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Laboratory observations on the larvicidal efficacy of three plant species against mosquito vectors of malaria, Dengue/Dengue Hemorrhagic Fever (DF/DHF) and lymphatic filariasis in the semi-arid desert

Author Details

S.K. Bansal (*Corresponding author*)	Desert Medicine Research Centre (ICMR), New Pali Road, Jodhpur - 342 005, India e-mail : bansalsk@dmrcjodhpur.org
Karam V. Singh	Desert Medicine Research Centre (ICMR), New Pali Road, Jodhpur - 342 005, India
Sapna Sharma	Desert Medicine Research Centre (ICMR), New Pali Road, Jodhpur - 342 005, India
M.R.K. Sherwani	Department of Chemistry, Jai Narayan Vyas University, Jodhpur - 342 005, India

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Abstract

Comparative larvicidal efficacy of aqueous and organic solvent extracts from seeds, leaves and flowers of three desert plants *viz. Calotropis procera* (Aiton), *Tephrosia purpurea* (L.) Pers. and *Prosopis juliflora* (Sw.) DC. was evaluated against *Anopheles stephensi* (Liston), *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say). For this purpose larvae of all the three mosquito species were reared in the laboratory and studies carried out on late 3rd or early 4th instars using standard WHO technique. Based on concentration mortality data 24 and 48 hr LC$_{50}$ and LC$_{90}$ values along with their 95% fiducial limits, regression equation, chi-square (χ^2)/ heterogeneity of the response were determined by log probit regression analysis. Experiments were carried out with different solvent extracts of seeds of *C. procera* which revealed that methanol (24 hr LC$_{50}$: 127.2, 194.8, 361.0) and acetone (229.9, 368.1, 193.0 mg l^{-1}) extracts were more effective with the three mosquito species, respectively. Petroleum ether extract was effective only on *An. stephensi* while aqueous extracts were not effective at all with any of the mosquito species (mortality <10-30%). Tests carried out with methanol extracts of fresh leaves (24 hr LC$_{50}$: 89.2, 171.2, 369.7) and flowers (24 hr LC$_{50}$: 94.7,617.3, 1384.0 mg l^{-1}) of *Calotropis* showed that preparations from fresh parts were 2-3 times more effective as compared to the stored plant parts. Efficacy was less than 10-30% with both *An. aegypti* and *Cx. quinquefasciatus* while *An. stephensi* was still susceptible to extracts from both leaves and flowers even after two years of storage. The 24 hr LC$_{50}$ values as observed for methanol extracts of seeds of *T. purpurea* and leaves of *P. juliflora* were 74.9, 63.2 and 47.0 and 96.2, 128.1 and 118.8 mg l^{-1} for the above three mosquito species, respectively. Experiments carried out up to 500 mg l^{-1} with leaves (*T. purpurea*) and seeds (*P. juliflora*) extracts show only up to 10-30% mortality indicating that active larvicidal principle may be present only in the seeds of *Tephrosia* and leaves of *Prosopis*. In general, anophelines were found more susceptible than the culicines to the plant derived derivatives. More studies are being carried out on some other desert plants found in this arid region. The study would be of great importance while formulating vector control strategy based on alternative plant based insecticides in this semi-arid region.

