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Evaluation of larvicidal activity from selected plant extracts against *Aedes aegypti*

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Abstract

Mosquitoes controlled by using synthetic chemical pesticides are creating numerous problems such as non-degradable, environmental pollution, and development of drug resistance etc., in this mind search for new approach based on biological active components. In this line, methanol solvent leaves extracts of *Colocasia esculenta* and *Wrightia tinctoria* were tested against second, third, fourth instar and pupa of *Aedes aegypti.* The results indicate that the LC_{50} values of second, third, fourth instar instars and pupa of *Ae. aegypti* for *C. esculenta* was 101.17ppm, 126.02ppm, 161.60ppm and 189.28 ppm. Likewise *W. tinctoria* LC_{50} values are 126.33 ppm for II instar, 149.90 ppm for III instar, 183.97 ppm for IV instar and 228.20 ppm for pupa respectively. Among the two plants methanolic solvent leaves extracts *C. esculenta* expose more effective than *W. tinctoria*.

Keywords: larvicidal activity, plant extracts, *Colocasia esculenta, Wrightia tinctoria, Aedes aegypti,* insecticidal activity

Introduction

Mosquitoes are main group of terrible insects which is spreading the life-threatening diseases such as Yellow fever, Filarial disease, Japanese encephalitis, Malaria, Dengue fever, Heamorragic fever, Chikungunya, Zika and West nile virus are the most terrific diseases transmitted by mosquitoes that show millions of deaths every years (Tolle, 2009; Venketachalam *et al.* 2010*;* Malar *et.al* 2021). WHO has acknowledged the mosquitoes are "public enemy's number one" (WHO, 2005).

Various chemical based methods used to control mosquitoes are facing a lot of problems due to harmful to other inhabitant organisms due to the continuous application of conventional synthetic insecticides (Govindarajan *et al.*, 2015). In this context a number of plant components are used against several of mosquito's species and these studies are reviewed from time to time (Sukumar *et al.*, 1999; Gosh *et al.*, 2012; Tehri and Sigh, 2015) . Green based insecticides has effective active compounds, biodegradable, non toxic to the environment. In this mind the present study finds out the larvicidal and pupicidal activity of methanol solvent leaves extract of *C. esculenta*, and *W.tinctoria* on *Ae.aegypti*. This study plants were selected based on their earlier report of their active compounds, medicinal, anti-microbial and larvicidal activity (Dhanraj, 2013; Tyagi and Agarwal, 2017; Thanigaivel *et al.*, 2017; Sakthivadivel, 2014).

Material and methods

Collection and preparation of extracts

The *C. esculenta* (Family: Asteraceae) and *W. tinctoria* (Family: Apocynaceae) leaves were collected from Karambayam village (10.4921° N, 79.3099° E), Thanjavur, Tamilnadu, India. The leaves were washed with tap water, shade dried for10-14 days at 27-37 °C.

C. esculenta and *W. tinctoria* 900 gms powder loaded in Soxhlet apparatus and extracted with methanol solvent. The solvent removed from crude extract with help of rotary vacuum evaporator and the crude extract powder obtained was stored at 4°C (Suman *et al.*, 2012; Govindarajan *et al.*, 2012). A stock solution prepare with 1 gm of crude extract mixed with 100 ml of acetone) (Kaushik *et al.*, 2008; Arivoli *et al.*, 2012). From this stock different concentrations were prepared and tested larvicidal bioassay.

GC- MS analysis

GC-MS analysis carried out from the crude extract. The components of the crude extract were identified by matching the peaks compared with the library present in the NIST library was the spectrum of detected compounds in the extract (Ghayal *et al.,* 2012).

Culture of Test Animal

Ae. aegypti eggs were obtained from vector control research centre, Madurai, Tamilnadu, India. The eggs were hatched out into the first instar emerged, the instar maintained in the laboratory at room temperature (27 ± 2 °C) and 75-85 % relative humidity. The larvae were fed with dog biscuit and yeast power in the ratio 3:1 (Sakthivadivel *et al.,* 2008). The second, third, fourth and pupa of *Ae. aegypti* was experimented for the present study.

Larvicidal Bioassay

The larvicidal activity evaluated as per direction of WHO protocols with slight modifications (WHO, 2005). Each 250ml containers filled with 200 ml of de chlorinated water. The test concentration ranging is 50 - 300ppm of *C. esculenta* and *W. tinctoria* methanol leaves extracts. A control also maintained separately adding 2ml of acetone in 200ml water in 250ml container. Each experiment 10 laevae were used. In order to prevent decomposition dead larvae were removed as soon as possible. A 24 hours larval stage and pupa mortality data was recorded against the two plant extracts. The corrected mortality was analyzed by using Abbott's formula (Abbott, 1925).

