robust, although we were unable to calculate the Lethal Time (LT_{50}).

Mortality Rate, mean mortality rate and regression line of mosquito larvae in boiled lemongrass extract (Decoction): The result from Table 5 indicates that from the varying concentrations by decoction techniques of lemongrass used, although mortality rate was recorded in all instances, a marked variation was recorded with those treated with 100ml volumes as more larvae died within the first 24 h as compared to other volumes.

Fig. 2. Regression line obtained when Probit values from infusion techniques were plotted against Log Concentration.

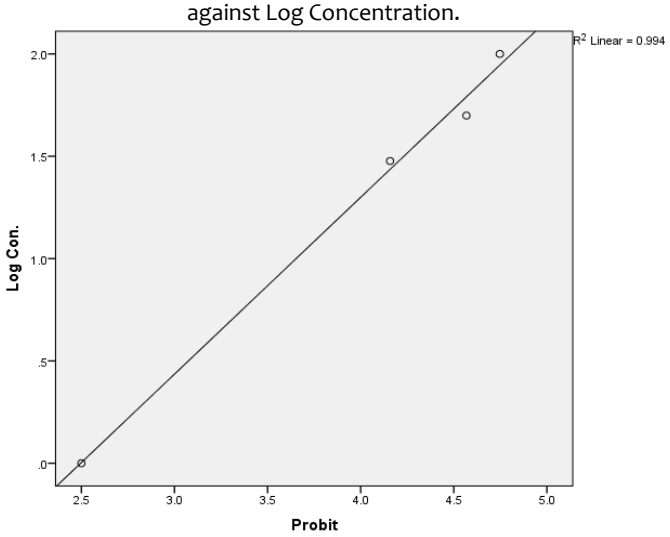


Table 6 showed the log concentration, percentage mean mortality and probit mean mortality for both the control and the various volumes of Decoction products applied. The results from Table 6 indicated that all larvae under the controlled environment survived the specified time period, but varying survival rate were recorded in all the volumes. Those treated with 100ml volumes were observed to die within Twelve hours as compared to the other volumes applied. This prompted the plotting of a scatter plot and a straight line was drawn to obtain the confidence interval as shown in Fig. 3. The results indicated that the confidence intervals are not wide apart; therefore the line is obviously highly reliable. There is a clear relationship: the regression line slope is significantly different from zero and it explains 94.3% of the variation in the uptake observations.

*Corresponding author

Table 5. Mortality rate of mosquito larvae in boiled lemongrass extract (Decoction).

	Time (h)											
	2 h			6 h			24 h			48 h		
Treatment (mL)	R1	R2	C	R1	R2	C	R1	R2	C	R1	R2	C
30	2	0	0	2	0	0	8	8	0	3	2	0
50	3	0	0	6	1	0	6	10	0	0	3	0
100	7	1	0	7	3	0	0	3	0	1	4	0

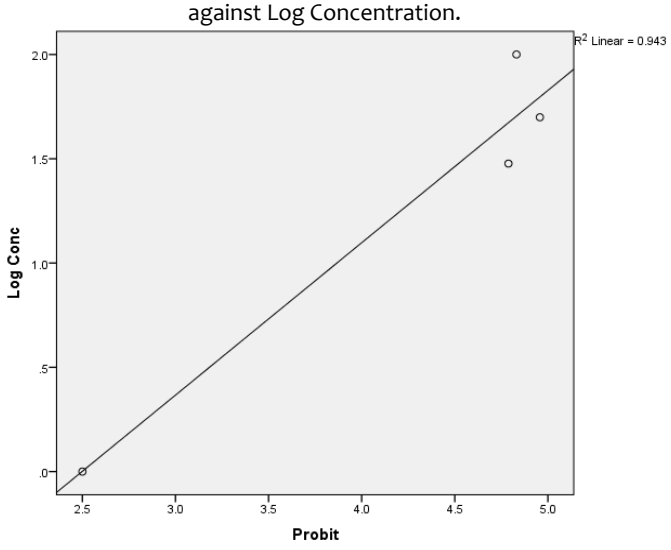
Table 6. Mean mortality rate of mosquito larvae in boiled lemongrass extract (Decoction).

Concentration (mL)	Log Conc.	No. of larvae in each replicate	Mortality in hours/time (mean)				Total mortality	Mean mortality (%)	Probit mortality
			2	6	24	48			
0.00	-	15	0	0	0	0	0	0	2.5
30	1.477	15	1.0	1.0	8.0	2.5	12.5	41.66	4.788
50	1.699	15	1.5	3.5	8.0	1.5	14.5	48.33	4.957
100	2.000	15	4.0	5.0	1.5	2.5	13.0	43.33	4.831

Table 7. Summary of larvicidal activity of different crude extracts of lemongrass against 4th instars larvae of *Culex* mosquito.

