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Evaluation of larvicidal efficacy of *Annona reticulata* (leaf) and *Psoralea corylifolia* (seed) extracts against larvae of *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* at Mysore.

Mohankumar, T. K. & Vijayan, V. A.

Department of Studies in Zoology, University of Mysore, Mansagangothri, Mysore-570006, Karnataka, India

Corresponding Author: *Mohankumar, T. K. Vector Biology Research Lab, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore- 570006, Karnataka, India.*

ABSTRACT

Mosquito borne diseases such as dengue, malaraia, lymphatic filariasis, yellow fever, Japanese encephalitis etc have intruded the globe since time immemorial. The present scenario for commanding the mosquitoes is aimed at application of target and stage specific, cost effective and biodegradable phytoproducts as these are reported to be safer for non- target organisms including man. It is in this regard, the present study was aimed to evaluate the efficacy of Annona reticulata leaf and Psoralea corylifolia seed extracts against the fourth instar larvae of Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi by employing the WHO bioassay method. The extraction process was carried out with a Soxhlet apparatus employing petroleum ether, chloroform, acetone, ethyl acetate and methanol as solvents. The results show that, the chloroform extracts of Annona reticulata was significantly effective against Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi with LC_{50} values of 58.73 ppm, 83.09 ppm and 100.83 ppm respectively ($P<0.05$). Likewise petroleum ether extracts of Psoralea corylifolia was significantly effective against Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi with LC_{50} values of 65.47 ppm, 58.40 ppm and 93.85 ppm respectively among all the above solvents ($P<0.05$). Thus the crude chloroform extracts of Annona reticulata and petroleum ether extracts of Psoralea corylifolia with good larvicidal efficacy could be considered for further characterization in order to explore the possible larvicidal efficacy on vectors.

Key words: *Annona reticulata, Psoralea corylifolia, Aedes aegypti, Culex quinquefasciatus, Anopheles stephensi, Efficacy, Bioactive, Larvicide.*

Introduction

Vector borne diseases have proved to be a major deterrent in the progress of man towards a better life, particularly in tropical and subtropical countries. Since many years arthropods have been shown to transmit human disease causing viruses, bacteria, protozoa, and helminthes by virtue of their hematophagous habits. In this regard mosquitoes are one of the most important group of vectors that affect the well being of humans and domestic animals world-wide. They are undeniably more detrimental to human health than any other group of arthropods as they transmit dreadful diseases such as malaria, dengue, chikungunya, filariasis, yellow fever, Japanese encephalitis etc.

Dengue and chikungunya are two mosquito-borne viral diseases of great public health concern in India as well transmitted by the same species of mosquito, *Aedes aegypti* and share spatiotemporal territories. Both the viruses (DEN and CHIK) are known to cause acute febrile illness with almost identical symptoms in the early phase of infection, although the clinical profiles differ as the infection progresses. The year 2015 was characterized by large dengue outbreaks worldwide, with the Philippines reporting more than 1,69,000 cases and Malaysia exceeding 111 000 suspected cases of dengue, representing a 59.5% and 16% increase in case numbers to the previous year, respectively (WHO, 2016). Brazil alone reported over 1.5 million cases in 2015, approximately 3 times higher than in 2014. Also in 2015, Delhi, in India, recorded its worst outbreak since 2006 with over 15,000 cases. An estimated 5, 00,000 people with severe dengue require hospitalization each year, a large proportion of who are children. About 2.5% of those affected die (WHO, 2016).

Chikungunya occurs in Africa, Asia and the Indian subcontinent. Human infections in Africa have been at relatively low levels for a number of years, but in 1999–2000 there was a large outbreak in the Democratic Republic of the Congo and in 2007 in Gabon. A large outbreak in India occurred during 2006 and 2007. Several other countries in South-East Asia were also affected. Since 2005, India, Indonesia, Maldives, Myanmar and Thailand have reported over 1.9 million cases (WHO, 2016). In 2007 transmission was reported for the first time in Europe, in a localized outbreak in north-eastern Italy. As of April 2015, over 13,79, 788 suspected cases of Chikungunya have been recorded in the Caribbean islands, Latin American countries, and the United States of America. 191 deaths have also been attributed to this disease during the same period. Canada, Mexico and USA have also recorded imported cases. WHO reported to small outbreaks of chikungunya in late 2015 in the city of Dakar, Senegal, and the state of Punjab, India (WHO, 2016).

