Advances in Research
3(6): 589-595, 2015, Article no.AIR.2015.054
ISSN: 2348-0394

SCIENCEDOMAIN ***international***
www.sciencedomain.org

Larvicidal Activity of *Pistia stratiotes* (Water Lettuce) against Larvae of *Aedes aegypti*

E. E. Ito[1*], J. C. Nmor[1*], J. E. G. Ake[1] and K. Utebor[1,2]

[1]Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria.
[2]Department of Biology, College of Education Agbor, Delta State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author EEI designed the study and carried out the experiments. Author JCN assisted with data analysis, interpretation and finalizing of the manuscript for intellectual contents. Authors JEGA and KU assisted with study design and review of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2015/13667
Editor(s):
(1) Antonio Mastino, Department of Biological and Environmental Sciences, University of Messina, Italy and Institute of Translational Pharmacology, National Research Council, Rome, Italy.
Reviewers:
(1) Anonymous, Brazil.
(2) Anonymous, Iran.
(3) Anonymous, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=758&id=31&aid=7363

Original Research Article

Received 27th August 2014
Accepted 10th November 2014
Published 17th December 2014

ABSTRACT

The present investigation assesses the larvicidal effect of *Pistia stratiotes* (water lettuce) against early 3rd and 4th instar larvae of *Aedes aegypti* in the laboratory. Mosquito larvae were sampled and larval population was monitored before and after application of plant aqueous extract. To assess the larvicidal activity, four different doses (0.5, 1.0, 1.5 and 2.0) mg/L respectively were used in the trials. Larval mortality was monitored after 24, 48 and 72 hours. At the highest dose, mortalities of 97.40, 98.80 and 99.80% for the third instar larvae and 99.70, 97.50 and 97.7% for the fourth instar larvae was observed. The percentage mortality for the fourth instar larvae decreased with hours of exposure. Analysis of variance (ANOVA) conducted on the data, showed that there was significant difference ($p>0.05$) in mortality between treatments and in hours of exposure. Further, the LC_{50} and LC_{90} for the 3rd were 0.11 and 1.44 mg/L while that of the 4th instar larvae were 0.22 and 1.51 mg/L respectively. The study indicated that (*P. stratiotes* is an effective insecticide against the larvae of *A. aegypti.* Measures to enhance its use for large scale mosquito control are essential.

**Corresponding author: E-mail: ito.eddie@yahoo.com, jcnmor@yahoo.com;*

Keywords: Larvicidal effect; aqueous botanical; Aedes mosquito larvae.

1. INTRODUCTION

It is well recognized that mosquito transmitted diseases constitute a significant public health problem globally. Species belonging to genera *Aedes, Anopheles* and *Culex* are vectors for the causative agent of various diseases like Dengue fever, Dengue haemorrhagic fever, Malaria, Japanese encephalitis and filariasis [1]. Among these diseases, malaria remains the most serious affecting some 300-500 million people and 1.4 to 2.6 million deaths annually throughout the world. More than 40% of the world's populations live in malarious areas [2]. In Nigeria, about 75% of the landmass is considered to be malarious and about two-thirds of the populations (over 40%) are at risk of the disease [3]. In addition to mortality, vector borne diseases cause morbidity in millions of persons thus, resulting in loss of man-days and causing economic loss [4].

A. aegypti, the vector of dengue and denuge haemorrhagic fever, is widely distributed in the tropical and subtropical zones. Dengue haemorraghic fever is endemic to South East Asia, the Pacific Islands, Africa and America [5]. One of the successful ways of reducing mosquito densities is by attacking the larval breeding sites with the use biopesticides [6].

Botanical products have been used traditionally by human communities and application of easily degradable plant compounds is considered to be one of the safest methods of control of insect pests and vectors. These plants are rich source of novel natural substances that can be used to develop environmental safe methods for insect control [7]. In all probability, these plants contained insecticidal phytochemicals that are predominantly secondary compounds produced by plants to protect themselves against herbivorous insects [8,9]. Development of resistance by pests and vectors against the botanicals has not been reported and botanical insecticides are generally pest or target specific, readily biodegradable, environmentally safe, target specificity, lower bioaccumulation and lack toxicity to higher animals, with low cost and can be used by individual and communities in specific situations [10-12].

Plant extracts or oils in general have been recognized as an important natural resource for the control of parasites and pest of public health importance [13-17]. Many researchers have reported the effectiveness of plant extract against mosquito larvae [12,18,19]. The use of plant crude-powder reduces the cost of extraction and thus would make the larvicides more accessible to the resource poor rural farming communities, especially in irrigation schemes.