Statistical Analysis

The larval mortality data were calculated by the log probit analysis method of Finney (Finney, 1971) chi-square values were calculated by using SPSS (SPSS, 2007). A two way ANOVA executed to discover the variations in mortality as a function of plant species and larval stages

Results

The result indicates that seven bio active compounds in *C. esculenta* methanolic extract and *W. tinctoria* methanolic extract were thirteen compounds identified by GC-MS analysis (Table 1, 2).

The crude methanol solvent leaves extracts of *C.esculenta* and *W. tinctoria* tested against II, III, IV larval stages and pupa of *Aedes aegypti*. The LC_{50} and LC_{90} values are observed and given the table 3. The LC_{50} values of II, III, IV instars and pupa of *Ae. aegypti* for *C. esculenta* was 101.17, 126.02, 161.60 and 189.28 ppm. In the case of *W. tinctoria* the LC_{50} value was 126.33 ppm for II instar, 149.90 ppm for III instar, 183.97 ppm for IV instar and 228.20 ppm for pupa respectively. There was no mortality in control. In this study inferred that II instar more ($P<0.05$) than other instars in two plant extracts used, even as the pupa is maximum tolerant. Among the two plant extract examined *C. esculenta* was highly effective than *W. tinctoria* studied. A manner ANOVA became executed to discover the distinction many of the plant species and larval stages on mortality, the evaluation suggests that there is a sizeable distinction ($P<0.01$) current among the larval mortality as a characteristic of larval stages and plant extracts used (Table 4).

Discussion

A couple of researcher have mentioned in the region of the larvicidal and pupicidal activities of various plant parts and their larvicidal potential (Naseem *et al.*, 2016; Kulkami, 2017; Mcgregor *et al.*, 2021). These results are also similar to previous reports of Dass and Mariappan 2016 reported *C. esculenta* highly effective against *Culex quinquefasciatus* larvae and pupa than *W. tinctoria* leave methanolic extract.

Hexane, ethyl acetate and methanol solvent leaves and park extract of *Ocimum gratissimum*, *Gleditsia triacanthos*, *Eucalyptus sglobulus* and *Azadirachta indica* larval activity against IV instar of *Culex quinquefasciatus* (Diptera: Culicidae) the activity of four medicinal plant extracts showed the highest toxic effect of the methanol extract of *Ocimum gratissimum* leaves LC_{50} was 43.00mg/l (Ammar *et al.,* 2020). The leaves of *Lantana camara*, *Ruta chalepensis*, *Rhazya stricta*, and *Acalypha fruticosa* exhibited the strongest larvicidal effects against *Culex pipiens* (Al. Solami *et al.*, 2021). Larvicidal potential of ethanolic leaf extracts of *Cyathocline purpurea*, *Blumea lacera*, *Neanotis lancifolia*, and *Neanotis montholonii* affected gut of *Ae. aegypti* (Torawane *et al.,* 2021; Rajmohan and Ramaswamy, 2007). The LC_{50} value obtained for the plants belonging to Asteraceae and Apocynaceae families were also reported in Table 5.

Conclusion

GC-MS analysis clearly shows that the compounds present in the leaves have larval activity. This result sustains the search for new bio-active compounds that offer alternatives to synthetic pesticides in plants. From the results of this study and from other reports, it is clear that the leaves of plants have strong larval potential and are safe for the environment.

The methanol leaves extract of *C.esculenta* and *W.tinctoria* can significantly contribute to the overall population density of *Ae.aegypti* and the reduction of environmental pollution. In addition, the toxic effects of certain plant compounds on larvae must be tested before they can be recognized as safe for the environment. As the concentration of the plant extract increases, so does the mortality rate of the larvae.