As of malaria in 2015, there were an estimated 4, 38,000 deaths (range 2, 36,000–6, 35,000) worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) (WHO, 2015). Other regions have achieved impressive reductions in their malaria burden. Since 2000, the malaria mortality rate declined by 72% in the Region of the Americas, by 65% in the Western Pacific Region, by 64% in the Eastern Mediterranean Region, and by 49% in the South-East Asia Region. For the first time, the European Region reported zero indigenous cases of malaria in 2015 (WHO, 2015). Children under five are particularly susceptible to malaria illness, infection and death. In 2015, malaria killed an estimated 3, 06,000 under-fives globally, including 2, 92,000 children in the African Region. Between 2000 and 2015, the mortality rate among children under five fell by 65% worldwide and by 71% in Africa (WHO, 2015).

Various measures have been employed to control mosquito menace and one such approach is by eliminating it at its larval stage. Even though chemical insecticides are effective in controlling mosquitoes these created many environment problems such as insecticide resistance and environmental hazards. Insecticides residual problem together with its resistance forced us to seek attention towards alternative methods (Macedo et. al., 1997). In this regard, Many herbal products have been evaluated and used as natural insecticides very early, even before the use of synthetic insecticides (Mittal and subbarao, 2003). The co-evolution of plants with insect has equipped them with plethora of chemical defense which can be used against insect (Arivoli et. al., 2012). These phytochemicals can be extracted and so a considerable amount of work has been done and the use of botanical derivatives against mosquitoes. Natural insecticides such as pyrethrum, rotenone and nicotine among others have been extensively for insect control (Balandrin, 1985). Natural products are generally preferred to control insect since they are harmless, have no effect on non-target organisms and bio-degradable as well **(**Sharma et. al., 2005 and Bowers et. al., 1995).

It is in this regard, plant derived products were assayed in the present study in order to test the insecticidal property of certain local plant species, at the Vector Biology Research Lab, Department of Studies in Zoology at University of Mysore.

Materials and Methods

Ae. aegypti, Cx. quinquefasciatus and *An. stephensi* larvae available at the mosquito colony maintained in Vector Biology Research Lab, Department of Studies in Zoology, University of Mysore by following standard rearing techniques were employed for the experiments. The larvae were reared in large enamel or plastic trays (30x24x5 cm) containing dechlorinated water and fed with finely powdered dog biscuits and dry yeast in the ratio of 2:1.

Plant material and extraction

Annona reticulata leaves and *Psoralea corylifolia* seeds were collected from in and around Mysore, Karnataka and were shade dried, powdered manually. These were subjected to Soxhlet extraction with five solvents such as acetone, chloroform, methanol, ethyl acetate and petroleum ether until exhaustion, to obtain non polar bioactive constituents. This was later dissolved in acetone and employed to prepare different concentrations.

Larval bioassay

Bioassays on mosquito larvae were performed on late third or early fourth instars, according to standard guidelines of WHO (2005). The required quantity of plant extract of different concentrations was prepared in acetone as solvent. One ml of each of the concentration was mixed thoroughly with 249 ml of dechlorinated water in 500ml glass beakers. Parallel control tests were also maintained by adding 1ml of the solvent to 249 ml of dechlorinated water. Twenty five early fourth instar larvae were transferred to each of the beakers. A minimum of three replicates were kept for each concentration along with the control. Observation for the dead or moribund larvae was carried out after 24 hr duration at 25±2 ºC and 75±5% of relative humidity (RH).

Data analysis

Larval mortality counts if any were adjusted in the control, by employing Abbott's formula (Abbott, 1925) to give an estimate of the plant extract attributable mortality. The corrected mortality data were subjected to regression analysis of probit mortality on log dosage (Finney, 1971). The significant difference in LC_{50} among different solvent extracts is based on the non-overlapping of 95% Fiducial limits.

