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Evaluation of mosquito larvicidal efficacy of leaf extract of a cactus plant, *Agave sisalana*

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ABSTRACT

The leaf extract of *Agave sisalana* was tested as a larvicide against three vector mosquito species *viz.,Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti.* Preliminary results showed that the2% dilution of the leaf extract produced 100% mortality of IIIrd instar larvae of *An. stephensi* and 1%dilution produced 100% mortality in case of *Cx. quinquefasciatus* and *Ae. aegypti*. For furthe r bioassays, the LC50 value of dried crude, methanol and petroleum ether extracts of *Ag. sisalana*leaves were determined against IIIrd/IVth instar larvae of *An. stephensi, Cx. quinquefasciatus* and *Ae.aegypti* and these values were 75, 86 & 76 ppm; 36, 82 & 220 ppm and 27, 51 & 31 ppm,respectively. The present study revealed that *Ag. sisalana* leaf extract possess larvicidal activity against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* and can be exploited for their control under integrated approach of vector control.

Keywords: Mosquito larvicide, *Agave sisalana, Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti.*

INTRODUCTION

During the last two decade, mosquito–borne diseases like malaria, filaria, dengue,chikungunya and Japanese encephalitis etc. are re-emerging in India and other tropical countries due to various factors **[1, 2, 3]**. The methods used to control these vectors for the interruption of disease transmission include indoor residual spray (IRS) with synthetic organic chemical insecticides, insecticides treated bed nets (ITBNs) and larvicides to control immature stages of these mosquitoes. Synthetic organic chemical insecticides have been in use since long for the control of these vector mosquitoes, which have resulted in problem of resistance and also these insecticides are harmful to environment and mankind **[4, 5, 6, 7, 8, 9]**. Vector-borne disease transmission depends on the degree of man-vector contact. For the protection against mosquitoes their population must be controlled by using effective methods. In recent years there has been much interest in natural insecticides derived from plants which are biodegradable, easily available at low cost, and safe for human health.

Various studies on the natural plant products as larvicides against mosquito vectors have been reported **[10, 11, 12, 13, 14, 15]**. However, more concerted efforts would be needed to make these environment friendly compounds viable for field use and for large scale vector control operations.

The genus *Agave* has more than 275 species distributed in different continents of the Word, of which *Agave sisalana, Agave cantala, Agave Mexicana, Agave veracruz* and *Agave americana* occur in India. Among these *Agave sisalana*, also known as sisal, is most prevalent species in India. Sisal was introduced in India by Portuguese in the 15th century.

Ag. sisalana plant has a stalk on which the succulent leaves are arranged spirally. Its dimensions are about 1 to 2 meters in height, with a diameter of about 20 cm. The lanceshaped leaf growing out from the stalk in a dense rosette, are fleshy and rigid, with dark green colour **[16]**. It can establish itself and easily grow in different states of our country covering sub humid to arid and semiarid regions. In India the plant is found mainly in the states of Orissa, Madhya Pradesh, Chhattisgarh, Andhra Pradesh, Bihar, Jharkhand, Maharashtra, Karnataka and Tamil Nadu including Delhi.

In India, this variety of cactus i.e. *Ag. sisalana* is planted in rows on the bunds, as a soil conservation and field protection plant against predatory animals. Sisal provides employment opportunities in off-seasons in remote tribal and forest areas for tribes. The leaves of sisal yield a strong fibre which is traditionally used for making ropes, cordage and twines. It is also being used to manufacture coarse fabrics, rugs, carpets, handicrafts, mats, fishing nets

etc [16]. The leaf contains several steroidal sapogenins. Sisal waste and the juice extracted from the leaf pulp by decortications of fresh leaves are the commercial source of sapogenins [17]. Preliminary pharmacological investigation showed that the juice of the leaf canbring down the blood pressure and it is also used as an antiseptic and is taken to stop the growth of bacteria in the stomach and intestine, stimulates their intestinal movements, treatment of indigestion, flatulence, constipation, jaundice and dysentery. The root is diaphoretic and diuretic and it is used in the treatment of syphilis. It possesses embolic properties and may be used as an abortifacient as it activates the uterine motility [17, 18].

The present communication reveals the mosquito larvicidal properties of *Ag. sisalana* leaf extract to test its role as a larvicide against three mosquito vectors species *viz*., *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*.