Appendix
ppm: Parts Per Million
hrs: Hours
LC_{50}: Lethal Concentration that kills 50% of the uncovered larvae
LC_{90}: Lethal Concentration that kills 90% of the uncovered larvae
UCL: Upper Confidence Limit
LCL: Lower Confidence Limit
°C: Degree Celsius
χ2: Chi-square
MW: Molecular weight
RT: Retention time

Table 1: GC-MS evaluation of methanolic solvent extract of *C. esculenta.*

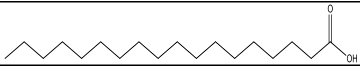
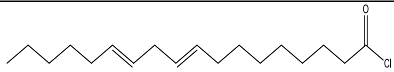
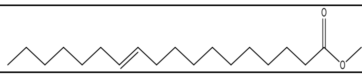
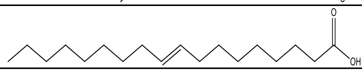
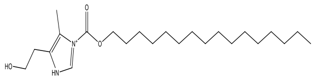
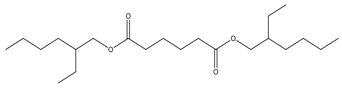
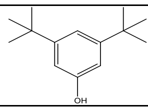
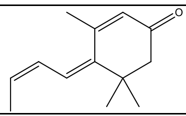
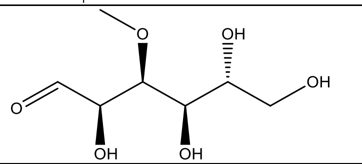
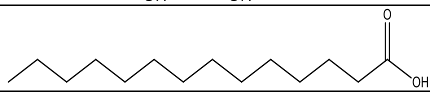
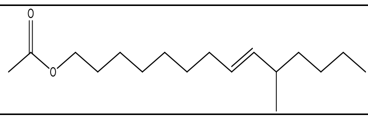
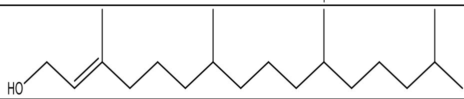
S. No	RT	Name of the compound	Molecular formula	MW	Peak area (%)	Structure
1	20.72	Octadecanoic acid	$C_{18}H_{36}O_2$	284	20.91	
2	21.83	9,12-Octadecadienoyl chloride	$C_{18}H_{31}ClO$	298	0.77	
3	21.918	11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	2.12	
4	22.677	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	64.37	
5	24.318	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl) 4-Methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409	1.36	
6	25.398	Hexanedioic acid, bis (2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370	1.36	
7	20.5	3,5-Di-t-butyl phenol	$C_4H_{22}O$	206	3.27	

Table 2: GC- MS analysis of methanol solvent extracts of *W.tinctoria*

S. No	RT	Name of the compound	Molecular formula	MW	Peak area (%)	Structure
1	11.85	Megastigmatrienone	$C_{13}H_{18}O$	190	1.16	
2	12.62	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	51.44	
3	13.36	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.88	
4	14.13	10-Methyl-8-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	2.80	
5	14.40	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.38	

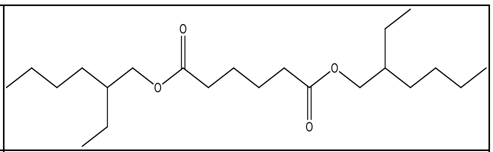
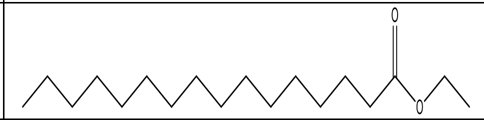
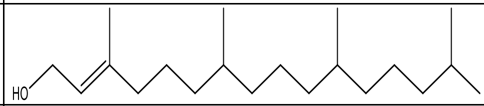
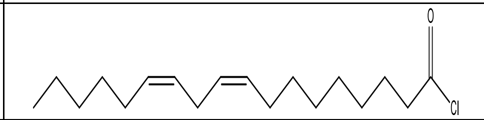
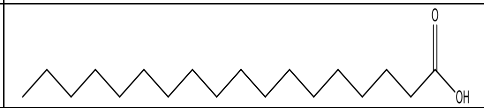
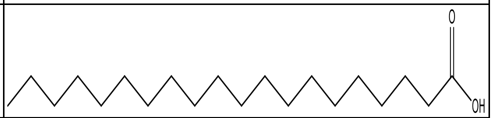
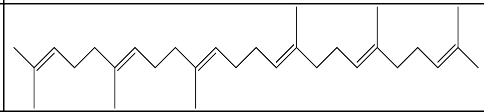
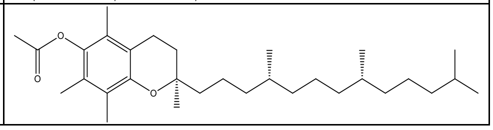
6	16.14	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.17	
7	16.44	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.64	
8	18.39	Phytol	$C_{20}H_{40}O$	296	4.47	
9	18.80	9,12-Octadecadienoyl chloride (Z,Z)-	$C_{18}H_{31}ClO$	298	4.31	
10	19.11	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.15	
11	22.13	Eicosanoic acid	$C_{20}H_{40}O_2$	312	1.39	
12	28.87	Squalene	$C_{30}H_{50}$	410	16.52	
13	35.84	Vitamin E acetate	$C_{31}H_{52}O_3$	472	3.13	

