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Relative larvicidal potential of *Pseudocalymma alliaceum* and *Allium sativum* against malaria vector, *Anopheles stephensi* (Liston)

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Abstract

Petroleum ether, hexane and methanol extracts of *Pseudocalymma alliaceum* and *Allium sativum* were evaluated against larvae of *Anopheles stephensi*. The hexane extract of *Pseudocalymma alliaceum* was found to be the most effective of the extracts tested followed by the hexane extract of *Allium sativum*. The LC_{50} values for hexane extract of *P. alliaceum* and *A. sativum* were 8.7 and 7.5 ppm after 24 h, and 10.8 and 7.6 ppm after 48 h of exposure. Additionally the LC_{90} values were 31.0 and 22.1 ppm after 24 h, and 25.0 and 15.4 ppm after 48 h of exposure.

Keywords: *Pseudocalymma alliaceum*, *Allium sativum*, *Anopheles stephensi*, Larvicidal action.

Introduction

Malaria is one of the most serious diseases of the tropical regions and accounts for 310-515 million clinical cases annually with 1.5-3.0 million deaths per year (Snow *et al*., 2005). In India, 2-3 million malaria cases and about 1,000 deaths are reported every year (Lal *et al*., 2010). According to the WHO (2011), there were 216 million cases of malaria and an estimated 655,000 deaths in 2010. Therefore, to minimize the losses due to malaria, the vector, *Anopheles stephensi* must be effectively controlled.

Different methodologies are currently employed including applications of synthetic pesticides, microbial pesticides and phytopesticides, of which it is considered that the synthetic pesticides are the most effective. As synthetic pesticides are non-biodegradable, environmental hazardous and non-target specific, it is considered preferable to replace these by phytopesticides which are biodegradable, less hazardous, target specific and importantly, have not been subject to the development of resistance. The present work advances the management of malaria vectors by evaluation of different extracts (petroleum ether, hexane and methanol) of *Pseudocalymma alliaceum* leaves and *Allium sativum* cloves.

Materials and methods

Collection of plant materials and preparation of extracts

Leaves of *Pseudocalymma alliaceum* (Figure 1a) were collected from the Botanical Garden, Department of Botany, Faculty of Science of Dayalbagh Educational Institute, Agra and cloves of *Allium sativum* (Figure 1b) were collected from the local market. Leaves were washed in running tap water and dried in the shade, crushed manually and then stored in glass containers at room temperature (30± 2ºC) until use. The cloves were separated manually and cut into small pieces, similarly dried in shade and then stored in airtight plastic containers. The dried plant material was subsequently subjected to Soxhlet extraction in petroleum ether, hexane and methanol in a Soxhlet apparatus (Borosil, Mumbai, India) independently for

72 h. The crude extracts were separated by vacuum rotary evaporator (Biocraft Scientific Industries Ltd. Agra, India) and a semisolid residue was obtained. The residue was stored in a glass beaker and covered with aluminium foil and maintained at 3ºC in a refrigerator until further use. The yields from dry leaves of *P. alliaceum* in petroleum ether, hexane and methanol extracts were 60.8, 2.5 and 216.4 g/kg, respectively. Likewise 1 kg of dry cloves of *A. sativum* yields in petroleum ether, hexane and methanol extracts were 2.7, 0.3 and 3.9 g, respectively.

Figure1a. *Pseudocalymma alliaceum*

Figure1b. *Allium sativum* cloves

Preparation of stock solutions

Stock solution of the petroleum ether extract of *P. alliaceum* was prepared by dissolving 1.6 g crude extract in 100 ml ethanol to obtain a final concentration of 16,000 ppm. For the hexane extract, 0.16 g extract was dissolved in 100 ml ethanol to obtain a final concentration of 1,600 ppm as a stock solution and for the methanol extract 2.0 g extract was dissolved in 100 ml of ethanol to obtain a final concentration of 20,000 ppm as stock solution.

Final concentrations of stock solutions were similarly obtained for *A. sativum* by dissolving 0.25 g of petroleum ether extract in 50 ml ethanol for 5,000 ppm, 0.31 g of the hexane extract in 50 ml ethanol for 6,200 ppm and 2.0 g of methanol extract in 50 ml ethanol for 40,000 ppm as stock solutions. These stock solutions were stored at 3ºC in a refrigerator and further diluted to prepare the range of desired test concentrations at the time of larval exposure.

