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Larvicidal and synergistic activity of *Anisomeles malabarica* and *Phyllanthus emblica* against the larvae of common malarial vector, *Anopheles stephensi* Liston. (Diptera : Culicidae)

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General Note

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ABSTRACT

In the present investigation, an attempt was made to assess the larvicidal activity of two indigenous plants, *Anisomeles malabarica* and *Phyllanthus emblica* against the larvae of economically important malarial vector, Anopheles stephensi under laboratory condition. Present experiment revealed that the methanol extract of both the plants showed significant larvicidal activity and also combined extracts (synergistic) exhibit highest larval mortality. Hence, the present research deciphers the possible utilization of naturally occurring plant based chemicals are potent enough to replace the synthetic chemical mosquitocidal agents in the intense vector control programme.

1. INTRODUCTION

Mosquito-borne diseases have health impact, including loss in commercial and labour outputs, particularly in developing countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases (Fradin and Day, 2002). Mosquitoes plays a major role in transmission of several diseases like, malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE) (James 1992; Gubler 1998; Peng et al., 1999). In India, malaria is one of the most important causes of direct or indirect infant, child and adult mortality. About two million confirmed malaria cases and 1,000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by the World Health Organisation (WHO) Southeast Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia (Kumar et al., 2007). Malaria infects more than 500 million humans each year, killing approximately 1.2 to 2.7 million per year. About 90% of all malaria cases occur in Africa, as do approximately 90% of the world's malaria-related deaths (Breman et al. 2004; Snow et al. 2005). JE is a disease caused by an arbovirus that is mainly transmitted by the bite of infected *Culex tritaeniorhynchus* mosquitoes. The annual incidence and mortality estimates for JE are 30,000 to 50,000 and 10,000, respectively (Solomon, 2004). Keiser et al. (2005) have reported that approximately 1.9 billion people currently live in rural JE-prone areas of the world, the majority of them in China (766 million) and India (646 million).

2. MATERIALS AND METHODS

The fresh leaves of *A. malabarica* and *P. emblica* were collected from Melvanakkambadi village, Chengam Taluk, Thiruvannamalai District of Tamilnadu, India. Voucher specimen has been deposited in the department laboratory. Mosquito culture was raised from the larvae collected from the field, and continuous care was taken until the emergence of the adult mosquito. Then they were allowed to lay fresh eggs in plastic and enamel trays containing tap water. They were maintained and all the experiments were carried out at 27±2°C and 75–85% relative humidity under 14:10 light and dark cycles. The freshly emerged larvae were fed *albeit*, dog biscuits. From this culture freshly moulted fourth instar larvae were employed in the experiments. For bioassay test, 25 larvae were taken in five batches in 249 mL of water and 1.0 mL of the desired plant extract concentration. The control was set up with respective solvent and DMSO (Elumalai et al., 2013). The numbers of dead larvae were counted after 24 h of exposure and the percentage of mortality was reported from the average of five replicates and the percent larvicidal activity was calculated.

2.1. Preparation of plant extracts

The dried leaf (200 g), was powdered by using mechanical blender and sequentially extracted with pentane, diethyl ether and methanol (1000 mL, Qualigens), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22–26 mmHg at 45°C by using rotary vacuum evaporator (Superfit, PBU 6) and the residue obtained was stored at 4°C.

2.2. Larvicidal bioassay

Larvicidal activity of the selected plant extracts were done as per the procedure prescribed by the WHO (1996) with some modification and as per the method of Elumalai et al. (2013). For bioassay test, larvae were taken in five batches of 25 in 249 mL of water and 1.0 mL of the desired plant extract concentration. The control was set up with respective solvent and DMSO. The numbers of dead larvae were counted after 24 h of exposure and the percentage of mortality was reported from the average of five replicates and the percent larvicidal activity was calculated by using Abott's corrected formula (Abott, 1925).

$$\text{Corrected \% mortality} = \frac{\%\ \text{mortality treated group} - \%\ \text{mortality control group}}{1 - \%\ \text{mortality control group}} X100$$

2.3. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits (lower and upper confidence limit) and chi-square values were calculated using the software SPSS (Version 17). Results with $p<0.05$ were considered to be statistically significant.

