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Larvicidal potential of wild mustard (*Cleome viscosa*) and gokhru (*Tribulus terrestris*) against mosquito vectors in the semi-arid region of Western Rajasthan

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Abstract

Cleome viscosa L. (Family: Capparaceae) commonly known as Tickweed or wild mustard and *Tribulus terrestris* L. (Family: Zygophyllaceae) commonly known as Gokhru, growing wildly in the desert areas in the monsoon and post monsoon season, are of great medicinal importance. Comparative larvicidal efficacy of the extracts from seeds of *C. viscosa* and fruits and leaves of *T. terrestris* was evaluated against 3rd or early 4th stage larvae of *Anopheles stephensi* (Liston), *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say) in different organic solvents. 24 and 48hr LC_{50} and LC_{90} values along with their 95% fiducial limits, regression equation, chi-square (χ^2)/ heterogeneity of the response was determined by log probit regression analysis. The 24hr LC_{50} values as determined for seeds of *C. viscosa* were 144.1, 99.5 and 127.1 (methanol); 106.3, 138.9 and 118.5 (acetone) and 166.4, 162.5 and 301.9 mg l^{-1} (petroleum ether extracts) for all the three mosquito species respectively showing that methanol and acetone extracts were a little bit more effective than the petroleum ether extracts. Experiments were carried out with fruits and leaves of *T. terrestris* with all the solvents and mosquito species. The 24hr LC_{50} values, as determined for fruits of *T. terrestris* were 70.8,103.4 and 268.2 (methanol); 74.0,120.5 and 132.0 (acetone) and 73.8, 113.5 and 137.4 mg l^{-1} (petroleum ether extracts) while the 24hr LC_{50} values for leaves were 124.3, 196.8 and 246.5 (methanol); 163.4, 196.9 and 224.3 (acetone) and 135.8,176.8 and 185.9 mg l^{-1} (petroleum ether extracts) for all the three mosquito species respectively. The results clearly indicate that fruit extracts of *T. terrestris* were more effective as compared to leaves extracts in the three solvents tested. Larvae of *An. stephensi* were found more sensitive to both fruit and leaves extracts of *T. terrestris* followed by larvae of *Ae. aegypti* and *Cx. quinquefasciatus.* Extracts from the seeds of *C. viscosa* were found less effective as compared to the fruit extracts of *T. terrestris* indicating that active larvicidal principle may be present in the fruits of this plant species. The study would be of great importance while formulating the control strategy, for vectors of malaria, dengue and lymphatic filariasis, based on alternative plant based insecticides in this semi-arid region.

Key words

C. viscosa, Mosquito larvicides, Semi-arid region, *T. terrestris*

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Introduction

Vector-borne diseases such as malaria, filaria, dengue and Japanese encephalitis contribute significantly to the disease burden, causing millions of deaths every year in many tropical and sub-tropical countries of the world (Jang *et al*., 2002). However, the control of these vector-borne diseases is becoming very difficult as the effectiveness of vector control has declined due to the precipitation of resistance in the vector population, against the insecticides being used in vector control operations (Nauen, 2007). It is also important to note that a limited number of insecticides are available for use in public health. Moreover, synthetic insecticides are very much toxic and adversely affect the bio-environment which can be detrimental to animals, including humans (Ghosh, 1991). As a result, researchers are investigating alternative ecofriendly approach of vector control.

One such approach is the exploration of botanical insecticides as they are easily available at competitive prices, non-toxic, biodegradable and show broad-spectrum target specific activities. Unlike conventional synthetic insecticides that are based on single active ingredient, botanical derivatives comprise blends of secondary metabolites, which act jointly on both behavioral and physiological processes (Rawani *et al*., 2009) These plant derived derivatives inhibit growth (Sharma *et al*., 2006) development and metamorphosis of insects (Mwangi and Rembold, 1986; Sukumar *et al*.,1991) and the chances of resistance development in vector mosquitoes to these derivatives are remote (Shaalan *et al*., 2005).

The two desert plants *viz*. *Cleome viscosa* L. (Family: Capparaceae) and *Tribulus terrestris* L. (Family: Zygophyllaceae) are widely distributed throughout the arid region and roots, leaves, fruits and seeds are well known for their multiple medicinal properties (Gupta and Dixit, 2009; Jameel *et al*., 2012; Krishnappa and Elumalai, 2013). *C. viscosa* commonly known as Tickweed or wild mustard is known to possess antihelminthic, antimicrobial, psychopharmacological, antidiarrhoeal and hepatoprotective activities while *T. terrestris* commonly known as Gokhru is indicated for the treatment of urinary disorders, kidney diseases, calculus affections, regulation of heart functions and diseases of the genito-urinary system (Krishnappa and Elumalai, 2013; Jameel *et. al*., 2012).