Bioassay

Bioassay was conducted independently for each extract in 500 ml capacity glass beakers containing 1.0 ml of the test concentration and 249 ml water in triplicates. Twenty larvae (3^{rd} instar) of *An. stephensi* were used for each test concentration of the extracts according to WHO standard procedures (World Health Organization, 2005). The controls were run parallel in triplicate with 1.0 ml of ethanol mixed with 249 ml of water at 27±2 C and 80-90% relative humidity (RH). Larvae were provided yeast powder at 0 and 24 h after exposure. The moribund and dead larvae in all the three replicates were combined and expressed as a percent larval mortality for each concentration. Experiments with >20% mortality in the controls were discarded and repeated. Mortality values ranging from 5-20% in the controls were corrected by using Abbott's formula (Abbot, 1925), as follows:

$$\text{Corrected \% mortality} = [T\text{-}C]/[100\text{-}C] \times 100$$

Where, T is the percent mortality in the test concentrations and C is the percent mortality in the control.

Data analysis

All the data observed were subjected to Probit analysis (Finney, 1971) for calculating concentrations that produced 50 and 90 % mortality i.e., LC_{50} and LC_{90} values and other statistics at 95% confidence levels using software developed by Reddy *et al*. (1992).

Results

The relative efficacy of different extracts of *P. alliaceum* leaves against *An. stephensi* larvae are shown in Table 1. The LC_{50} values of petroleum ether extract were 18.2 ppm and 17.5 ppm after 24 and 48 h of exposure, respectively. The LC_{90} values were 35.0 ppm and 30.7 ppm after 24 and 48 h of exposure, respectively. The LC_{50} values of the hexane extract were 8.7 ppm and 7.5 ppm after 24 and 48 h of exposure, respectively, and the LC_{90} values were 31.0 ppm and 22.1 ppm after 24 and 48 h of exposure, respectively. The LC_{50} values of methanol extract were 170.2 ppm and 94.5 ppm after 24 and 48 h of exposure, respectively and LC_{90} values were 538.9 ppm and 334.5 ppm after 24 and 48 h of exposure, respectively (Figure 2).

Extraction solvent	Exposure period (hours)	Regression equation	Chi-square	LC_{50} ± S.E. (Fiducial limit) ppm	Relative toxicity	LC_{90}± S.E. (Fiducial limit) ppm	Relative toxicity
Petroleum ether	24	4.49x-5.16	1.31	18.17±2.23 22.53-13.82	0.106	35.03±4.05 42.97-27.07	0.065
	48	5.26x-6.79	0.66	17.50±1.93 21.28-13.72	0.185	30.67±3.52 37.56-23.78	0.092
Hexane	24	2.31x+0.52	1.53	8.65±1.36 11.32-5.99	0.050	31.00±7.05 44.81-17.19	0.057
	48	2.73x-0.11	4.49	7.49±1.08 9.60-5.38	0.079	22.11±3.93 29.82-14.39	0.066
Methanol	24	2.58x-3.28	9.72	170.3±22.54 214.5-126.1	1	538.9±90.05 715.4-362.4	1
	48	2.34x-1.94	10.87	94.47±16.20 126.22-62.71	1	334.5±55.29 442.9-226.1	1

Table 1. Larvicidal efficacy of different extracts of *Pseudocalymma alliaceum* leaves against *Anopheles stephensi.*