3. RESULT AND DISCUSSIONS

Larvicidal activity of pentane extract of *A. malabaria* showed in figure 1. At 25 ppm concentration the maximum larval mortality of 23.2% was recorded in diethyl ether extract followed by pentane (18.6%) and 17.6% of larvae were found dead in methanol extract. Similarly at 50ppm concentration, the maximum larval mortality was recorded from the pentane extract followed by methanol and diethyl ether extracts. The similar trend was also observed at 100ppm concentration of the extracts. The maximum larval mortality in *A. malabarica* was noted from pentane extract (97.8%) followed by ethanol (96.8%) and diethyl ether extract (95.6%). Among the

three extracts tested against the fourth instar larvae of *A. stephensi*, diethyl ether extract showed minimum LC50 value of 46.62ppm with 43.08 (LCL) and 59.95 (UCL). In the same way, the minimum LC90 value of 123.41ppm was recorded from the pentane extract of *A. malabarica* with 99.47ppm (LCL) and 153.11ppm (UCL) (Table 1). Similarly, *P. Emblica* showed maximum larvicidal activity in methanol extract at higher concentration (200mg/l) with 97.2% larval mortality (Table 2; figure 2). Besides, synergistic effect of selected plants showed statitstically significant larval mortality of 97.4% at 200mg/l concentration (table 3 & figure 3). The LC_{50} value of 34.53 mg/l was noted with methanol extract of *P. emblica* and the LC90 value was found to be 201.62mg/l (regression value= y = 3.634x - 1.003).

Table 1

Lethal concentrations of different extracts of *Anisomeles malabarica* tested against fourth instar larvael of *Anopheles stephensi*

Solvents used	LC_{50}	95% Fiducial limit		LC_{90}	95% Fiducial limit		Regression	Slope	x^2
		LCL	UCL		LCL	UCL			
Pentane	48.66	43.08	54.95	123.41	99.47	153.11	y = 4.318x - 2.247	4.3188	42.609
Diethyl ether	46.62	39.07	55.62	169.95	113.54	254.11	y = 3.61x - 1.099	3.6100	62.797
Methnol	51.30	45.78	57.50	127.77	102.63	159.06	y = 4.156x - 2.044	4.1560	42.25

LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; x^2= Chi-square test.

Table 2

Lethal concentrations of different extracts of *Phyllanthus emblica* tested against fourth instar larvael of *Aedes aegypti*

Solvents used	LC_{50}	95% Fiducial limit		LC_{90}	95% Fiducial limit		Regression	Slope	x^2
		LCL	UCL		LCL	UCL			
Pentane	37.10	29.37	46.86	137.83	98.15	193.57	y = 3.660x - 1.047	3.6600	52.88
Diethyl ether	45.88	39.49	53.33	135.11	102.72	177.77	y = 3.796x - 1.385	3.7959	47.997
Methnol	34.53	24.54	48.59	201.62	107.51	378.11	y = 3.634x - 1.003	3.6346	74.257

LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; x^2= Chi-square test.

Table 3

Synergistic effects of *Anisomeles malabarica* + *Phyllanthus emblica* (lethal concentrations) tested against fourth instar larvael of *Aedes aegypti*

Solvents used	LC_{50}	95% Fiducial limit		LC_{90}	95% Fiducial limit		Regression	Slope	x^2
		LCL	UCL		LCL	UCL			
Pentane	42.02	35.22	50.13	132.59	99.58	176.55	y = 3.885x - 1.462	3.8858	46.13
Diethyl ether	37.36	29.18	47.82	151.22	101.95	224.30	y = 3.643x - 1.029	3.6433	48.035
Methanol	35.30	27.36	45.55	136.39	96.48	192.79	y = 3.936x - 1.452	3.9367	62.611

LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; x^2= Chi-square test.

Earlier authors reported that the methanol extracts of *C. nigricans* showed 100% larval mortality against *O. triseriatus* (Georges et al. 2008). Jang et al. (2002) have reported that the methanol extracts of *C. obtusifolia, C. tora* and *V. tetrasperma* exhibited more than 90% larval mortality at 200 ppm *on A. aegypti* and *C. pipiens*. The larvicidal activity of ethanolic extract of *Caesalpinia bonduc (Family:*