Insecticidal activities of different plant essential oils have been reported against different mosquito species. For example, Tare et al. [20] reported the larvicidal activity of essential oils of 11 plants grown in the Himalayan region against *A. aegypti* larvae. Likewise, Pitasawat et al. [21] screened the larvicidal effects of ten plant species and found three plant essential oils (*Kaempferia galanga, Illicium verum* and *Spilanthes acmella*) to have larvicidal properties against *Culex quinquefasciatus*. The identification and eventual use of local plants in the control of mosquito larvae may be very valuable for developing countries. As a result, there has been an increased interest in developing potential alternative or additional control methods/materials that are effective against the target vector species [22,23]. The present investigation assessed the larvicidal effect of *P. stratiotes* against early 3rd and 4th instar larvae of *A. aegypti* in the laboratory.

2. MATERIALS AND METHODS

The research was conducted at the Department of Animal and Environmental Biology at Delta State University, Abraka, Nigeria (Long. 6º10 E, Lat. 5º79 N) between October and November, 2012. The materials used for this study were: Electric blender, white plastic container, *A. aegypti* mosquito larvae and dry leaves of water lettuce (*P. stratiotes*). Water lettuce(*P. stratiotes*) is a branched, smooth, succulent, herb, found in open, damp and waste places. It is more or less purplish herb (10-50 cm) in height, with somewhat 4-angled stems. The plant has analgesic [24], anti-inflammatory and antiarthritic activity [25].

2.1 Plant Collection and Preparation

Green leaves of water lettuce (*P. stratiotes*) were collected from Ogbe-Ijoh swamp in Delta State, Southern Nigeria and allow to air dried under shade for four weeks. The dry dark leaves were separated from the leaf petioles and ground into a fine powder by an electric motor driven blender. The ground material was further sieved severally through a fine mesh (1mm) to give the fine powder used for the bioassay.

2.2 Test Mosquitoes

Tests were carried out on Laboratory reared *A. aegypti* mosquitoes were used as test mosquitoes. These mosquitoes were free of exposure to insecticides and pathogens. Cyclic generation of *A. aegypti* was maintained at 25-29ºC and 80-90 percent R.H (relative humidity) in insectariums following the methods of [26].

Commercially prepared feed (powdered dog biscuit and yeast in the ratio of 3:1) was used in feeding the larvae. For adult mosquitoes, they were fed using10% glucose solution. Also, to aid egg production, adult female mosquitoes were periodically blood-fed using restrained albino mice [26]. Mosquito larvae were collected from the insectarium along the bank of River Ethiope in Delta State. Each habitat was first inspected for the presence of mosquito larvae. The mosquito larvae when present were sampled by the standard dipping technique as described by Service [27].

2.3 Bioassays Test

A total of 30 circular white plastic containers of 10cm in diameter and depth of 6cm were used for each treatment. The plastic basins with a capacity of 100 ml were filled with fresh aquatic water to mimic the natural environment of the mosquito larvae. The doses of the plant powder used for the test were 0.5, 1.0, 1.5 and 2.0 mg/L respectively. Each dose was placed in a plastic container and 100ml of fresh water was added. Ten active 3rd instar larvae of the mosquito were introduced into the container. Similar treatment was carried out for the fourth (4th) instar larvae of the mosquito. In the control, 100ml of distilled water were placed in a plastic container to which ten (10) 3rd and 4th instar larvae were separately added. Three replicated were set up for each dose including the control.

Mortality observations were made over 72 hours, after which the larvae were introduced into distilled water to notice recovery. A recovery time of 72 hours was allowed as recommended by WHO. Larvae were counted as dead when they were not coming to the surface for respiration and were probe insensitive [28].
In all tests, percentage reduction of larvae was determined and the percentage mortality calculated indirectly using Abbott's formulae [29], taking into account mortality in the controls. In this study, standard methods for testing the susceptibility of mosquito larvae to insecticides were employed following the methods described by WHO, [30].

$$\text{Mortality} = \frac{\begin{array}{c}\text{\% Mortality in treated} - \\ \text{\% Mortality in control}\end{array}}{100 - \text{\% Mortality in control}} \text{X } 100$$

2.4 Statistical Analysis

Descriptive statistics was performed using graph pad statistical package version 5 and Microsoft Excel 2003 was used for graphical presentation of data. Analysis of variance (ANOVA) was performed to test for significant difference in mortality with hours of exposure.Regression equation was derived to determine the LC_{50} for both 3rd and 4thinstar larvae.