Key words

Vector mosquitoes, Larvicidal, Desert plants, Semi-arid region

Introduction

Mosquitoes are well known group of insects, which transmit many dreadful diseases causing serious health problems to human civilization. Controlling of these vectors by synthetic insecticides has resulted into environmental hazards through development of insecticides resistance in vector population, persistence and accumulation of non-biodegradable chemicals in the ecosystem, biological magnification through the food chains and finally toxic effects in human health and non-target organisms. Consequent to the realization of these inimical impacts of most of the synthetic insecticides, efforts have gain impetus in recent times to explore alternative ecofriendly methods of vector control. Biologically active plant extracts have been well documented for evolving an ecologically sound and environmentally acceptable mosquito control programmes. Phytochemical insecticides, in this regard, have received much attention as they are considered to be more environmentally safe and biodegradable than synthetic insecticides and highlighted the need for the development of new strategies for selective mosquito larval control. Furthermore, unlike commercial insecticides that are based on single active component, phyto-derivatives comprise hundreds of secondary metabolites, which act unitedly on both behavioral and physiological processes (Mwangi and Rembold, 1986; Sukumar *et al*., 1991). Thus the chances of resistance development in vector mosquitoes to these phytochemicals are remote (Shaalan *et al*., 2005).

The present investigation has been carried out to explore the mosquito larvicidal efficacy of different parts of three desert plants *viz*. *Calotropis procera* (Aiton) (Fam. Asclepiadaceae), *Tephrosia purpurea* (L.) Pers. (Fam. Papilionaceae /Fabaceae) and *Prosopis juliflora* (Sw.) DC. (Fam. Mimosoideae /Fabaceae) after extraction in aqueous and other organic solvents and identification of the active larvicidal ingredients present there in. All these three plants are widely distributed throughout the arid region and roots, leaves, fruits and seeds are well known for their multiple medicinal uses.

Anopheles stephensi, *Aedes aegypti* and *Culex quinquefasciatus* are the important vectors of malaria, DF/DHF and lymphatic filariasis, respectively. All these mosquito species have been identified as the primary vectors in this region of Rajasthan (Bansal and Singh, 1993). Hence, their control either by conventional or plant derived insecticides is the primary requirement for planning an effective vector control strategy. Several studies have been done on the insecticide susceptibility status of adult mosquitoes (Bansal and Singh 1995; 1996) and larvae (Bansal and Singh, 2004; 2006) in this region, the level of susceptibility of their larvae to different phytochemicals is not yet explored extensively in this semi-arid desert.

Materials and Methods

Plant material was collected from different habitats in and around the city and help from Botanical Survey of India (BSI) was taken for identification after depositing the sample specimen in the herbarium. The samples of leaves, flowers, fruits and seeds were washed thoroughly with distilled water, chopped and shade dried separately at a temperature between 30-40ºC for 15 days. Dried plant material was then powdered separately and stored at a temperature range of 15-20ºC. Seeds were taken out from the dried fruits and powdered separately. Powdered plant material (100 g) each from leaves, flowers and seeds was extracted separately using 400 ml of distilled water/ acetone/ methanol/ petroleum ether on a hot plate at a temperature of 40±2ºC for 2 days with constant stirring and finally filtered, air dried, weighed and stored in glass vials in a refrigerator until use. The percent yield for seeds of *C. procrea* were 4.3, 7.2, 9.8 and 6.5 % for the aqueous, acetone, methanol and petroleum ether extracts while for leaves and flowers in methanol it was 8.1 and 5.4 %, respectively. The yield for leaves and seeds of *T. purpurea* and *P. juliflora* in methanol was 8.6 and 6.6 and 10.1 and 3.4%, respectively. Stock solutions from the residual extracts (5 g 50 ml^{-1} of solvent) were prepared in distilled water for aqueous extracts while in ethanol for other organic solvents. Test concentrations from 10-500 mg l^{-1} for aqueous and other organic solvent extracts were used during the experimentation.

Efficacy experiments with various solvent extracts were carried out on different mosquito larvae as per protocol described by WHO (1981). Late 3rd and early 4th instars of different mosquito larvae were reared in the laboratory and used for the tests. Serial dilutions of the stock solution were prepared and added to 249 ml of tap water in a 500 ml beaker to obtain the test concentrations. Controls with the same amount of solvent (1 ml ethanol) were kept side by side. Healthy (20-25) late 3rd or early 4th instar wrigglers were kept in different test concentrations and mortality noted after 24 and 48 hr. All treatments were replicated 5 times and carried out at a controlled room temperature of 28±2°C and relative humidity (RH) 75±5%. The percent-corrected mortality was calculated if mortality was between 5-20% in control experiments. The LC_{50} and LC_{90} values were computed using probit regression analysis (Finney, 1971).