Table 3: The LC_{50} and LC_{90} values of *C.esculenta*, and *W.tinctoria* methanol solvent extract against the II, III, IV instar and pupa of *Ae.aegypti* in 24 hrs exposure

Plant species	Larval stages	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	$\chi 2$	Regression equation
Colocasia esculenta	II instar	101.17 (72.06 -127.95)	212.89 (164.07-351.91)	3.320	Y= -0.73 + 0.03 X
	III instar	126.02 (94.35-156.77)	257.17 (199.88-428.04)	1.932	Y= -0.6 + 0.037 X
	IV instar	161.60 (128.88 - 193.85)	283.91 (228.80-460.49)	1.737	Y= -2.05 + 0.04 X
	Pupa	189.28 (154.08 - 230.74)	335.63 (264.66-613.60)	.450	Y= -2.26 + 0.03 X
Wrightia tinctoria	II instar	126.33 (95.71-156.24)	250.13 (195.76 -407.13)	3.982	Y= -8.56 + 0.38 X
	III instar	149.90 (115.03-188.83)	317.43 (239.09-466.646)	5.697	Y= -2.21 + 0.041 X
	IV instar	183.97 (150.42-220.72)	314.55 (253.21-439.13)	.485	Y= -2.40 + 0.038 X
	Pupa	228.20 (190.45-292.29)	392.48 (302.17-958.76)	.497	Y= -1.86 + 0.021 X

Table 4: Two way ANOVA to check the validity if relationship in mortality (LC50) as a characteristic of plant extracts and larval and pupa

Source of variation	**SS**	***df***	**F**	**P-Value**	**F-scrit**	Significant
Plant species	10914.375	3	3638.125	292.02341	0.00033	***
Larval stages	1326.125	1	1326.125	106.44481	0.00194	**
Error	37.375	3	12.45833333			
Total	12277.875	7				

Significant at $P < 0.05$ level

Table 5: Summary of IV instars activity of Asteraceae and Apocynanceae family.

Sl. No	Plant species	Mosquito species	Solvent	LC 50 (ppm)	Reference
Family: Asteraceae					
1	*Ageratina adenophora*	*Cx.quinquefasciatus*	Acetone	227.2	Kamaraj *et al.*, 2010)
2	*Chrysanthemum indicum. L*	*Cx.quinquefasciatus*	Methanol	39.98	
3	*Tridax procumbens*	*Cx.quinquefasciatus*	Acetone	51.57	
4	*Sphaeranthus indicus*	*Cx.quinquefasciatus*	Hexane	1007.18	
			Diethy ether	211.42	
			Dichloromethane	1470.73	
			Ethyl acetate	1229.74	
5	*Tagetes minuta*	*Ae. fluviatilis*	Ethanol	15.5	
6	*Artemisia annua*	*An. stephensi*	Petroleum ether	16.85	(Sharma *et al.*, 2006
7	*Spilanthes acmella*	*Ae. albopictus*	Methanol	54.10	(Nath *et al.*, 2006)
8	*Eclipta alba*	*Ae.aegypti*	Benzene	151.38	(Govindarajan and Karuppannan, 2011)
			Hexane	165.10	
			Ethyl acetate	154.88	
			Methanol	127.64	
			Chloroform	146.28	
9	*Eclipta prostrata*	*Cx.quinquefasciatus*	Methanol	114.25	(Dass and Mariappan, 2016)
10	*Colocasia esculenta*	*Cx.quinquefasciatus*	Methanol	165.69	
11	*Colocasia esculenta*	*Ae.aegypti*	Methanol	161.60	Present study
Family: Apocynanceae					
12	*Solenostemma argel*	*Cx. pipens*	Methanol	0.31	Al-Doghairi *et al.*, 2004
13	*Calotropis gigantea*	*Ae. albopictus*	Methanol	467.00	(Nath *et al.*, 2006)
14	*Catharanthus roseus*	*Cx.quinquefasciatus*	Methanol	92.42	(Nazar *et al.*,2009)
15	*Catharanthus roseus*	*Ae.aegypti*	Acetone	207.83	(Remia and Logaswamy, 2011)
16	*Nerium oleander*	*Cx.quinquefasciatus*	Hexane	102.54	(Raveen *et al.*, 2014)
			Aqueous	2758.80	
18	*Eria coronaria*	*Cx.quinquefasciatus*	Methanol	72.41	(Mathivanana *et al.*, 2010)
		Ae.aegypti	Methanol	65.67	
		An. stephensi	Methanol	62.08	
19	*Wrightia tinctoria*	*Cx.quinquefasciatus*	Methanol	210.0	(Sakthivadivel *et al.*, 2014)
			Aqueous	3700	
20	*Wrightia tinctoria*	*Cx.quinquefasciatus*	Petroleum ether	210.29	(Dass and Mariappan, 2016)
21	*Wrightia tinctoria*	*Ae.aegypti*	Methanol	183.98	Present study