Solvent	Concentration (mL)			LC50	95% Confident limit		LC95	95% Confident limit	
	30	50	100		LCL	UCL		LCL	UCL
Wet	1.0±1.2	1.7±2.1	2.0±2.6	79.4	75.5	81.7	380.2	379.3	382.5
Dried	2.5±5.8	2.5±5.8	2.5±5.8	7.8	7.2	12.2	49.0	48.4	53.4
Boiled	2.1±3.0	2.4±3.3	2.2±2.7	52.5	51.3	55.7	186.2	185.0	189.4

Fig. 3. Regression line obtained when Probit values from decoction techniques were plotted against Log Concentration.



The 95% confidence intervals are quite close to the best-fit line confirming that the relationship is robust, although we were unable to calculate the Lethal Time (LT50).

Larvicidal activity of different crude extracts of lemongrass against 4th instars larvae of Culex mosquito: As discussed in materials and methods, the results of relative toxicity of different lemongrass extracts are presented in Table 7.

In the present study, larvicidal activity of crude extracts of lemongrass tested against larvae of Culex mosquito. It was evident from Table 7 that all the tested crude extracts demonstrated significant larvicidal activity against lemongrass. Present results revealed that ethanol extract of dried lemongrass were more potent followed by boiled lemongrass and then wet lemongrass extract. The highest larval mortality was found in ethanol extract of dried lemongrass 100% (LC 50 7.8 and LC95 49.0), followed by boiled extract at 88.9% (LC5052.5 and LC95 185.2) and then wet extract at 62.2% (LC50 79.4 and LC95 380.2). Chi-square value was significant at P<0.05 Level of significance.

Discussion

The present study showed that different solvent extract of lemongrass have significant larvicidal activity against Culex mosquitoes. The study further revealed that lowest mortality was recorded in wet extract of lemongrass (LD50 62.2%). The results of present study are comparable with similar reports of earlier workers. For example, in a study by Tandon and Sirohi (2010), on the larvicidal efficacy of aqueous extracts of seeds of *Azardirachta indica*, leaves of *Gymnema sylvestre* R.Br, bark and leaves of *Nerium indicum* Mill and leaves of *Datura metal* L. against fourth instar of *Culex quinquefasciatus* larvae, they noted that *Azardirachta indica* elicited 70-99% mortality, followed by *Gymnema sylvestre* 44-89%, *Nerium indicum* 44-74 % and *Datura metal* 19-54%.

*Corresponding author

Although the present report was specific on the leaves, it has been reported that different parts of plants contain a complex of chemicals with unique biological activity (Farnsworth and Bingel, 1977) which is thought to be due to toxins and secondary metabolites, which acts as attractants or deterrents (Fisher, 1991). A good example is the study carried out by Jeyasankar and Ramar (2015) on the toxicity of ethyl acetate leaf extract of *Breyenia vitis* where the results showed the LC50 value of 98.2, 107.79 and 115.8 ppm respectively were obtained. Similarly, Sharma *et al.* (2009) reported that, petroleum ether extract of *Ageratum conyzoides* leave exhibited larvicidal activity with LC50 value of 425.60 and 267.90 ppm after 24 and 48 h of exposure. The dried lemongrass extract proved to be more active such that within two hours of application, all the larvae were dead. This result is in agreement with previous work done by Sosan *et al.* (2013) which showed that *Cymbopogon citratus* and *Ageratum conyzoides* used against *Ae. aegypti* did achieved 100% mortality at 120, 200, and 300 mg/L concentrations respectively. The biological activity of these plants extracts might be due to various compounds, including phenolic, flavonoids, saponins, and alkaloids existing in plant. These compounds may jointly or independently contribute to the larvicidal activity against both species of mosquitoes (Ravikumar *et al.*, 2010). The crude leaf extract of lemongrass with different solvents, viz. ethanol, boiled water, and pure water when tested for larvicidal activity against *Culex* mosquito species indicated that the LC50 value were 7.8, 52.5 and 79.4 respectively while the LC95 were 49.0, 186.2, and 380.2 respectively. The results indicated that the solvent crude leaf extract of lemongrass possesses remarkable larvicidal activities against *Culex* mosquito species. This is in agreement with previous studies done by Tiwary *et al.* (2007) on the analysis of the chemical composition and larvicidal activities of the essential oil of *Zanthoxylum amatum* DC (Rutaceae) against *Aedes aegypi, Culex quinquefasciatus* and *Anopheles stephensi* where they were able to show that *Culex quinquefasciatus* was the most sensitive followed by *Aedes aegypti* and *Anopheles stephensi* with LC50 values of 49, 54 and 58 ppm.