Results and Discussion

The results of the larvicidal activity with extracts from different plant parts of *C. procera*, *T. purpurea* and *P. juliflora* against larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* are given in tables 1-3. Experiments carried out with methanol and acetone extracts of seeds of *C. procera* showed that these were more effective, the 24 hr LC_{50} being 127.2, 194.8, 361.0 mg l^{-1} for the methanol extracts and 229.9, 368.1, 193.0 mg l^{-1} for the acetone extracts to all the three mosquito species, respectively (Table 1). However, with petroleum ether extracts the 24 hr LC_{50} was 267.5 mg l^{-1} for *An. stephensi* while a mortality of less than 20-30% was observed with *Ae. aegypti* and *Cx. quinquefasciatus*. A mortality of less than 20-30% was also observed with aqueous extracts of seeds of this plant species (Table 1) showing that methanol extracts were much effective followed by acetone, petroleum ether and aqueous extracts to all the three mosquito species tested. *An. stephensi* was found to be more susceptible as compared to the other two species. Singh and Bansal (2003), Mohan *et al*. (2005) and Bansal *et al*. (2009a, 2009b) also showed that organic solvent extracts from fruits of *Solanum xanthocarpum* were very much effective against the larvae of *An. stephensi* followed by *Ae. aegypti* and *Cx. quinquefasciatus.* The 48 hr LC_{50} values were much less as compared to 24 hr LC_{50} values showing that plant derived insecticides are much slower in their insecticidal action unlike the conventional insecticides. Organic solvent extracts of some plant species and their oil cakes have also been reported to be quite effective against larvae of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* (Srivastava *et al*., 2008; Maurya *et al*., 2008 Shanmugasundaram *et al*., 2008).

Giridhar *et al*. (1984) and Shahia *et al*. (2008) also determined the efficacy of alcoholic extracts of whole plant and latex of the milkweed (*C. procera*) and showed that *An. stephensi* larvae

Table 1 : Comparative efficacy of seeds of *Calotropis procera* against larvae of different mosquito vectors in different organic solvents

Mosquito species	Solvent	Regression equation	χ^2 (DF)	LC_{50} 24 hr (mg l^{-1})	LC_{50} 48 hr (mg l^{-1})
An. stephensi	Methanol	Y = 2.82x -0.93	0.14(3)	127.2(101.7-159.5)*	89.8(70.0-115.3)*
Ae. aegypti		Y = 3.18x -2.29	0.58(3)	194.8(159.2-238.5)	115.4(91.0-146.4)
Cx. quinquefasciatus		Y = 1.74x +0.55	0.05(3)	361.0(256.4-508.4)	141.5(109.1-183.3)
An. stephensi	Acetone	Y = 1.25x +2.06	1.45(3)	229.9(142.8-370.5)	119.9(88.0-163.6)
Ae. aegypti		Y = 1.42x +1.35	0.25(3)	368.1(243.6-556.3)	146.9(99.1-217.5)
Cx. quinquefasciatus		Y = 1.39x +1.81	0.12(3)	193.0(108.1-198.9)	126.4(89.8-177.8)
An. stephensi	Petroleum Ether	Y = 2.04x +0.04	1.22(3)	267.5(180.5-396.7)	181.2(125.9-260.9)
Ae. aegypti		- - -	- - -	**24.1**	**27.2**
Cx. quinquefasciatus		- - -	- - -	**23.3**	**25.3**
An. stephensi	Aqueous	- - -	- - -	**15.0**	**16.8**
Ae. aegypti		- - -	- - -	**12.2**	**16.3**
Cx. quinquefasciatus		- - -	- - -	**18.0**	**22.1**