3. RESULTS

The larvicidal activity of *P. stratiotes* (water lettuce) against *A. aegypti* mosquitos' larva is presented in Tables 1 and 2. The results indicated that in 24 hours duration, the percentage mortality of the 3rd and 4th instar larvae caused by 0.5g of *P. stratiotes* powder was 18.8 and 23.60% respectively. In 48 hours, the percentage mortality was 55.8 and 55.2 for the 3rd and 4th instar larvae respectively. At 72 hours of exposure, the mortality at 0.5g increased to 80.1 and 67.8%, for the 3rd and 4th instar larvae respectively.

At the application of 1.0 g, the mortality increased from 86.2, 87.60 and 92.30% for the 3rd instar larvae of the mosquito. A similar trend was also observed for the 4th instar larvae with a percentage mortality of 92.40, 83.00 and 91.70% at 24, 48 and 72 hours of exposure. At 1.5g of the powder of *P. stratiotes*, the mortality of *A. aegypti* for the 3rd instar larvae was 97.90, 97.20 and 99.40% (Table 1). Although there was a twist in the order of mortality in the 4th instar larvae with a decreasing mortality of 99.0, 85.0 and 93.60% at the same dose of 1.5g of the plant powder (Table 2).

At the highest powder dose of 2.0g, mortalities of 97.40, 98.80 and 99.80% was observed for the 3rd instar larvae as shown in Table 1. Still, there was fluctuation in mortality for the 4th instar larvae with mortality of 99.70, 97.50 and 97.7%

respectively (Table 2). The percentage mortality for the fourth instar larvae was observed to be decreasing with hours of exposure. Analysis of variance (ANOVA) conducted on the data, showed that there was significant difference (*P*<0.05) in mortality between treatment and in hours of exposure at 95% confidence limit. The highest mortality was observed in the highest application dose of the powder producing 98.80% and 99.80% (48 and 72h respectively) for the third instar larvae. While 97.50%, 97.70% and 99.70% was observed at 24, 48 and 72h of exposure respectively for the fourth instar larvae.

Analysis of the regression equations in Figs. 1 and 2 respectively shows that theLC_{50} andLC_{90} for the 3rd were 0.11 and 1.44mg/L respectively while that of the 4th instar larvae were 0.22 and 1.51 mg/L respectively.

Table 1. Cumulative mortality of 3rd instar larvae of *A. aegypti* exposed to *P. stratiotes* powder over 72 hours

Treatment dose (mg/L)	Mean percentage mortality (± S.E)*			LC (mg/L)	
	Exposure periods (Hrs)			LC_{50}	LC_{90}
	24	48	72		
0.5	18.80±1.44	55.80±1.31	80.10±1.17		
1.0	86.20±1.37	87.60±0.29	92.30±0.59	0.11	1.44
1.5	97.90±1.04	97.20±1.02	99.80±1.04		
2.0	97.40±1.27	98.80±0.60	99.80±0.14		

**Each value is mean of triplicate observations with 10 larval per replicate*

Table 2. Cumulative mortality of 4th instar larvae of *A. aegypti* exposed to *P. stratiotes* powder over 72 hours

Treatment dose (mg/L)	Mean percentage mortality (± S.E)*			LC(mg/L)	
	Exposure periods (Hrs)			LC_{50}	LC_{90}
	24	48	72		
0.5	23.60±1.17	54.20±1.27	67.80±1.69		
1.0	92.40±1.75	83.00±1.20	91.70±1.33	0.2	1.51
1.5	99.0±0.44	85.00±1.44	93.60±1.63		
2.0	99.70±0.17	97.50±0.41	97.70±1.29		