Results and discussion

The results depicting the efficacy of extracts of *Annona reticulata* leaf and the *Psoralea corylifolia* seeds obtained with different solvents tested against three mosquito species are presented in Table 1 and 2 along with the log dose-probit mortality responses in Fig 1 and 2 respectively. Out of five organic solvents, chloroform extracts of *Annona reticulata* and petroleum ether extracts of *Psoralea corylifolia* were significantly effective against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* mosquito species ($P<0.05$). The results show that the chloroform extracts of *An. reticulata* was significantly effective ($P<0.05$) against *Ae. Aegypti*, *Culex quinquefasciatus* and *An. Stephensi* with LC_{50} values of 58.73 ppm, 83.09 ppm, 100.83 ppm and LC_{90} values 116.01 ppm, 135.43 ppm, 219.70 ppm respectively, (Table 1). Likewise the petroleum ether extracts of *P. corylifoia* were found to be significantly effective ($P<0.05$) against *Ae. Aegypti*, *Cx. quinquefasciatus* and *An. Stephensi* with LC_{50} values of 65.47 ppm, 58.40 ppm, 93.85 ppm and LC_{90} being 130.23 ppm, 108.95 ppm, 211.04 ppm respectively (Table 2).

In another study carried out in Brazil ethanolic leaf extract of *An. squamosa* exhibited promising larvicidal activity against *Cx. quinquefasciatus* larvae (Das et al., 2007). The larvicidal and growth regulating activities of the related species *An. squamosa* and *Syzygium cumini* have been established against *An. stephensi* and other mosquitoes (Saxena et al. 1993 and Kaushik & Saini, 2008). In Tanzania Magadula et al., (2009) have reported significant activity by the extracts of *A. squamosa* and *A. senegalensis* with strong killing effects against insects particularly mosquitoes and from Brazil such a study indicated larvicidal effect against *Aedes abopictus* and *Cx. quinquefasciatus* (Das et al., 2007) and against *An. stephensi* (Saxena et al., 1993). In Tamilnadu Selvakumar et al., (2015) have reported larvicidal, ovicidal and pupicidal activity with different solvent extracts of *An. reticulata* against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* mosquitoes. An earlier study indicated that the crude extracts of stem bark of *An. reticulata* showed excellent larvicidal potency at very low concentrations against *Cx. quinquefasciatus* (Mallick and Chandra 2015). Govindarajulu et al., (2015) too have reported that the aqueous, ethanol and methanol extracts of *An. reticulata* leaf has exhibited larvicidal acticity against *Ae. aegypti*. Mohankumar et al., (2016) too have earlier reported that the methanol extracts of *An. reticulata* leaf showed significantly more efficacy against larvae of *Ae. aegypti* and *An. stephensi* mosquitoes ($P<0.05$).

P. corylifolia an annual herb is an important medicinal plant with clinical application and found in many traditional Chinese medicine formulas for the treatment of diseases such as leucoderma and other skin diseases, cardiovascular diseases, nephritis, osteoporosis, and cancer. Phytochemical studies indicated that coumarins, flavonoids, and meroterpenes are the main components of *P. corylifolia*, and most of these components are present in the seeds or fruits. The extracts and active components of *P. corylifolia* demonstrated multiple biological activities, including estrogenic, antitumor, anti-oxidant, antimicrobial, antidepressant, anti-inflammatory, osteoblastic, and hepatoprotective activities (Zhang et al., 2016). Kiran et al., (2011) also have showed the antifugal activity of bioactive compound isolated from seeds of *P. corylifolia* in Bangalore. Inspite of all these properties of this plant not much work has been reported with regard to the insecticidal activity. No study has been done on mosquitoes except, Dua et al., 2013 who have reported larvicidal and adulticidal activity of essential oil extracted from the *P. corylifolia* against larvae of *Cx. quinquefasciatus* at Uttrakhand. Thus the present study gains importance in exploiting the larvicidal activity of this plant species against three medically important mosquito species.

Many reports are available regarding the efficacy of non polar phyto constituents against mosquitoes. For instance, the reports of Madhumathy et al., (2007), and Lata and Ammini (2000) have indicated that petroleum ether extracts are more effective among organic solvents which is in accordance with our results. Raghavendra et al., (2011) who have reported the larval bioassay employing crude extracts of *Eugenia jambolana* by different solvents against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* mosquito species, all the organic extracts of *Eugenia jambolana* were bioactive, however significant larvicidal activity was observed