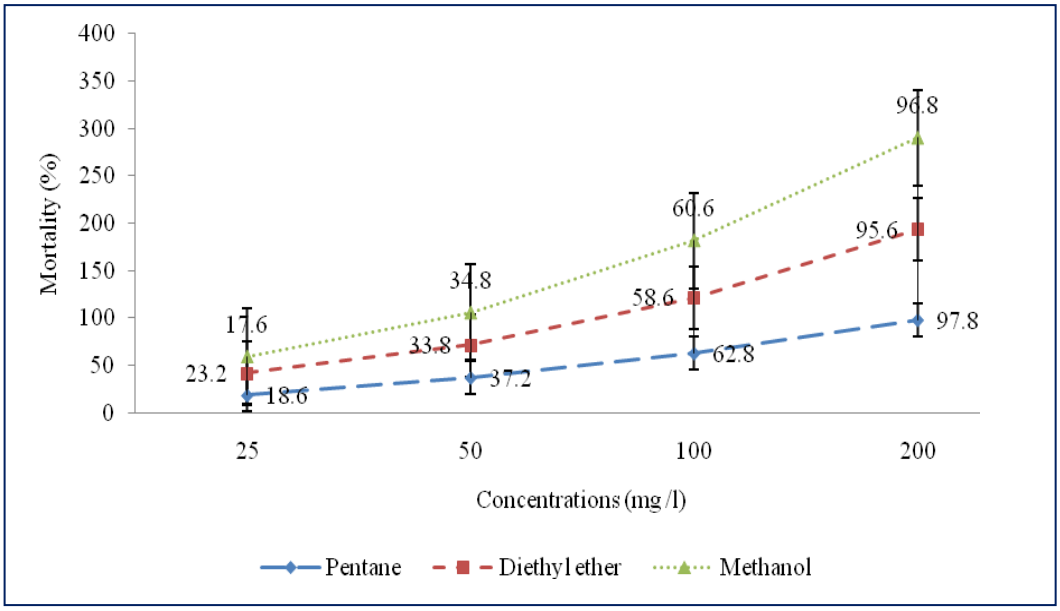

Figure 1

Larval mortality of different extracts *Anisomeles malabarica* tested against fourth instar larvael of *Anopheles stephensi*

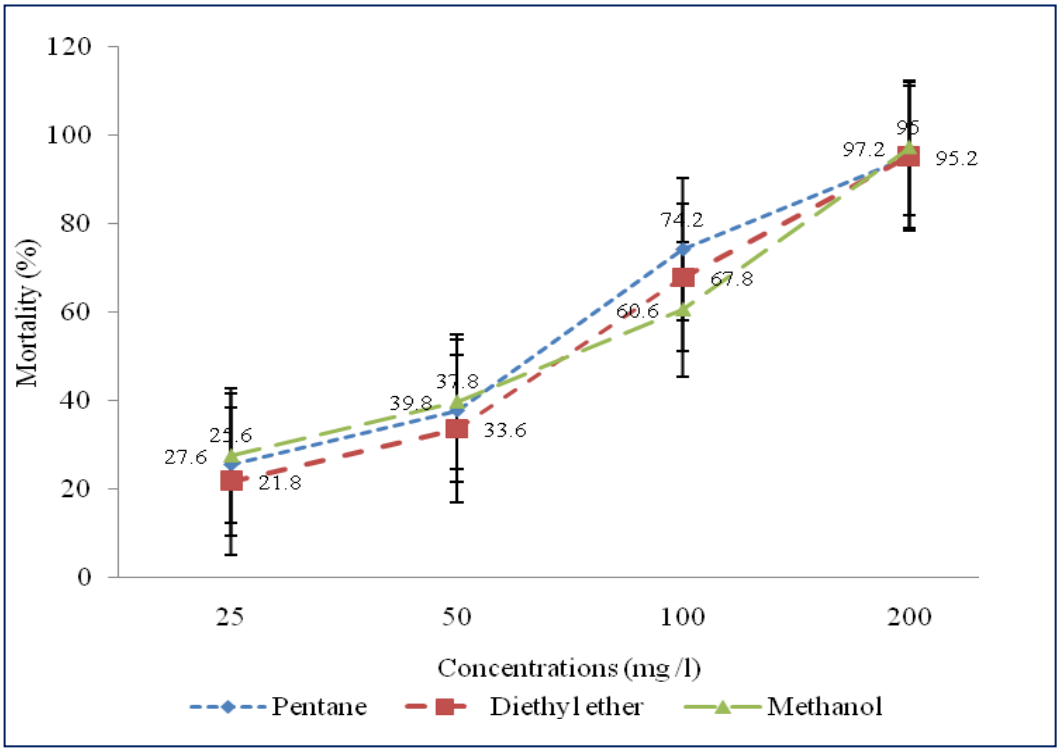

Figure 2

Larval mortality of different extracts *Phyllanthus emblica* tested against fourth instar larvael of *Anopheles stephensi*

The isolation and purification of crude extract of the selected plants l are in progress.