**Each value is mean of triplicate observations with 10 larval per replicate*

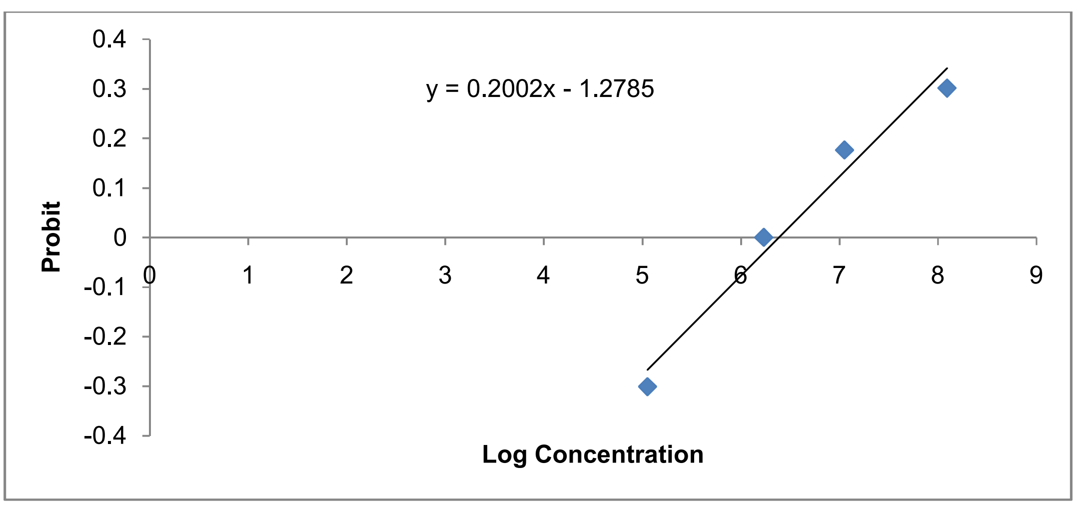

Fig. 1. Percentage mean mortality of 3rd instar larvae of *A. aegypti* exposed to *P. stratiotes* powder: regression equation inclusive

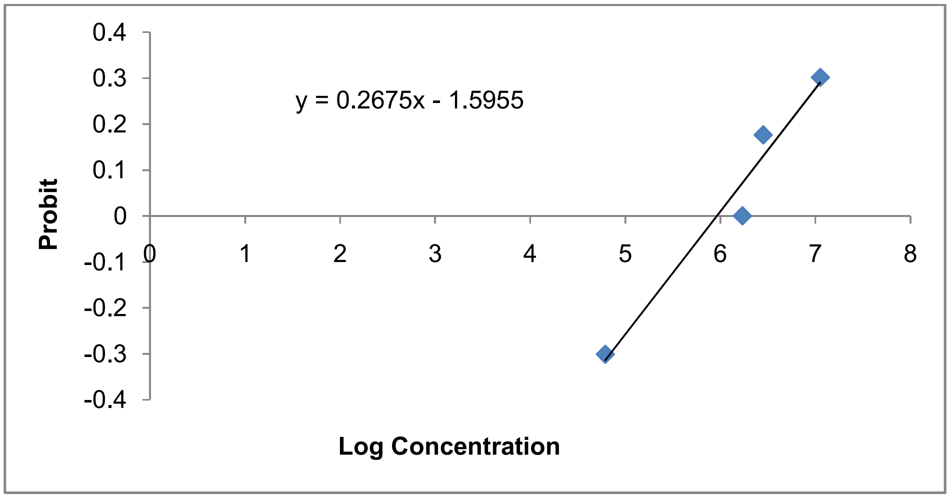

Fig. 2. Percentage mean mortality of 4th instar larvae of *A. aegypti* exposed to *P. stratiotes* powder: regression equation inclusive

4. DISCUSSION

Water lettuce, *P. stratiotes* L. is a free-floating aquatic weed that is well distributed in the tropical and subtropical region of Africa. Studies have shown that the plant possesses some medicinal properties. For example, the plant is considered antitubercular, antiseptic, antidysentric, antimicrobial and antifungal [31, 32]. It is no doubt why the Gambian, extracts from the leaves of *P. stratiotes* are used in treating eczema, leprosy, ulcers, piles and syphilis among other chronic skin diseases [33].

Despite the vast recognition of *P. stratiotes* as a medicinal plant with wide scale applicability, reports on the use of the plant as a possible candidate for mosquito larval control in Nigeria are lacking. This study assessed the larvicidal potential of *P.* stratiotes (water lettuce) against 3rd and 4th instar larvae of *Aedes aegypti.* It was observed that percentage mortality increased proportionally with increased in concentration of *P. stratiotes* extract. This suggest that *P. stratiotes* possess active compounds which are responsible for insecticidal. That the level of larvicidal activity was found to be dose dependent is not unexpected as result of previous study using plant crude extracts have also shown that larval mortality of *An. gambiae* and *Cx. quinquefarciatus* exposed to crude extracts of *Lepidagathis alopecuroides* and *Azadirachta indica* (neem) increased with time of exposure and concentration [34].