with petroleum ether followed by ethyl acetate, acetone and methanol extracts. Sakthivadivel et al., (2012) have also have shown that petroleum ether extract of *Argemone mexicana* have toxic effect against *Cx. quinquefasciatus* in India. Petroleum ether extract of *Jatropha curcas* leaf was also shown to have larvicidal effects on vector mosquitoes including *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* (Sakthivadivel and Daniel, 2008). Ahmed (2009) has proved that the toxic effect of petroleum ether extract of *Phragmites australis* leaf against *Cx. pipiens* larvae and adults. Madhumitha and Mary (2012) too have shown significant larval mortality in petroleum ether extract of *Crossandra infundibuliformis* leaf against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. Prejwltta et al., (2012) have also examined that the crude petroleum ether of *Ocimum basilicum* showed promising larvicidal activity against *An. stephensi*. The present report too agrees that the petroleum ether extracts of *P. corylifolia* seed has got significant larvicidal efficacy against *Ae. Aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Thus the present findings on *An. reticulata* and *P. corylifolia* showed promise for further chemical isolation of the active ingredient in future and it could be considered as a potent resource for an integrated control of mosquito larvae.

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References

1. ABBOTT, W. S. (1925) 'A method for computing the effectiveness of an insecticide'. Journal of Economic Entomology, vol. 07, pp. 154-156.
2. Ahmed, S. B. (2009) 'Toxicity and repellent activity of Phragmites australis extracts against the mosquito vector *Culex pipiens'*. Tunisian Journal of Plant Protection, vol. 4(2), pp.157-172.
3. Arivoli, S., John Ravindran, K and Samuel, T. (2012) 'Larvicidal Efficacy of Plant Extracts against the Malarial Vector *Anopheles stephensi* Liston (Diptera: Culicidae)'. World Journal of Medical Sciences, vol. 7(2), pp. 77-80.
4. Balandrin, M. F., Klocke, J, A., Wurtele, E. S and Bollinger, W. H. (1985) 'Natural plant chemicals sources of industrial and medicinal materials'. Science (Washington) vol. 228, pp. 1154-1160.
5. Bowers, W. S., Sener, B., Evans, P. H., Bingol, F and Erdomgan, I. (1995) 'Activity of Turkish medicinal plant against mosquitoes *Aedes aegypti* and *Anopheles gambiae'*. Insect Science and its Application. Vol. 16, pp. 339-342.
6. Das, N. G., Goswami, D., Rabha, B. (2007) 'Preliminary Evaluation of Mosquito Larvicidal Efficacy of Plant Extracts'. Journal of Vector Borne Diseases, vol. 44, pp. 145.
7. Dua, V. K, Kumar, A., Pandey, A. C and Kumar, S. (2013) 'Insecticidal and genotoxic activity of *Psoralea corylifolia* Linn. (Fabaceae) against *Culex quinquefasciatus* Say, 1823'. Parasites and Vectors, vol. 6(30), pp. 1-8.
8. Finney, D. J. (1971) Probit analysis. 3rd edn. Cambridge University Press, Cambridge.
9. Govindarajulu, B., Srimathi, A., Bhuvana, R and Karthikeyan, J. (2015) 'Mosquito larvicidal efficacy of the leaf extracts of *Annona reticulata* against *Aedes aegypti'*. International Journal Current Microbiology Applied Science, vol. 4(8), pp. 132-140.
10. Kaushik, R and Saini, P. (2008) 'Bioefficacy of *Syzygium cumini* (Jamun) leaf extracts against larval stages of *Aedes aegypti* (Diptera: Culicidae)'. Indian Journal of Environmental Science, vol. 12(2), pp. 99–102.
11. Kiran, B., Lalitha, V and Raveesha,.K. A. (2011) 'Antifungal activity of bioactive compound of
12. seeds of *Psoralea corylifolia* L. against seed borne *Fusarium* species of maize'. Journal
13. of Applied Pharmaceutical Science, vol. 01(08), pp. 161-164.
14. Lata, C and Ammini, J. (2000) 'Curcuma raktakanda is a potential larvicide for mosquito control'. Journal of Pharmaceutical Biolology, vol. 38, pp. 167-170.
15. Macedo, M. E., Consoli, T. M. S., Grandi, A. M. G., Anjos, A. B., Oliveira, N. M and Mendes, R. O. (1997) 'Screening of Asteracea plant extract for larvicidal activity against *Aedes fluviatilis'*, Memerias do Institute Oswaldo Cruz, vol 92, pp. 565-570.
16. Madhumathy, A. P., Aivazi, A. A and Vijayan, V. A. (2007) 'Larvicidal efficacy of *Capsicum annum* against *Anopheles stephensi* and *Culex quinquefaciatus'*. Journal of Vector Borne Diseases, vol. 44, pp. 223-226.
17. Madhumitha, G and Mary, S. A. (2012) 'Screening of larvicidal activity of *Crossandra infundibuliformis* extracts against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus'*. International Journal of Pharmacy and pharmaceutical Sciences, vol. 4(1), pp. 485-487.
18. Magadula, J. J., Innocent, E and Otiewo, J. N. (2009) 'Mosquito larvicidal and cytotoxic activities of 3 *Annona* species and isolation of active principles'. Journal of Medicinal Plants Research, vol. 3(9), pp. 674 – 680.
19. Mallick, S and Chandra, G. (2015) 'Larvicidal activities of extracts of stem bark of *Annona reticulata* against filarial vector *Culex quinquefasciatus'*. International Journal of Pharmacy and Biological Sciences, vol. 6(3), pp. 1347 – 1356.