χ^2 = Chi-square, LC_{50} = Lethal concentration, DF = Degree of freedom. Values in bold are the % mortalities at the highest concentration (500 mg l^{-1}). *Fiducial limits at P 0.05

Table 2 : Comparative efficacy of methanol extracts of fresh and old leaves and flowers of *Calotropis procera* against larvae of different mosquito vectors

Mosquito species	Leaves (fresh)		Leaves (2 year old)	
	LC_{50} 24 hr (mg l^{-1})	LC_{50} 48 hr (mg l^{-1})	LC_{50} 24 hr (mg l^{-1})	LC_{50} 48 hr (mg l^{-1})
An. stephensi	89.2(66.5-119.6)*	65.0(48.8-86.6)*	135.5(104.1-176.4)*	102.8(79.5-132.7)*
Ae. aegypti	171.2(114.3-256.5)	118.7(82.0-171.7)	**25.4**	**30.2**
Cx. quinquefasciatus	369.7(276.8-493.9)	259.3(209.4-321.1)	**31.0**	**35.0**
	Flowers (fresh)		Flowers (2 Year old)	
An. stephensi	94.7(73.8-121.5)	68.7(54.8-86.2)	253.0(195.8-327.1)	143.6(113.2-182.4)
Ae. aegypti	617.3(357.6-1066.0)	361.7(264.6-494.4)	**15.2**	**25.2**
Cx. quinquefasciatus	1384.0(557.2-3438.0)	616.2(359.2-1057.0)	**24.2**	**33.1**

DF- Degree of freedom. Values in bold are the % mortalities at the highest concentration (500 mg l^{-1}). *Fiducial limits at P 0.05

Table 3 : Comparative efficacy of methanol extracts of *Tephrosia purpurea* and *Prosopis juliflora* against larvae of different mosquito vectors

Mosquito species	Plant part	Regression equation	χ^2 (DF)	LC_{50} 24 hr (mg l^{-1})	LC_{50} 48 hr (mg l^{-1})
An. stephensi	*T. purpurea*	Y = 1.81x +1.61	1.68 (3)	74.9(54.8-102.3)*	382.3(156.9-931.7)*
Ae. aegypti	(Seeds)	Y = 2.15x +1.13	2.01 (3)	63.2(48.8-81.9)	249.6(133.6-466.0)
Cx. quinquefasciatus		Y = 2.35x +1.08	2.17 (3)	47.0(35.7-61.9)	165.1(90.0-303.0)
	(Leaves)	_	_	**12.6**	**20.5**
An. stephensi	*P. juliflora*	Y = 2.31x +0.41	1.54(3)	96.2(73.0-126.8)	344.2(192.9-614.4)
Ae. aegypti	(Leaves)	Y = 2.44x -0.15	2.47(3)	128.1(101.7-161.4)	428.5(254.8-721.1)
Cx. quinquefasciatus		Y = 2.65x -0.50	2.11(3)	118.8(93.7-150.7)	361.4(212.4-614.8)
	(Seeds)	_	_	**10.4**	**14.8**

χ^2 = Chi-square, LC_{50} = Lethal concentration, DF = Degree of freedom. Values in bold are the % mortalities at the highest concentration (500 mg l^{-1}). *Fiducial limits at P 0.05