Conclusion

Lemongrass is a safe and natural insect repellants that is just as effective as the commercial chemical product. With Dried lemongrass in ethanol extract a significantly higher mortality of 100% of Culex larvae were recorded after 48 h as compared to other treatments. Base on the findings, the study recommends that the plant extract (lemongrass) should be tested at lower concentration as many of the extracts recorded 100% mortality at higher concentrations. We were unable to study the biological activity of these plants extracts to ascertain if their mortality was due to various compounds, including phenolic, flavonoids, saponins, and alkaloids existing in plant, as these compounds may jointly or independently contribute to the larvicidal activity against both species of mosquitoes.

Acknowledgements

We are grateful to the Department of Zoology, University of Jos for the kind support and supply of Oocysts for the research work. The study was conceived and finalized at the Applied Entomology and Parasitology Unit of the Department of Zoology, University of Jos, Nigeria. There were no financial or non-financial competing interests influencing the interpretation of data or presentation of information to our work. ONG conceived and designed the study and wrote the manuscript. GDA, AOF, EKT, NN, AIA, KD, AYM, MHB conducted the research work and also contribute to the writing of the manuscript. GD, NN, KD and AYM aided in the laboratory plant extraction, collection and identification of larvae and data analysis.

References

1. Alto, B.W. 2011. Interspecific larval competition between invasive *Aedes japonicas* and native *Aedes triseriatus* (Diptera: Culicidae) and adult longevity. *J. Med. Entomol.* 48(2): 232-242.
2. Alto, B.W., Lounibos, L.P., Higgs, S. and Juliano, S.A. 2005. Larval competition differentially affects arbovirus infection in *Aedes* mosquitoes. *Ecol. 86*(12): *3279–3288*.
3. Alto, B.W., Lounibos, L.P., Mores, C.N., and Reiskind, M.H. 2008. Larval competition alters susceptibility of adult *Aedes* mosquitoes to dengue infection. *Proc. the Royal Soc. B: Biol. Sc.* 275(1633):463-71.
4. Amer A. and Mehlhorn, H. 2006. Larvicidal effects of various essential oils against Aedes, Anopheles, and *Culex* larvae (Diptera, Culicidae). *Parasitol Res.* 99:466-472.
5. Bevins, S.N. 2007. Invasive mosquitoes, larval competition, and indirect effects on the vector competence of native mosquito species (Diptera: Culicidae). *Biol. Invasion.* 10(7): 1109-1117.
6. Briegel, H., Knusel, I. and Timmermann, S.E. 2001. *Aedes albopictus* (Diptera: Culicidae): physiological aspects of development and reproduction. *J. Med. Entomol.* 38(4): 566-571.
7. Center for Disease Control and Prevention (US). 1993. Emergency mosquito control associated with Hurricane Andrew-Florida and Louisiana, 1992. *MMWR Mord Mortal Weekly Rep.* 42(13):240-242.
8. Clements, A.N. 1992. *The biology of mosquitoes Volume 1. Development, nutrition and reproduction.* London: United Kingdom: Chapman and Hall.
9. Cunha, I.B.S., Sawaya, A.C.H.F., Caetano, F.M., Shimizu, M.T., Marcucci, M.C., Drezza, F.T., Povia, G.S. and Carvalho, P.O. 2004. Factors that influence the yield and composition of Brazilian propolis extracts. *J. Braz. Chem. Soc.* 15(6): 964–970.
10. Farnsworth, N.R. and Bingel, A.S. 1977. Natural products and plant drugs with pharmacological, biological or therapeutic activity. *Springer, Berlin, 1977.*
11. Fisher, P.R. 1991. The role of gaseous metabolities in phototaxis by *Dictyostelium discoideum* slugs. *FEMS Microbiol. Letters.* 77(1): 117-120.