Anopheles stephensi (Liston), *Aedes aegypti (Linnaeus)* and *Culex quinque- fasciatus* (Say) have been identified as the primary vectors of malaria, dengue fever and lymphatic filariasis respectively, in this part of the desert (Bansal and Singh 2006; 2007). Although several insecticide susceptibility studies have been done on the adult vector mosquitoes (Kulkarni *et. al*., 1992; Dorta *et. al*., 1993; Bansal and Singh 2003; 2006) and larval instars (Vijayan *et. al*., 1993; Dorta *et. al*., 1993; Bansal and Singh 2004; 2006; 2007), the status of susceptibility of their larvae to different plant derived derivatives is not yet explored in this region. Hence, for an effective vector control strategy, the determination of the level of susceptibility of these vector mosquitoes to the synthetic or plant derived insecticides is of utmost importance. The present study was therefore undertaken to evaluate the mosquito larvicidal potential of seeds of *C. viscosa* and leaves and fruits of *T. terrestris*.

Materials and Methods

Seeds of *C. viscosa* and leaves and fruits of *T. terrestris* were collected from different habitats in and around the city and help from Botanical Survey of India was taken for identification. Seeds were taken out from the dried pods of *C. viscosa* and powdered separately while leaves and fruits from *T. terrestris* were washed three to four times with distilled water, dried in an oven at a temperature of 40ºC for 2-3 days, powdered separately and stored at a temperature of 15-20ºC. 100g of the powdered plant material each from leaves, fruits and seeds was extracted separately using 400 ml of methanol/ acetone/ 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. Stock solutions from the residual extracts (5g /50 ml of solvent) were prepared in ethanol for other organic solvents. Different test concentrations from 25-500 mg l^{-1} for all organic solvent extracts were used during the experimentation.

Susceptibility tests with different 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 insectary and used for the bioassay 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 desired test concentrations. Controls with the same amount of solvent (1 ml ethanol) were kept side by side. 20-25 healthy late 3rd or early 4th instar larvae were kept in different test concentrations and mortality noted after 24 and 48hr during which no food was given. All treatments were replicated four times and carried out at a controlled room temperature of 28±2ºC and RH 75±5%. Larvae were considered dead if appendages did not move when probed with a needle in the siphon or cervical region. Larvae incapable of rising to the surface or not showing any characteristic diving movement when water was disturbed were considered moribund/ dead for calculating percent mortality. If mortality was between 5-20% in the control experiments, the percent corrected mortality was calculated using Abbott's formula. 24 and 48hr LC_{50} and LC_{90} values were computed using probit regression analysis (Finney, 1971).

Results and Discussion

The percent yield for seeds of *C. viscosa* was 8.7, 6.4 and 5.5 % for the, methanol, acetone and petroleum ether extracts while for leaves and fruits of *T. terrestris* it was 9.6 and 7.9; 8.8 and 5.7 and 8.3 and 5.0 % for the above three solvents, respectively. Results obtained with methanol and acetone extracts from the seeds of *C. viscosa* showed that these were more effective, the 24 hr LC_{50} being 144.1, 99.5, 127.1 mg l^{-1} for the methanol extracts and 106.3, 138.9, 118.5mg l^{-1} for the acetone extracts to all the three mosquito species, respectively (Table 1). However, with petroleum ether extracts, the 24hr LC_{50} were 166.4, 162.5 and 301.9 mg l^{-1} for all the three mosquito species, respectively showing that the methanol and acetone extracts performed in a better way than the petroleum ether extracts. Methanolic extracts from whole plant of *C. viscosa* (Nazar *et al*., 2009) were also found quite effective where 100% mortality for *Cx. quinquefasciatus* was observed at 80 mg l^{-1}. In the present study, 24hr LC_{50} for petroleum ether seed extracts for *Cx. quinquefasciatus* was 301.9 mg l^{-1} showing that this species was less sensitive to this plant extract. Petroleum ether extracts from fruits of *C. gynandra* were also not much effective as the 24hr LC_{50}

for these three mosquito species was greater than 200 mg l^{-1} (Sakthivadivel and Daniel, 2008). 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.* 48hr LC_{50} values were much less as compared to 24hr LC_{50} values showing that plant derived phytochemicals are much slower in

Table 1 : Comparative efficacy of seeds of *Cleome viscosa* against larvae of major mosquito vectors in different organic solvents