MATERIALS AND METHODS

Ag. sisalana leaves were collected from the plants located in north east area of Delhi. These leaves were washed with tap water, cleaned thoroughly, cut into small pieces and immediately ground using a pestle and mortar. The leaf extract was filtered through a muslin cloth and filtrate was used for the experiments. Preliminary experiment with this extract was undertaken to determine the larvicidal activity by making serial dilutions *viz*., 2, 1, 0.5, 0.25, 0.125 and 0.0625%(ml/100ml) which were used for larval bioassayagainst I/ II and III/ IV larval stages of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*.

For further bioassay, 560ml leaf extract obtained from 733.35gram fresh leaves of *Ag. sisalana* after grinding, was divided in to three parts (160, 200 and 200ml). First part of the leaf extract was dried to obtain 22.87gm dried crude extract which was used after making dilutions viz., 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 ppm (mg $l^{-1)}$ into distilled water. Second and third part of the leaf extract wasextracted three times with 600 ml each of methanol and petroleum ether solvents using 200ml separator funnel and was dried with the help of rotary vacuum evaporator to obtain the active moiety in crude dried form as methanol extract (17.29 g) and petroleum ether extract (15.76 g). Different test dilutions viz., 500, 250, 125, 62.5, 31.25, 15.62 and 7.81ppm were prepared in distilled water separately for methanol and petroleum ether extracts. Each bioassay was done separately to determine the efficacy of the driedcrude extract and the methanol and petroleum ether extract againstIII/ IV larval stages of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. 250 ml of each dilution was taken into a 500 ml plastic bowl and 20-25 larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* were added separately. Three replicates for each concentration and the control were used for larval bioassay as per WHO procedure [19]. The experiments were conducted at room temperature of 27±1^{o}C and 70% humidity. The larval mortality in each concentration and control was recorded after 24 hours of continuous exposure. The corrected mortality was determined using Abbott's formula whenever required [20]. The dose mortality data was analysed by log-probit method of Finney [21] and lethal concentrations for 50% and 90% mortality were calculated byusing the software SPSS for windows.

In addition, *Mesocyclos* a non-target predator of mosquito larvae were also exposed to 10, 5, 2, 1, 0.5, 0.25 and 0.125% dilutions of the leaf extract to determine the probable toxicity of *Agave sisalana* leaves on these organisms. Twenty five *Mesocyclops* werekept into 250 ml of each dilution in 500 ml capacity plastic bowl. *Mesocyclops* were collected from aquatic habitats mainly ponds, pools and ditches in Burari village area of north east at Delhi.

RESULTS AND DISCUSSION

Preliminary results showed 100% mortality of III/ IV instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* with 1% dilution of the original leaf extract and 100% mortality of III/ IV instars larvae of *An. stephensi* with 2% dilution. The LC50 value of the leaf extract of *Ag. sisalana* against I/II instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* were 0.112, 0.160 and 0.046% and against III/ IV instar larvae it was 0.173, 0.27 and 0.128% respectively. The LC90 value of the leaf extract of *Ag. sisalana* against I/ II instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and*Ae. aegypti* were 0.183, 0.480 and 0.066% and against III/ IV instar larvae it was 0.73, 0.42 and 0.356% respectively (Table 1).

Table 1: Efficacy of *Ag. sislana* crude extract against mosquito larvae

Mosquito species	Larval stage	% Concentration(ml/100ml)	
		LC50	LC90
An. stephensi	I/II	0.112	0.183
	III/IV	0.173	0.73
	I/II	0.16	0.48

Cx. quinquefasciatus	III/IV	0.27	0.42
Ae. aegypti	I/II	0.046	0.066
	III/IV	0.128	0.356