Caesalpiniaceae) showed 100% mortality *(Saravanan et al.* 2007). Plant extracts found to have potent larvicidal activity against various vector mosquitoes have also been reported by Rahuman et al. (2008a-e). Kannathasan et al. (2008) have reported that the fatty acid methyl ester extract of *V. trifolia* showed the highest larvicidal activity with an LC_{50} value of 9.25 ppm followed by *V. altissima* (14.82 ppm) and *V. negundo* (18.64 ppm) against early fourth instar larvae of *C. quinquefasciatus.* Chowdhury et al. (2008a&b) have been reported that methanol extracts of mature leaves of *S. villosum* showed the LC_{50} value for all instars between 24.20 and 33.73 ppm after 24 h and between 23.47 and 30.63 ppm after 48 h of exposure period against *A. subpictus*. The hexane fraction of *Kaempferia galangal* was found to exhibit the highest larvicidal effect with the LC_{50} of 42.33 ppm against *C. quinquefasciatus* (Choochote et al. 1999). In conclusion, an attempt has been made to evaluate the role of medicinal plant extracts' larvicidal bioassay against *A. stephensi* and the results reported in this study open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts.

4. CONCLUSION

Application of synthetic mosquitocides caused several unwanted consequences such as allergic reactions and side effects. Thus, it paves the way for further exploration of phytopesticide that are easily available in nature and also environmentally safer to non-target organisms like mammals. Hence, the present investigation was under taken to assess the combined effect of selected two plants against the fourth instar larvae of *A. stephensi*, a common malarial vector. Certainly, in the near future, these plants would replace the chemicals and will occupy the real moment in the intense vector control programme.

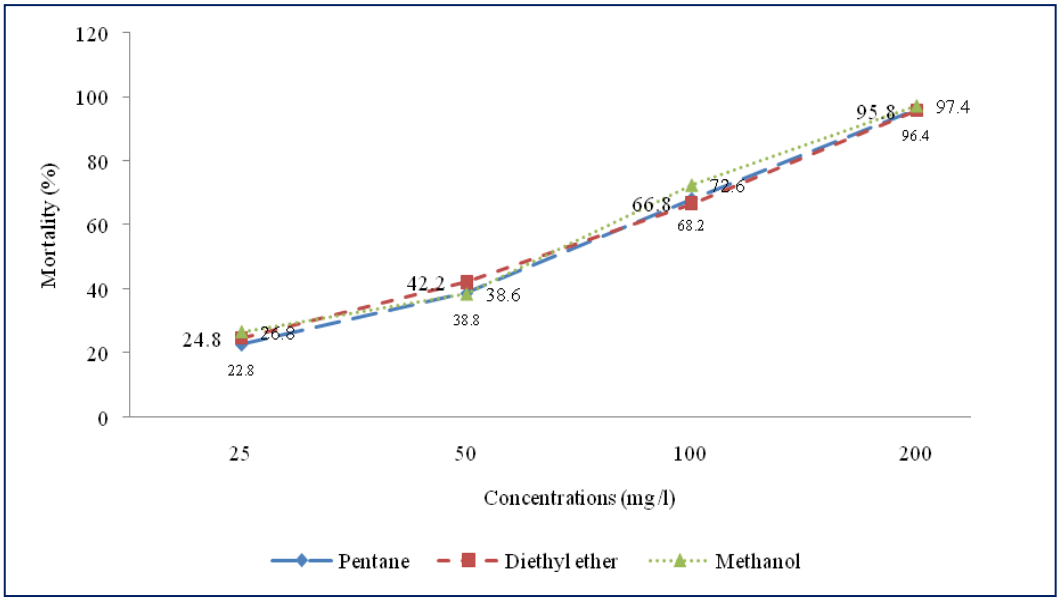

Figure 3

Synergistic effects of Anisomeles *malabarica* +*Phyllanthus emblica* (larval mortality) tested against fourth instar larvael of *Aedes aegypti*