***Corresponding author**

12. Goddard, L.B., Roth, A.E., Reisen, W.K. and Scott, T.W. 2002. Vector competence of California mosquitoes for West Nile Virus. *Emerg. Infect. Dis.* 8(12): 1385-1391.
13. Grimstad, P.R., and Walker, E.D. 1991. *Aedes triseriatus* (Diptera: Culicidae) and La Crose Virus. Nutrition deprivation of larvae affects the adult barriers to infection and transmission. *J. Med. Entomol.* 28(3): 378-386.
14. Helmenstine, A.M. 2011. Natural mosquito repellent. Strategies that work. Retrieved from http://chemistry.a.htm.
15. Helmerdinger, A., Olivo, C.J., Molento, M.B., Agnolin, C.A., Ziech, M.F., Scaravelli, L.F., Skonieski, F.R., Both, F.R. and Charao, P.S. 2006. Alcoholic extract of lemongrass (*Cymbopogon citratus*) on the control of *Boophilus microplus* in cattle. *Rev. Bras. Parasitol. Vet.* 15(1): 37-39.
16. Jeyasankar A. and Ramar G. 2015. Ovicidal and pupicidal activity of *Andrographis paniculata* (Acanthaceae) against vector mosquitoes (Diptera: Culicidae)Larvicidal properties of Breyeniavitis –idaea (Burm.F) Fischer (Euphorbiaceae) against important vector mosquitoes (Diptera: Culicidae). *Int. J. Curr. Res. Med. Sci.* 1(3): 53–61.
17. Klowden, M.J., Blackmer, J.L. and Chambers, G.M. 1988. Effects of larval nutrition on the host-seeking behaviour of adult *Aedes aegypti* mosquitoes. *J. Amer. Mosquito Control Assoc.* 4(1): 73-75.
18. Muturi, E.J., Blackshear, M. and Montgomery, A. 2012. Temperature and density dependent effects of larval environment on *Aedes aegypti* competence for an alphavirus. *J. Vector Ecol.* 37(1): 154-161.
19. Nasci, R.S. 1991. Influence of larval and adult nutrition on biting persistence in *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 28(4): 522-526.
20. Phrompittayarat, W., Putalun, W., Tanaka, H., Jetiyanon, K., Wittaya-areekul, S. and Ingkaninan, K. 2007. Comparison of various extraction methods of *Bacopa monnier*. *Naresuan Univ. J.* 15(1): 29-34.
21. Ponlawat, A. and Harrington, L.C. 2007. Age and body size influence male sperm capacity of the Dengue Vector *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 44(3): 422-426.
22. Pushpanathan, T., Jebanesan, A. and Govindarajan, M. 2008. The essential oil of *Zingiber officinale* Rosc (Zingiberaceae) as a mosquito larvicidal and repellent agent against the filarial vector *Culex quinquefasciatus* (say) (Diptera: Culicidae). *Parasitol. Res.* 102(2): 1289-1291.
23. Ravikumar, S., Ramanathan, G., Inbaneson, S.J. and Ramu, A. 2011. Antiplasmodial activity of two marine polyherbal preparations from *Chaetomorpha antennina* and *Aegiceras corniculatum* against *Plasmodium falciparum*. *Parasitol. Res.* 108: 107–113.
24. Reiskind, M.H. and Lounibos, L.P. 2009. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Med. Vet. Entomol.* 23(1): 62-68.
25. Sasidharan, S., Darah, I. and Jain, K. 2008. *In Vivo* and *In Vitro* toxicity study of *Gracilaria changii*. *Pharm. Biol.* 46(6): 413–417.
26. Schmidt, T.L., Barton, N.H., Rasić, G., Turley, A.P., Montgomery, B.L., Iturbe-Ormaetxe, I., Cook, P.E., Ryan, P.A., Ritchie, S.A., Hoffmann, A.A., O'Neill, S.O. and Turelli, M. 2017. Local introduction and heterogeneous spatial spread of dengue-suppressing *Wolbachia* through an urban population of *Aedes aegypti*. *PLoS Biol.* 15(5):1-28.
27. Sharma, P., Mohan, L. and Srivastava, C.N. 2009. Anti-juvenile activity of *Azadirachta indica* extract on the development and morphometry of filarial vector, *Culex quinquefasciatus* (Diptera: Culicidae) Say. *Parasitol. Res.* 105: 1193-1203.
28. Sosan, M.B., Adewoyin, F.B. and Adewunmi, C.O. 2001. Larvicidal properties of three indigenous plant oils on the mosquito *Aedes aegypti*. *Niger J. Natl. Prod. Med.* 5: 30-33.
29. SPSS 9.0 for Windows SPSS, Chicago; 2007.
30. Sukhdev, S.H., Suman, P.S.K., Gennaro, L. and Dev, D.R. 2008. Extraction technologies for medicinal and aromatic plants, International centre for science and high technology. pp.1-10.
31. Tandon, P. and Sirohi, A. 2010. Assessment of larvicidal properties of aqueous extracts of four plants against *Culex quinquefasciatus* larvae. *Jord. J. Biol. Sci.* 3: 1-6.
32. Telang, A., Qayum, A.A., Parker, A., Sacchetta, B.R. and Byrnes, G.R. 2012. Larval nutritional stress affects vector immune traits in adult yellow fever mosquito *Aedes aegypti* (*Stegomyia aegypti*). *Med. Vet. Entomol.* 26(3): 271-281.
33. Tiwary M., Naik S. N., Tewary, D. K., Mittal P. K. and Yadav, S. 2007. Chemical Composition and Larvicidal activities of the essential oil of *Zanthoxylum armatum.* DC (Rutaceae) against three mosquito vectors. *J. Vector Borne Dis.* 44(3): 198-204.
34. Woisky, R.G. and Salatino, A. 1998. Analysis of propolis: some parameters and procedures for chemical quality control. *J. Apicult. Res.* 37(2): 99–105.
35. World Health Organization. 2005. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13.Geneva.

***Corresponding author**