20. Mittal, P. K and Subbarao, S. K. (2003) 'Prospects of using herbal products in the control of mosquito vectors'. ICMR Bulletin, vol. 33(1), pp. 1-10.
21. Mohankumar, T. K., Kumuda, S. S and Vijayan, V. A. (2016) 'Screening of methanolic plant extracts against larvae of *Aedes aegypti* and *Anopheles stephensi* in Mysore'. Journal of Arthropod Borne Diseases, vol. 10(3), pp. 303-314.
22. Prejwltta, M., Preeti, S., Lalit, M., Manish, M. V and Chand, N. S. (2012) 'Larvicidal efficacy of *Ocimum basilicum* extracts and its synergistic effect with neonicotinoid in the management of *Anopheles stephensi'*. Asian Pacific Journal of Tropical Diseases, pp. 110-116.
23. Raghavendra, B. S, Prathibha, K. P and Vijayan, V. A. (2011) 'Larvicidal efficacy of Eugenia jambolana Linn. extracts in three mosquito species at Mysore'. Journal of Entomology, pp. 1-6.
24. Sakthivadivel, M and Daniel, T. (2008) 'Evaluation of certain insecticidal plants for the control of vector mosquitoes viz. *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti'*. Applied Entomology and Zoology, vol. 43, pp. 57-63.
25. Sakthivadivel, M., Eapen, A and Dash, A. P. (2012) 'Evaluation of toxicity of plant extracts against vector of lymphatic filariasis, *Culex quinquefasciatus'*. Indian Journal of Medical Research, vol. 135, pp. 397-400.
26. Saxena, R. C., Harshan, V., Saxena, A., Sukumaran, P., Sharma, M. C and Lakshanakumar, M. (1993) 'Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi'*. Journal of the American Mosquito Control Association' vol. 9(1), pp. 84-87.
27. Sharma, P., Mohan, L and Srivastava, C. N. (2005).' Larvicidal potential of *Nerium indicum* and *Thuja orientalis* extract against malaria and Japanese encephalitis vector'. Journal of Environmental Biology, vol. 26(4), pp. 657-660.
28. Selvakumar, B., Gokulakrishnan, J., Elanchezhiyan, K and Deepa, J. (2015) 'Mosquito larvicidal, ovicidal and pupicidal activities of *Annona reticulata* Linn. (Annonaceae) against *Aedes aegypti*, *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say. (Diptera: Culicidae)'. International Journal of Recent Scientific Research, vol. 6(2), pp. 2690-2696.
29. WHO, (2005) 'Guidelines for laboratory and field testing of mosquito larvicides'. *WHO/CDC/WHOPES/GCDPP/2005.13.*
30. WHO, (2015) World malaria report. http://www.who.int/malaria/media/world-malaria-report-2015/en/
31. WHO, (2016) 'Fact sheet'. int/mediacentre/factsheets/fs327/en/
32. Zhang, X., Zhao, W., Wang, Y., Lu, J and Chen, X. (2016) 'The Chemical Constituents and Bioactivities of *Psoralea corylifolia* Linn'. A Review, vol. 44(1), pp. 35-60.

Table. 1. Efficacy of different solvent extracts of *Annona reticulata* leaf against the larvae of three mosquito species.