The efficacy of crude, methanol and petroleum ether extracts of *Ag. sisalana* against III/IV instar larvae of three mosquito vector species, *An. stephensi*, *Cx. quinquefasciatus* and *Ae. Aegypti* are shown in Table 2. The crude extract showed 100% mortality of III/ IV instar larvae of *Cx. quinquefasciatus* and *An. stephensi* with 500ppm concentration. LC_{50} values of crude extract against *Cx.quinquefasciatus, Ae. aegypti* and *An. stephensi* were 86, 76 & 75ppm and were almost same against all the three mosquito species, but with some variation in LC_{90} values, which were 163, 179 & 212ppm respectively (Table 2). Petroleum ether extract of *Ag. sisalana* showed 100% mortality of III/ IV instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* with 500 and 250 ppm. concentrations of petroleum ether extract. The LC_{50} and LC_{90} values of petroleum ether extract of *Ag. sisalana* against III/ IV instar larvae of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* were 51, 31 and 27ppm and 151, 77 and 221 ppm respectively. Methanol extract produced 100% mortality of III/ IV instar larvae of *An. stephensi* and *Cx. quinquefasciatus* with 500 ppm concentration. The LC_{50} and LC_{90} values of methanol extract of *Ag. sisalana* against III/ IV instar larvae of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*, were 82, 220 and 36 ppm and 250, 586 and 109 ppm respectively (Table 2).

Table 2: Efficacy of different solvent extracts of Ag. *sislana* leaves against mosquito larvae

Mosquito species	Concentration (ppm)								
	Crude extract			Methanol extract			Petroleum ether extract		
	LC50	LC90	X^2(df)	LC50	LC90	X^2(df)	LC50	LC90	X^2(df)
Cx. quinquefasciatus	86 (54-149)	163 (117-367)*	11.262(4)	82 (57-121)	250 (161-597)	7.387(4)	51 (42-0.061)	151 (118-212)	3.49(4)
Ae. aegypti	76 (53 -106)	179 (124 -368)	7.802(4)	220 (185 -267)	586 (447-887)	4.119(5)	31 (26 -36)	77 (62-105)	2.42(5)
An. Stephensi	75 (55-133)	212 (134-558)	8.92(5)	36 (30-44)	109 (86-151)	4.614(5)	27 (19-36)	221 (149-391)	5.69(5)

LC_{50} – Lethal concentration for 50% mortality, LC_{90} – Lethal concentration for 90% mortality X^2 – Chi-square, df – Degree of freedom.
*Fiducial limits at P 0.05.

These results showed that the efficacy (LC_{50}) of dried crude extract was more or less same against all the species, while the efficacy of petroleum ether and methanol extracts was more against *An. Stephensi* than *Cx. quinquefasciatus* and *Ae. aegypti*. Methanol extract was most effective against *An. stephensi* (LC_{50} 36ppm) followed by *Cx. quinquefasciatus* (LC_{50} 82ppm) and *Ae. aegypti* (LC_{50} 220ppm). Similarly, petroleum ether extract was most effective against *An. stephensi* (LC_{50} 27ppm) followed by *Ae. aegypti* (LC_{50} 31ppm) and *Cx. quinquefasciatus* (LC_{50} 51ppm).

The results also indicated that all the fractions possessed larvicidal effect but the activity of different fractions varied differently against different species. This may be due to the presence of different active moieties in the three fractions having different mode of action. Plants of the family Agavaceae are rich in secondary metabolites such as saponins, tannins, terpenoids, alkaloids and flavonoids, which have been found to have antimicrobial, immune boosting and anti-inflammatory properties *in vitro* [22]. Saponins are useful for soap making and have pharmacological importance [18]. The crude extract of *Ag. attenuata* was evaluated for some activity against *Bulinus africanus, Daphnia pulex, An. arabiensis* and *Oreochromis mossambicus* demonstrating molluscicidal, piscicidal and larvicidal properties [23]. Pizarro *et al*. (1999) reported that the waste of sisal had insecticidal properties particularly against larvae of vector mosquitoes, which transmit malaria, filarial and dengue [24]. In the present study, leaf extract of *Ag. sislana* showed no toxic action (zero mortality up to 72 hours observation) against non- target organism like *Mesocyclops* but was found to be toxic against mosquito larvae of three urban mosquito vectors *An. stephensi*, *Ae.aegypti* and *Cx. quinquefasciatus* indicating its possible use in the integrated vector control.

CONCLUSION

Further studies are required for the identification of the active ingredients responsible for larvicidal activity. leaf extract of *Ag. sisalana* had no toxic action (zero mortality up to 72 hours observation) against non-target organism like *Mesocyclops*. Hence, the larvicidal action of the leaf extract of *Ag. sisalana* could be exploited in integrated control of mosquito.

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