References

1. Abbott WS. A method of computing the effectiveness of insecticides. J Econ Entomol,1925:18:267–269
2. Al-Doghairi M, El-Nadi A, El hag E, Al-Ayedh H. Effect of *Solenostemma argel* on oviposition, egg hatchability and viability of *Culex pipiens* L. larvae. Phytother Res,2004:18:335-338.
3. Al-Solami HM. Larvicidal activity of plant extracts by inhibition of detoxification enzymes in *Culex pipiens*. Journal of King Saud University-Science.2021:33-101371.
4. Ammar U, Amina D, Mohammad S. Larvicidal activity of some selected medicinal plant extracts against the vector of filariasis. Journal of Experimental Sciences, 2020, 41-43.
5. Arivoli S, John Ravindran K, Raveen R, Tennyson S. Larvicidal activity of botanicals against the filarial vector *Culex quinquefasciatus* say (Diptera: culicidae). Inter J Res Zool,2012:2(1):13–17.
6. Dass KP, Mariappan P. Larvicidal activity of *Colocasia esculenta, Eclipta prostrata* and *Wrightia tinctoria* leaf extract against *Culex quinquefasciatus.* Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci,2016:86:139-143. DOI 10.1007/s40011-014-0423-7

7. Dhanraj NB, Kadam MS, Patil KN, Mane VS. Phytochemical screening and antibacterial activity of western region wild leaf *Colocasia esculenta.* Intl J Biol Sci,2013:2(10):18–23.
8. Finney DJ. Probit Analysis, III edn. Cambridge University Press, London, 1971
9. Ghayal N, Toro V, Biware M, Padhye A. Larvicidal Effects of GC-MS Fractions from Leaf Extracts of *Cassia uniflora* Mill Non Spreng. Int. J. Pharm. Sci. Rev. Res,2020:63(1):149-155
10. Ghosh A, Chowdury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J Med Res,2012:35:581-598
11. Govindarajan M, Karuppannan P. Mosquito larvicidal properties of Eclipta alba (L) Hassk (Asteraceae) against Chikungunya vector, *Ae. aegypti* (Linn) (Diptera:Culicidae). Asian Pac J Trop Med,2011:4(1):24–28.
12. Govindarajan M, Rajeswary M, Hoti SL, Bhattacharyya A. Benelli G. *Eugenol pinene* and *Plectranthus barbatus* essential oil as eco-friendly larvicides against malaria, dengue and *Japanese encephalitis* mosquito vectors. Parasitol. Res,2015:115:807-815.
13. Govindarajan M, Rajeswary M, Sivakumar R. Mosquito larvicidal and ovicidal activity of *Delonix elata L Gamble* against *Culex quinquefasciatus* say (Diptera: Culicidae). Asian Pac J Trop Dis, 2012, 571–573
14. Kamaraj C, Rahuman AA, Mahapatra A, Bagavan A, Elango G. Insecticidal and larvicidal activities of medicinal plant extract against mosquitoes. Parasitol Res,2010:107:1337–1349. doi: 10.1007/s00436-010-2006-8.
15. Kaushik R, Saini P. Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. J Vector Borne Dis,2008:45:66-69.
16. Kulkarni SJ. Review on mosquito control: surveys, analysis and investigations. International Journal of Research and Review,2017:4:10-3.
17. Malar J, Ejeta D, Asme A, Asefa A. Insecticidal effect of ethnobotanical plant extracts against *Anopheles arabiensis* under laboratory conditions. Malaria Journal,2021:20:466. doi: 10.1186/s12936-021-04004-6.
18. Mathivanana T, Govindarajan M, Elumalai, Kuppusamy, Kaliyamoorthy, Krishnappa, Ananthan A. Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* Stapf. (Family: Apocynaceae). Journal of vector borne diseases,2010:47:178-80.
19. McGregor BL, Connelly CR. A review of the control of Aedes aegypti (Diptera: Culicidae) in the continental United States. Journal of Medical Entomology,2021:58(1):10-25.