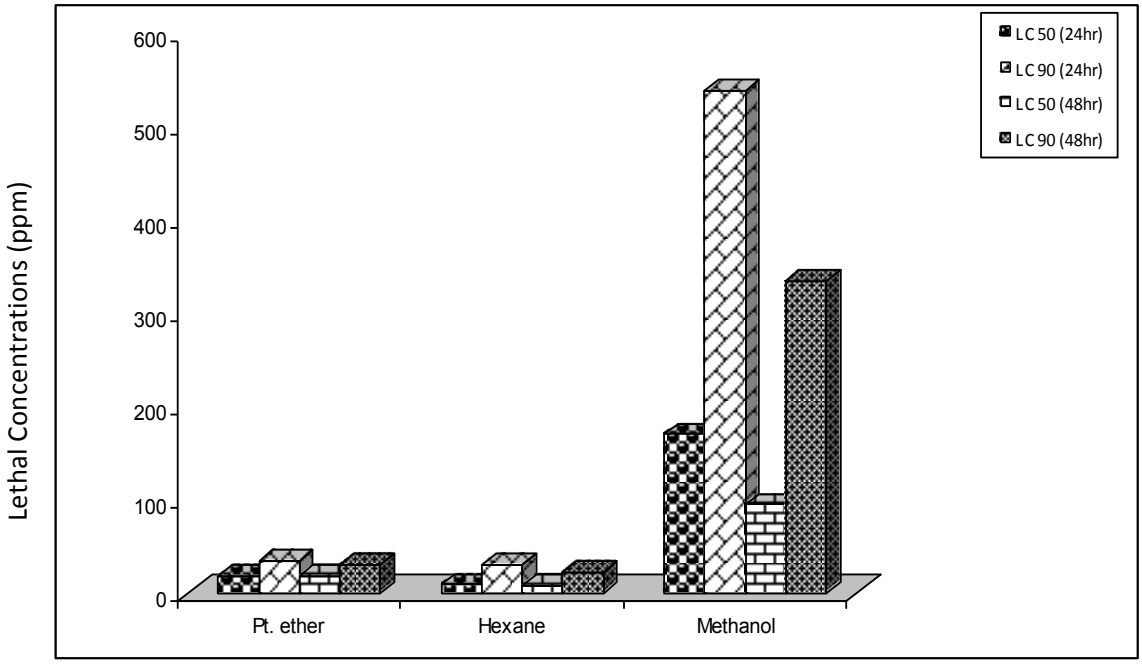

Figure 2. Bioefficacy of different extracts of *Pseudocalymma alliaceum* leaves against the larvae of *Anopheles stephensi*.

The relative efficacy of *A. sativum* against *An. stephensi* larvae are shown in Table 2. The LC_{50} values of petroleum ether extract were 22.6 ppm and 17.9 ppm after 24 and 48 h of exposure, respectively and LC_{90} values were 95.4 ppm and 49.8 ppm after 24 and 48 h of exposure, respectively. The LC_{50} values of hexane extract were 10.8 ppm and 7.6 ppm after 24 and 48 h of exposure, respectively and the LC_{90} values were 25.0 ppm and 15.4 ppm after 24 and 48 h of exposure, respectively. The LC_{50} values of methanol extract were 295.1 ppm and 197.9 ppm after 24 and 48 h of exposure, respectively and LC_{90} values were 2105.8 ppm and 684.9 ppm after 24 and 48 h of exposure, respectively (Figure 3).

Extraction solvent	Exposure period (hours)	Regression equation	Chi-square	LC_{50} ± S.E. (Fiducial limit) ppm	Relative toxicity	LC_{90}± S.E. (Fiducial limit) ppm	Relative toxicity
Petroleum Ether	24	2.04x+0.18	14.27	22.57±3.38 29.20-15.94	0.076	95.40±22.26 139.03-51.77	0.045
	48	2.88x-1.50	21.65	17.91±2.48 22.78-13.08	0.091	49.77±6.98 63.47-36.07	0.073
Hexane	24	3.52x-2.16	4.79	10.82±0.96 12.71-8.93	0.037	25.02±2.97 30.84-19.19	0.012
	48	4.20x-2.91	6.97	7.65±0.68 8.97-6.32	0.039	15.44±1.62 18.62-12.26	0.023
Methanol	24	1.50x-0.21	6.64	295.1±73.15 438.5-151.7	1	2105.8±1213.8 4484.7-2.73.2	1
	48	2.38x-2.83	28.39	197.8±27.54 251.8-143.9	1	684.9±183.1 1043.8-326.2	1

Table 2. Larvicidal efficacy of different extracts of *Allium sativum* cloves against *Anopheles stephensi.*

Discussion

The hexane extract of *Pseudocalymma alliaceum* was found to be the more effective against *Anopheles stephensi* larvae with LC_{50} value 8.7 ppm as compared to the other tested extracts of *Pseudocalymma alliaceum* and *Allium sativum*. Many researchers worked on the malaria vector with different plant extracts to control them (Table 3).