Sl. No	Mosquito species	Extraction solvents	LC_{50} (ppm)	95% FL	LC_{90} (ppm)	95% FL	Slope±SE	Heterogeneity (df)	Regression equation
1	*Aedes aegypti*	Petroleum ethe	135.63	83.54-215.28	316.51	203.96-1897.77	3.48±0.44	6.77 (3)	Y=3.48X±2.42
		Ethyl acetate	95.24	46.83-139.69	262.64	172.23-931.30	2.90±0.30	5.20 (3)	Y=2.90X±0.75
		Chloroform*	58.73	54.53-63	116.01	104.67-132.28	4.33±0.02	1 (2)	Y=4.33X±2.66
		Methanol	156.27	97.78-212.48	398.48	272.06-1425.59	3.15±0.36	4.08 (3)	Y=3.15X±1.91
		Acetone	150.61	105.58-188.43	293.71	226.88-572.02	4.41±0.28	4.19 (3)	Y=4.41X±4.4
2	*Anopheles stephensi*	Petroleum ethe	129.73	92.63-167.18	315.21	227.62-709.53	3.32±0.24	3.12 (3)	Y=3.32X±2.02
		Ethyl acetate	99.12	67.29-127.89	234.84	173.70-466.96	3.42±0.23	3.31 (3)	Y=3.42X±1.83
		Chloroform*	100.83	57.74-137.33	219.70	156.85-635.89	3.78±0.38	5.67 (3)	Y=3.78X±2.59
		Methanol	115.82	105.03-126.66	294.11	254.75-356.59	3.16±0.02	1 (3)	Y=3.16X±1.53
		Acetone	157.87	144.97-171.43	375	325.14-454.78	3.41±0.29	1 (3)	Y=3.41X±2.49
3	*Culex quinquefasciatus*	Petroleum ethe	96.42	89.39-103.01	182.93	167.31-205.29	4.60±0.02	1 (3)	Y=4.60X±4.14
		Ethyl acetate	118.94	112.53-125.20	198.60	184.41-218.27	5.75±0.02	1(3)	Y=5.75X±6.94
		Chloroform*	83.09	67.45-96.75	135.43	113.51-196.27	6.04±0.20	3.37(3)	Y=6.04X±6.59
		Methanol	109.01	99.18-118.70	256.30	226.39-301.23	3.45±0.02	1(3)	Y=3.45X±2.03
		Acetone	70.30	62.98-77.40	176.16	155.13-207.29	3.21±0.02	1 (3)	Y=3.21X±0.93

Note: LC_{50} - median lethal concentration; FL - fiducial limits; LC_{90} - lethal concentration; df – degree of freedom.
*Difference in LC_{50} among other solvent extracts is significant based on non-overlapping 95% fiducial limits ($P < 0.05$).

Table. 2. Efficacy of different solvent extracts of *Psoralea corylifolia* seed against the larvae of three mosquito species.

Sl. No	Mosquito species	Extraction solvents	LC_{50} (ppm)	95% FL	LC_{90} (ppm)	95% FL	Slope±SE	Heterogeneity (df)	Regression equation
1	*Aedes aegypti*	Petroleum ethe	65.47	49.71-80-76	130.23	100.72-239.90	4.29±0.23	3.37 (3)	Y=4.29X±2.79
		Ethyl acetate	107.34	95.72-119.05	315.39	267.07-394.81	2.73±0.03	1 (3)	Y=2.73X±0.56
		Chloroform	122.67	112.27-133.23	287.02	251.77-341.32	3.47±0.02	1 (3)	Y=3.47X±2.25
		Methanol	110.67	101.27-119.95	245.09	218.66-283.72	3.71±0.02	1 (3)	Y=3.71X±2.28
		Acetone	114.13	104.25-123.98	263.25	232.73-309.09	3.53±0.02	1 (3)	Y=3.53X±2.26
2	*Anopheles stephensi*	Petroleum ethe	93.85	56.74-125.10	211.04	153.61-477.43	3.64±0.30	4.58 (3)	Y=3.64X±2.18
		Ethyl acetate	109.95	100.30-119.57	246.36	219.71-284.62	3.65±0.02	1 (3)	Y=3.65X±2.4
		Chloroform	99.04	89.54-108.26	234.46	207.81-273.74	3.42±0.02	1 (3)	Y=3.42X±1.83
		Methanol	113.24	102.32-124.23	286.93	250.36-342.40	3.17±0.02	1 (3)	Y=3.17X±1.51
		Acetone	108.16	60.26-153.96	326.28	214.95-100.43	2.67±0.29	4.01 (3)	Y=2.67X±0.43
3	*Culex quinquefasciatus*	Petroleum ethe	58.40	42.01-74.66	108.95	83.00-232.19	4.73±0.31	5.04(3)	Y=4.73X±3.36
		Ethyl acetate	68.79	46.25-91.01	142.94	104.03-388.63	4.03±0.35	4.98(3)	Y=4.03X±2.41
		Chloroform	97.46	60.28-136.69	213.51	147.95-926.75	3.76±0.44	5.75(3)	Y=3.76X±2.48
		Methanol	65.58	49.33-83.46	136.38	102.91-247.68	4.03±0.19	3.53(3)	Y=4.03X±2.32
		Acetone	110.02	84.11-137.84	212.68	161.93-456.77	4.47±0.28	3.89(3)	Y=4.47X±4.13