20. Naseem S, Malik MF, Munir T. Mosquito management: A review. Journal of Entomology and Zoology Studies,2016:4(5):73-79.
21. Nath DR, Bhuyan M, Goswami S. Botanicals as Mosquito larvicides. Defence Sci J 2006: 56(4):507–511.
22. Nazar S, Ravikumar S, Williams PG, Syed Ali M, Suganthi P. Screening of Indian coastal plant extracts for larvicidal activity of *Culex quinquefasciatus*. Indian J Sci Technol,2009:2:24 -27.
23. Rajmohan D, Ramaswamy M. Evaluation of larvicidal activity of the leaf extract of a weed plant, *Ageratina adenophora* against two important species of mosquitoes, *Ae. aegypti* and *Culex quinquefasciatus*. African J Biotech,2007:6(5):631–638.
24. Raveen KT, Kamakshi M, Deepa M, Arivoli S, Tennyson S. Larvicidal activity of *Nerium oleander* L (Apocynaceae) flower extracts against *Culex quinquefasciatus* say (Diptera: Culicidae). Intl J Mosq Res,2014:1(1):38-42.
25. Remia KM, Logaswamy S. Larvicidal efficacy of leaf extract of two botanical against the mosquito vector *Ae. aegypti* (Diptera:Culicidae). Indian J Nat Prod Resour,2011:1(2):208–212.
26. Sakthivadivel M, Daniel T. Evaluation of certain insecticidal plants for the control of vector of vector mosquitoes viz., *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. Appl. Entomol. Zool,2008:43:57-63.
27. Sakthivadivel M, Gunasekaran P, Annapoorani JT, Samraj DA, Arivoli S, Tennyson S. Larvicidal activity of *Wrightia tinctoria* R. BR. (Apocynaceae) fruit and leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pac J Trop Dis 2014: 4 (Suppl 1): S373-S377. doi: 10.1016/S2222-1808(14)60473-4.
28. Sharma P, Mohan L, Srivastava CN. Phytoextract-induced developmental deformities in malaria vector. Bioresource Technol,2006:97:1599–1604.
29. SPSS. SPSS for windows, version 11.5. SPSS Chicago, IL 2007.
30. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc,1999:7:210-237.
31. Suman T, Elumalai D, Vignesh A, Kaleena P, Murugesan K. Evaluation of larvicidal activity of the aerial extracts of a medicinal plant, *Ammannia baccifera*(Linn) against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. Asian Pacific Journal of Tropical Disease, 2012, 352-355.
32. Tehri K and Singh N. The role of botanicals as green pesticides in integrated mosquito management - A review. International Journal of Mosquito Research,2015:2(1):18-23.
33. Thanigaivel A, Srinivasan P, Senthil-Nathan S, Edwin E, Ponsanka A, Chellappandian M, Rani S, Escaline J and Kalaivani. Impact of *Terminalia chebula* Retz. against *Aedes aegypti* L. and non-target aquatic predatory insects. Ecotoxicology and Environmental Safety,2017:137:210-217.
34. Tolle MA. Mosquito-borne disease. Curr Probl Pediatr Adolesc Healthc,2009:39(4):97-140.

35. Torawane S, Andhale R, Pandit R, Mokat D, Phuge S. Screening of some weed extracts for ovicidal and larvicidal activities against dengue vector *Aedes aegypti*. The Journal of Basic and Applied Zoology,2021:82:36, 1-9. https://doi.org/10.1186/s41936-021-00233-y.
36. Tyagi T, and Agarwal M. Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes*. International journal of basic and applied medical sciences,2017:7(1):14-21.
37. Venketachalam MR., Jebasan A. Larvicidal activity of *Hydrocotyl javanica* thumb extract against *Culex quinquefasciatus*. J. Expt. Zool. India,2010:4:99-101.
38. WHO Communicable Disease Tool kit, Sudan. World Health Organization, 2005 WHO/CDS/2005.26.
39. WHO. Guideline for laboratory and filed-testing of mosquito larvicides. 2005: WHO/CDS/WHOPES/GCDPP/.13