Although not significant, the percentage mortality of the fourth instar larvae slightly decreased with hours of exposure. The reason for this is unclear. However, given that the $LC_{50}$for the 4th instar larvae was higher than that of the 3 instar larvae, it is reasonable to infer that the 3rd instar larvae are more susceptible to *P. stratiotes.* Unrelated study using plant extract have shown that second instar larvae of *Cx. quinquefasciatus* were relatively more susceptible than fourth instar larvae as shown by higher level of mortality [35,36]. Further investigation on the larvicidal effect of *P. stratiotes* against early 3rd and 4th instar larvae of *A. aegypti would* be needed to unveil the pattern of mortality among the 4th instar larvae with reference to exposure.

In this study, it was observed that extract of *P. stratiotes* rendered the *A. aegypti* larvae inactive and motionless. Furthermore, disturbance in the normal behaviour of the larvae was observed in treatments of *A. aegypti* suggesting that the plant possesses larvicidal properties that may affect the either behavioural or physiology of the larvae. This possibly may be interterm to the study of Vincent et al. [37] which reported a decrease in the feeding behavior in *Anopheles* and *Culex* after treatment with neem extract.

5. CONCLUSION

The current results obtained in this study have important implications in the practical control of mosquito larvae in polluted aquatic ecosystem. The plant used in this study is available in large quantities in several aquatic habitats in the tropics. It can be concluded that nature possesses numerous medicinal plants, which may be useful for control of vector borne diseases. Therefore, the derived materials could be useful for managing field populations of mosquitoes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. Malaria fact sheets No. 94. WHO Report. Geneva: WHO media centre; 2010.
2. Ghai OP, Gupta P. Essential preventive medicine: A clinical and applied orientation, Ansari Road, New Delhi. 2000;969p.
3. Ghebreyesus TA, Deressa W, Witten KH, Getachew A, Seboxa T. Malaria. In: Berhane Y, Hailemariam D, Kloos H, (eds.). Epidemiology and Ecology of Health and Disease in Ethiopia, Shama Books, Addis Ababa. 2005;556-576.
4. Dhiman RC, Pahw S, Dhillon GP, Dash AP. Climate change and threat of vector-borne diseases in India: Are we prepared? Parasitol Res. 2010;106(4):763–773.
5. Senthilkumar A, Kannathasan K, Venkatesalu V. Chemical constituents and larvicidal property of the essential oil of *Blumea mollis* (D. Don) Merr. Against *Culex quinquefasciatus*. Parasitology Research. 2008;103:959-962.
6. Knio KM, Usta J, Dagher S, Zournajian H, Krediyyeh S. Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, *Ochlerotatus caspius*. Bioresource Technology. 2008;99:763-768.
7. Mandal S. Mosquito vector management with botanicals–the most effective weapons in controlling mosquito-borne diseases. Asian Pacific Journal of Tropical Biomedicine. 2012;336.
8. Grodner ML; 1997. Available: http://aapse.ext.vt.edu/archives/97AAPCO_report.
9. Casida JE, Quistad GB. Golden age of insecticide research: Past, present and future? Annu. Rev. Entomol. 1998;43:1-16.
10. Wariko R, Ray A, Sandhu JK, Samal R, Wahab N, Kumar S. Larvicidal and irritant activities of hexane leaf extracts of *Citrus sinensis* against the dengue vector *Aedes aegypti* L. Asian Pacific Journal of Tropical Biomedicine. 2012;152-155.
11. Govindarajan M, Sivakumar R. Mosquito adulticidal and repellent activities of botanical extracts against the malaria vector, *Anopheles stephensi* Liston (*Diptera: Culicidae*). Asian Pacific Journal of Tropical Medicine. 2011;941-947.
12. Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. Parasitol Res. 2008a;102(2):289–292.
13. Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res. 2009;104(6):1365–1372.
14. Schmahl G, Al-Rasheid KA, Abdel-Ghaffar F, Klimpel S, Mehlhorn H. The efficacy of neem seed extracts (Tre-san, MiteStop on a broad spectrum of pests and parasites. Parasitol Res. 2010;107(2):261–269.
15. Semmler M, Abdel-Ghaffar F, Al-Rasheid K, Klimpel S, Mehlhorn H. Repellency against head lice (*Pediculus humanus capitis*). Parasitol Res. 2010;106(3):729–731.
16. Elango G, Rahuman AA. Evaluation of medicinal plant extracts against ticks and fluke. Parasitol Res. 2011;108(3):513–519.9
17. Walldorf V, Mehlhorn H, Al-Quraishy S, Al-Rasheid KA, Abdel-Ghaffar F, Mehlhorn J. Treatment with a neem seed extract of beetle larvae parasitizing the plumage of poultry. Parasitol Res; 2011.
DOI: 10.1007/s00436-011-2535-9
18. Kovendan K, Murugan K, Naresh Kumar A, Vincent S, Hwang JS. Bio-efficacy of larvicidal and pupicidal properties of *Carica papaya* (*Caricaceae*) leaf extract and bacterial insecticide, spinosad against chikungunya vector, *Aedes aegypti* (*Diptera: Culicidae*). Parasitol Res. 2012a;110:669–678.
19. Kovendan K, Murugan K, Vincent S. Evaluation of larvicidal activity of *Acalypha alnifolia* Klein ex Willd. (*Euphorbiaceae*) leaf extract against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and *Bancroftian filariasis* vector, *Culex quinquefasciatus* (*Diptera:*