Note: LC_{50} - median lethal concentration; FL - fiducial limits; LC_{90} - lethal concentration; df – degree of freedom.
*Difference in LC_{50}, among other solvent extracts is significant based on non-overlapping 95% fiducial limits (P< 0.05).

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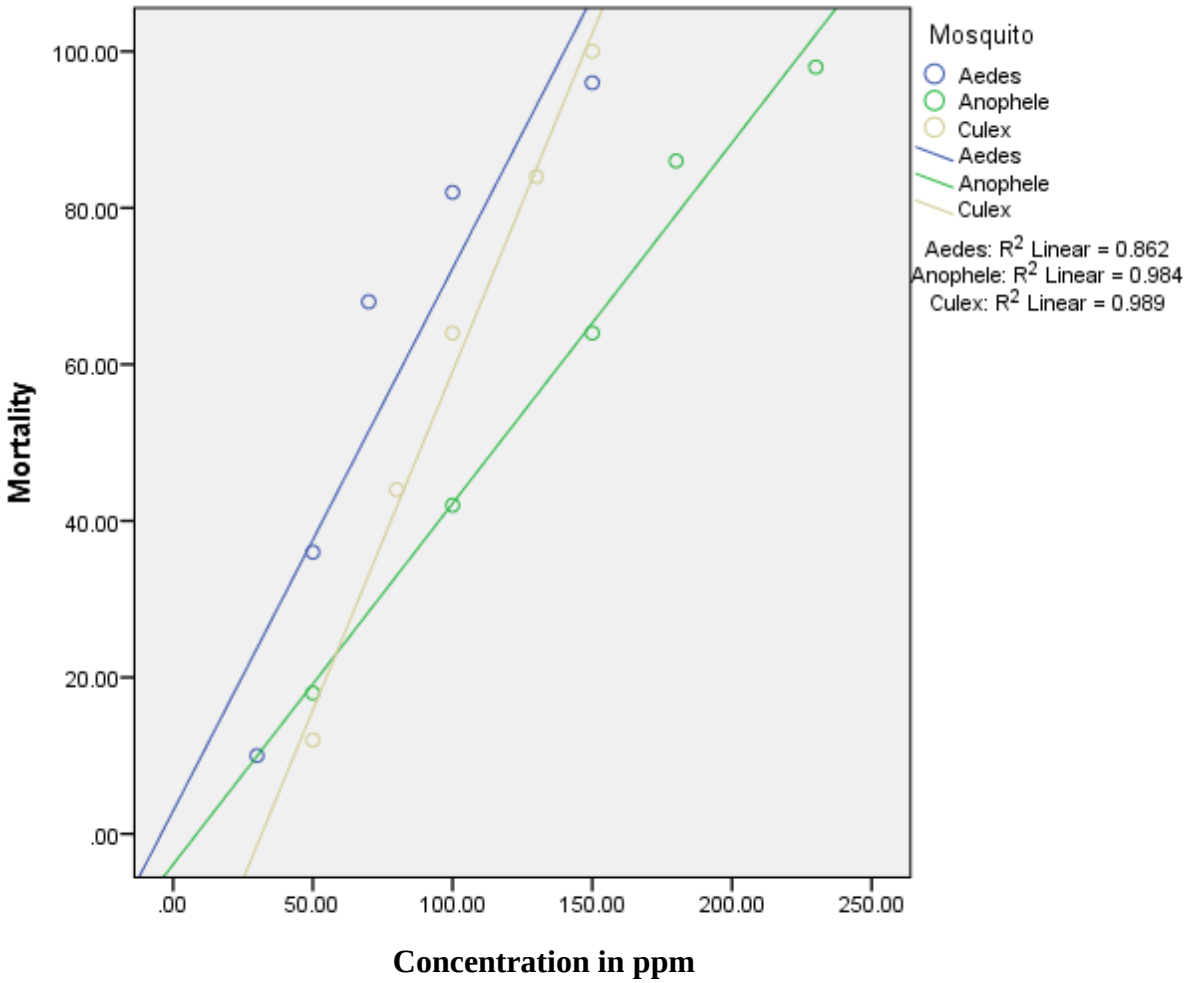

Fig. 1. Effect of chloroform leaf extract of *Annona reticulata* against *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* larvae.