were more susceptible than *Cx. quinquefasciatus* to both the extracts. The alcoholic extracts of the plant were less toxic (LC_{50}, 109.71 and 387.93 mg l^{-1}) than extracts from latex (13.06 and 86.47 mg l^{-1} respectively) in both the species. Singhi *et al*. (2006) also studied the larvicidal impact of latex of this plant after dissolving in different organic solvents and observed that methanolic extracts were quite effective against all these three mosquito species. Markouk *et al*. (2000) also studied larvicidal efficacy of *C. procera* and obtained an LC_{50} of 28 mg l^{-1} with aqueous latex against *An. labranchiae*, while ethanolic extract of root showed an LC_{50} of 315 mg l^{-1} against this *Anopheles*. Experiments have been carried out on the larvicidal efficacy of fresh and two years stored leaves and flowers of *C. procera* in the present investigation (Table 2) which revealed that fresh parts were 2-3 times more effective as compared to the old stored parts. The methanolic extracts from old stored leaves and flowers were effective only on *Anopheles* while *Aedes* and *Culex* showed <20-30% mortality. The 24 hr LC_{50} values of the fresh leaves and flowers were 89.2, 171.2, 369.7 and 94.7, 617.3, 1384.0 mg l^{-1} for the three mosquito species respectively showing that *Anopheles* is far more susceptible than the other two species. Singh *et al*. (2005) and Rahuman *et al*. (2009) also evaluated the larvicidal properties of the leaves of milkweed (*C. procera*) and showed that methanolic extracts were much more effective than the fresh leaves extract against larvae of *Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus*.

Efficacy experiments were also carried out with methanol extracts of leaves and seeds of *T. purpurea* and *P. juliflora* (Table 3). The LC_{50} s' were 74.9, 63.2 and 47.0 for the seeds of *T. purpurea* and 96.2, 128.1 and 118.1 mg l^{-1} for the leaves of *P. juliflora* for the three mosquito species respectively showing that seeds of *Tephrosia* were much effective larvicide as compared to the leaves of *Prosopis*. However, leaves of *T. purpurea* and seeds of *P. juliflora* were not found effective at all (Table 3) with any of the mosquito species tested which revealed that active larvicidal component may be present only in the seeds of *T. purpurea* and leaves of *P. juliflora*. Senthilkumar *et al*.(2009) also evaluated the larvicidal and adulticidal efficacy of 10% leaves extracts of *P. juliflora* and showed that these were quite effective against larvae (LC_{50}: 9.3 mg l^{-1}) and adults (LT_{50}: 193.04 min)) of *An. stephensi.* Petroleum ether extracts of flowers of *P. juliflora* were also quite effective against *Cx. quinquefasciatus* followed by *An. stephensi* and *Ae. aegypti* (Sakthivadivel and Daniel, 2008). Nazar *et al*. (2009) also screened many Indian coastal plants and found that shoots of *T. purpurea* didn't show any efficacy against larvae of *Culex.* In our studies leaves were not found effective with any of the mosquito species, but seed extracts were quite effective (LC_{50}<100 mg l^{-1}) against these mosquitoes showing that active component is present in the seeds of *Tephrosia* and not in its leaves.

Results of the present study with the extracts from different parts of these plants exhibited variable larvicidal efficacy that warrants further investigations. These larvicidal activities vary according to the plant species, plant part used, geographical location of the plant, photosensitivity of some of the compounds in the plant extract and finally the solvent of extraction and the species responses to the specified extracts (Sukumar *et al*., 1991). Keeping in view the above variables , it will be prudent to study the variations in efficacies of extracts and also to characterize the key component present in different tropical and sub tropical plants with a view to discover and develop new insecticidal phytochemicals (Berenbaum, 1989). Many plants from different families possess promising phytochemicals for mosquito control (Jacobson, 1989) which are much economical and environmental friendly (Jang *et al*., 2002, Sivagnaname and Kalyanasundaram, 2004). These phytochemicals like phenolics, alkaloids and terpenoids exist in plants (Wink, 1993) which may jointly or independently contribute to the generation of mosquito larvicidal activities (Hostettmann and Potterat, 1997).

The results of the present study suggest that chemical composition of extracts differ not only from plant to plant but also within parts of the same plant require thorough exploration of the active components present therein. For their successful application, therefore, it is obligatory to understand the mechanisms of their action in the target insects as well as the spectrum of insects affected by them. Further, work on these biologically active components of plants is in progress for developing them into effective formulations to be utilized in integrated vector control and in exploration of the multiple medicinal properties inherited by them.

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