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REFERENCE

1. Abbott WS. A method of computing the effectiveness of an insecticides. *J Econ. Entomol.,* 1925. 18: 265-267.
2. Breman JG, Martin AS, Mills A. Conquering the intolerable burden of malaria: what's new, what's needed: a summary. *Am J Trop Med Hyg*., 2004, 71(2):1–15
3. Choochote W, Kanjanapothi D, Panthong A, Taesotikul T, Jitpakdi A, Chaithong U, Pitasawat B. Larvicidal, adulticidal and repellent effects of *Kaempferia galanga*. *Southeast Asian J Trop Med Public Health*. 1999, 30(3):470–476
4. Chowdhury N, Chatterjee SK, Laskar S, Chandra G Larvicidal activity of *Solanum villosum* Mill (Solanaceae: Solanales) leaves to *Anopheles subpictus* Grassi (Diptera:Culicidae) with effect on non-target *Chironomus circumdatus* Kieffer (Diptera: Chironomidae). J Pest Sci., doi: 2008a,10.1007/s10340-008-0213-1
5. Chowdhury N, Ghosh A, Chandra G. Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. *BMC Complement Altern Med*., 2008b, 8:10
6. Krishnappa K, Mathivanan T, Elumalai, A, Jeyasankar A, DhanasekaranS, Elumalai K. Evaluation of *Cissus quadrangularis* and *Combretum ovalifolium* medicinal plants extracts against medically important human malarial vector mosquito *Anopheles stephensi* Liston (Diptera:Culicidae). *Int J of Interdisci Res and Revs*. 2013. 1(4): 11-18
7. Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquitoes bites. *N Engl J Med*., 2002, 347:13–18
8. Georges K, Jayaprakasam B, Dalavoy SS, Nair MG. Pestmanaging activities of plant extracts and anthraquinones from *Cassia nigricans* from Burkina Faso. *Bioresour Technol*., 2008, 99 (6):2037–2045
9. Gubler DJ. Resurgent vector borne diseases as a global health problem. *Emerg Infect Dis.,* 1998, 4:442–450
10. James AA. Mosquito molecular genetics: The hands that feed bite back. *Science*., 1992, 257:37–38
11. Jang YS, Baek BR, Yang YC, Kim MK, Lee HS. Larvicidal activity of leguminous seeds and grains against *Aedes aegypti* and *Culex pipiens pallens*. *J Am Mosq Control* Assoc., 2002, 18(3):210–213
12. Kannathasan K, Senthilkumar A, Venkatesalu V, Chandrasekaran M. Larvicidal activity of fatty acid methyl esters of *Vitex* species against *Culex quinquefasciatus*. *Parasitol Res*., 2008, 103 (4), 999–1001.
13. Keiser J, Maltese MF, Erlanger TE, Bos R, Tanner M, Singer BH, Tzinger J. Effect of irrigated rice agriculture on Japanese encephalitis, including challenges and

opportunities for integrated vector management. *Acta Trop*., 2005, 95:40–57

14. Kumar A, Valecha N, Jain T, Aditya P. Dash burden of malaria in India: retrospective and prospective view. Am J Trop Med Hyg.,2007,77(6):69–78
15. Peng Z, Yang J, Wang H, Simons FER. Production and characterization of monoclonal antibodies to two new mosquito *Aedes aegypti* salivary proteins. *Insect Biochem Mol Biol.,* 1999, 29:909–914
16. Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K. Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) Sweet. *Parasitol Res.,*2008a, 102(5):981–988
17. Rahuman AA, Venkatesan P, Gopalakrishnan G Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. *Parasitol Res*., 2008b, 103 (6):1383–1390
18. Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K, Bagavan A. Mosquito larvicidal activity of isolated compounds from the rhizome of *Zingiber officinale*. *Phytother Res*., 2008c, 22 (8):1035–1039
19. Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K. Larvicidal activity of some Euphorbiaceae plant extracts against Aedes aegypti and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res*., 2008d, 102(5):867–873
20. Rahuman AA, Bagavan A, Kamaraj C, Vadivelu M, Zahir AA, Elango G, Pandiyan G. Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res*., 2008e, doi:10.1007/s00436-008-1240-9
21. Raja N, Elumalai K, Jayakumar M, Jeyasankar A, Muthu C, Ignacimuthu S. Biological activity of different plant extracts against armyworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *J Entomol Res*., 2003, 27(4):281–292
22. Saravanan KS, Periyanayagam K, Ismail M. Mosquito larvicidal properties of various extract of leaves and fixed oil from the seeds of *Caesalpinia bonduc* (L) Roxb. *J Commun Dis*., 2007, 39(3):153–157
23. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI .The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*., 2005, 434(7030):214–217
24. Solomon T. Flavivirus encephalitis. *N Engl J Med*., 2004, 351:370– 378
25. WHO. Report of the WHO informal consultation on the evaluation on the testing of insecticides. CTD/WHO PES/IC/.1996,96.1, p 69.