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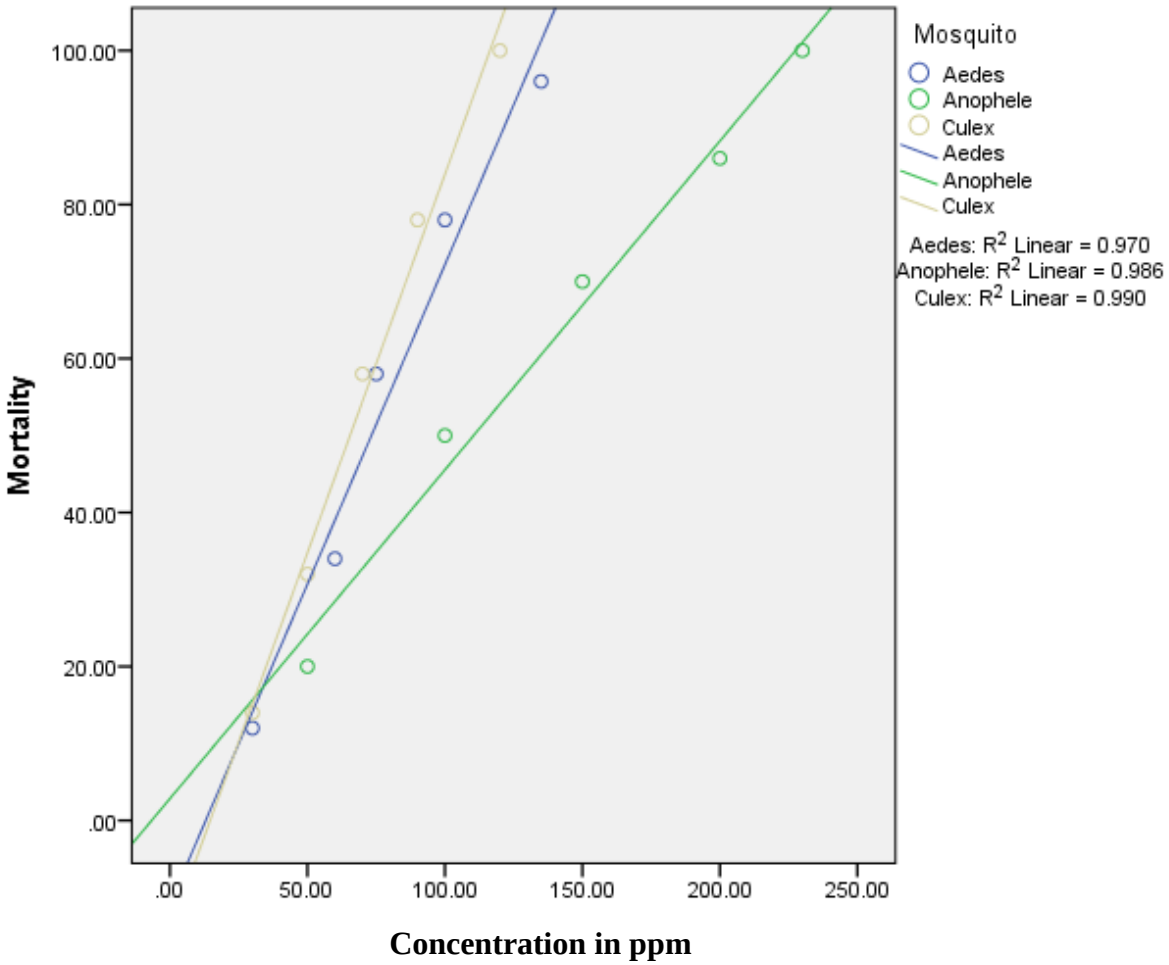

Fig. 2. Effect of petroleum ether seed extract of *Psoralea corylifolia* against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* larvae.