Table 3 shows the hexane extract of *P. alliaceum* to be more effective against anopheline larvae as compared to other plant extracts discussed earlier. Thus, this plant extract has shown remarkably effective mosquito larvae control properties and has a bright future in vector management as a phytolarvicide.

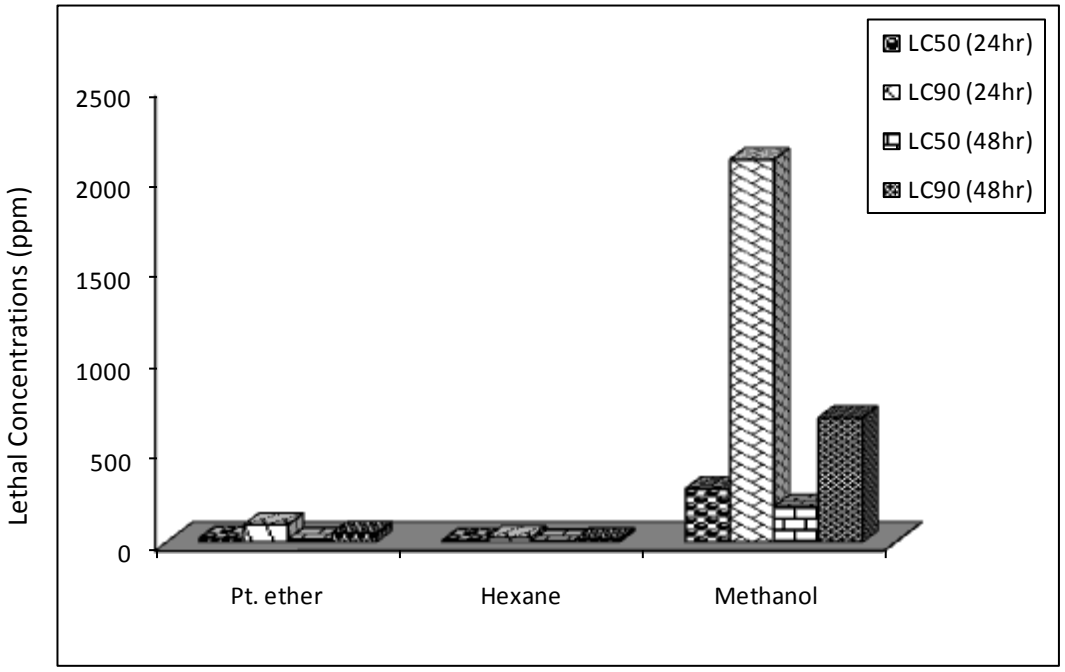

Figure 3. Bioefficacy of different extracts of *Allium sativum* cloves against the larvae of *Anopheles stephensi.*

Source	Plant	Solvent	LC_{50}	Target mosquito species
Sivagnaname & Kalyanasundaram, (2004)	*Atlantia monophylla*	Methanol	2.03 mg/l	*An. stephensi*
Singh *et al*., (2006)	*Momordica charantia*	Hexane	66.05 ppm	*An. stephensi*
Batabyal *et al.,* (2007)	*Azadirachta indica*	Methanol	15.25 ppm	*An. stephensi*
Maurya *et al*., (2007)	*Aloe barbadensis*	Carbon tetrachloride	15.58 ppm	*An. stephensi*
Mullai *et al*., (2008)	*Citrullus vulgaris*	Benzene	18.56 ppm	*An. stephensi*
Arivoli & Tennyson, (2011)	*Murraya koenigii*	Hexane	418.7 ppm	*An. stephensi*
Prabhu *et al*., (2011)	*Moringa oleifera*	Methanol	72.45 ppm	*An. stephensi*
Dhandapani & Kadarkarai, (2011)	*Cassia occidentalis*	Ethanol	70.56%	*An. stephensi*
Bagavan & Rahuman, (2011)	*Abrus precatorius*	Ethyl acetate	19.31 µg/ml	*An. vagus*
Senthilkumar *et al.,* (2012)	*Coccinia indica*	Methanol	161 mg/l	*An. stephensi*

Table 3. Larvicidal activity of different phytoextracts against *Anopheles* species studied by different workers.

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