Culicidae). Parasitol Res. 2012b;110:571–581.

20. Tare V, Deshpande S, Sharma RN. Susceptibility of two different strains of *Aedes aegypti* (*Diptera: Culicidae*) to plant oils. Journal of Economic Entomology. 2004;97:1734-1736.
21. Pitasawat B, Choochote W, Kanjanapothi D, Panthong A, Jitpakdi A, Chaithong U. Screening for larvicidal activity of ten Carminative plants. Southeast Asia J. Trop. Med. Public Health. 1998;29:660-662.
22. Redwane A, Lazrek HB, Bouallam S, Markouk M, Amarouch H, Jana M. Larvicidal activity of extracts from Quercus Lusitania var. infectoria galls (oliv). J. Ethnophamacology. 2002;79:261-263.
23. Monzon RB, Alvior JP, Luezon LLC, Morales AS, Mutuo FES. Larvicidal potential of five Philippine plants against *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say). Southeast Asian J. Trop. Med. Public Health. 1994;25:755-759.
24. Amritphal S, Samir M, Ravi S. Anti-inflammatory and Analgesic Agents from Indian medicinal plants IJIB. 2008;131p.
25. Halstead SB. Global perspective on Dengue Research. Dengue Bulletin. 2000;24:77-82.
26. Arivoli S, Samuel T. Mosquitocidal activity of *Hyptis suaveolens* (l.) poit (*lamiaceae*) extracts against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* (*diptera: culicidae*). International Journal of Recent Scientific Research. 2011;I2(5):143 -149.
27. Service MW. Mosquito Ecology. Field Sampling Methods, Elsevier Applied Science. 2nd Edn, London. 1993;988.
28. Sivagnaname N, Kalyanasundaram M. Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutacea) against immature stages of mosquitoes and non-target organisms. Mem. Inst. Oswaldo. 2004;99(1):115-118.
29. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925;18:265-267.
30. WHO. Malaria entomology and vector control: Guide for participants. World Health Organization; 2013.
31. Achola KJ, Indalo AA. Pharmacologic activities of *Pistia stratiotes*. Pharmaceutical Biology. 1997;35(5):329-333.
32. Prem-kumar VG, Shyamsundar D. Antidermatophytic activity of *Pistia stratiotes*. Indian J Pharmacol. 2005;37:127-128.
33. Kirtikar KK, Basu BD. The Indian medicinal Plants. Dehradun: Oriental Enterprises. 2001;3576-3579.
34. Obomanu FG, Ogbalu OK, Gabriel UU, Fekarurhobo GK, Adediran BI. Larvicidal properties of *Lepidagathis alopecuroides* and *Azadirachta indica* on *Anopheles gambiae* and *Culex quinquefasciatus*. African Journal of. Biotechnology. 2006;5:761-765.
35. Ferreira PM, Carvalho AF, Farias DF, Cariolano NG, Melo VM, Queiroz MG, Machado-Neto JG. Larvicidal activity of the water extract of Moringa oleifera seeds against *Aedes aegypti* and its toxicity upon laboratory animals. Annals of the Brazilian Academy of Sciences. 2009;81(2):207-216.
36. Ashfaq M, Ashfaq U. Evalauation of mosquitocidal activity of water extract of Moringa Oleifera seeds against *Culex quinquefasciatus* (*Diptera: Culicidae)* in Pakistan. Pak Entomology. 2012;34:21-26.
37. Vincent I. Cycling to the finish. Neurobiol. Aging. 2000;21:757-760.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=758&id=31&aid=7363