Mosquito species	Solvent	Exposure Time (hrs)	Regression equation	Chi Square (χ^2)	LC_{50} Fiducial limits (ppm)	LC_{90} Fiducial limits (ppm)
An. stephensi	Methanol	24	Y= 2.51x-0.41	3.79	144.1±1.15(109.0-190.4)	467.2±1.38(248.8-877.1)
		48	Y= 2.85x-0.67	0.50	97.6±1.13(76.8-123.9)	274.4±1.29(165.6-454.9)
Ae. aegypti		24	Y=2.32x+0.36	0.91	99.5±1.17(72.9-135.7)	354.1±1.45(169.9-738.2)
		48	Y=2.41x+0.63	0.45	65.3±1.17(48.3-88.2)	221.7±1.43(109.4-449.0)
Cx. quinque-		24	Y=2.38x-0.02	0.46	127.1±1.14(97.8-165.3)	473.8±1.35(242.4-790.5)
fasciatus		48	Y=3.11x-1.09	2.29	90.2±1.12(72.1-112.8)	232.4±1.27(145.0-372.7)
An. stephensi	Acetone	24	Y=2.08x+0.79	0.24	106.3±1.15(80.7-140.1)	439.4±1.38(232.3-831.1)
		48	Y=2.49x+0.62	0.26	57.6±1.13(45.0-73.7)	188.3±1.30(112.0-316.7)
Ae. aegypti		24	Y=2.05x+0.61	0.53	138.9±1.16(103.0-187.4)	584.6±1.39(307.1-1112.7)
		48	Y=2.44x+0.16	0.32	96.2±1.13(75.6-122.5)	321.6±1.30(192.8-536.2)
Cx. quinque-		24	Y=1.93x+1.00	0.22	118.5±1.15(90.7-154.9)	546.8±1.38(292.0-1023.7)
fasciatus		48	Y=0.02x+0.97	0.20	99.4±1.14(77.0-128.4)	428.4±1.36(235.1-780.9)
An. stephensi	Petroleum Ether	24	Y=1.72x+1.18	0.96	166.4±1.19(117.5-235.7)	924.2±1.53(400.2-2134.6)
Ae. aegypti		48	Y=2.23x+1.19	0.17	51.4±1.14(39.4-66.9)	193.2±1.35(107.0-348.7)
		24	Y=2.06x+0.44	1.47	162.5±1.19(116.3-227.0)	678.6±1.48(314.4-1464.7)
		48	Y=2.41x-0.15	2.11	136.4±1.16(102.7-181.0)	462.3±1.37(248.9-858.7)
Cx. quinque-		24	Y=2.12x+0.25	1.38	301.9±1.53(228.5-398.9)	1214.4±1.38(648.4-2274.4)
fasciatus		48	Y=2.64x-1.20	3.49	221.2±1.14(172.6-283.6)	674.9±1.29(409.9-1111.1)

Values are mean of replicates ±SE

Table 2 : Comparative efficacy of fruits of *Tribulus terrestris* against larvae of major mosquito vectors in different organic solvents

Mosquito species	Solvent	Exposure Time (hrs)	Regression equation	Chi Square (χ^2)	LC_{50} ± SE Fiducial limits (ppm)	LC_{90} ± SE Fiducial limits (ppm)
An. stephensi	Methanol	24	Y=2.41x-0.54	0.41	70.8±1.15(53.5-93.7)	240.4±1.39(126.4-457.4)
		48	Y=2.61x+0.44	0.63	55.7±1.15(42.7-72.9)	172.3±1.37(92.9-319.7)
Ae. aegypti		24	Y=2.44x+0.09	0.59	103.4±1.14(80.3-133.1)	346.4±1.31(202.7-592.0)
		48	Y=2.51x+0.30	0.81	74.9±1.37(58.3-96.2)	242.8±1.33(139.3-423.1)
Cx. quinque-		24	Y=1.72x-0.81	1.21	268.2±1.18(194.5-369.6)	148.5±1.51(662.8-3316.1)
fasciatus		48	Y=1.97x+0.55	0.90	179.1±1.15(135.8-236.3)	796.8±1.41(408.4-1554.3)
An. stephensi	Acetone	24	Y=2.18x+0.92	0.51	74.0±1.14(56.9-96.3)	285.2±1.35(159.3-510.6)
		48	Y=2.69x+0.56	0.14	44.5±1.12(35.4-55.9)	132.8±1.27(83.1-212.4)
Ae. aegypti		24	Y=2.05x+0.74	0.49	120.5±1.17(87.9-165.2)	508.3±1.44(248.6-1039.1)
		48	Y=2.32x+0.65	0.32	74.6±1.15(56.4-98.6)	265.5±1.38(141.3-498.8)
Cx. quinque-		24	Y=1.87x+1.03	0.76	132.0±1.17(97.6-178.5)	638.4±1.44(312.9-1302.5)
fasciatus		48	Y=2.13x+1.02	0.92	74.3±1.15(56.9-97.0)	297.2±1.38(157.4-561.0)
An. stephensi	PetroleumEther	24	Y=1.81x+1.61	1.27	73.8±1.17(54.2-100.3)	374.6±1.43(185.6-756.1)
		48	Y=2.59x+0.60	1.08	50.0±1.13(39.1-63.9)	156.0±1.29(94.3-258.1)
Ae. aegypti		24	Y=2.35x+0.16	0.38	113.5±1.14(88.2-146.1)	397.1±1.30(237.4-664.3)
		48	Y=2.13x+1.26	1.93	57.0±1.14(44.0-73.8)	227.3±1.32(131.1-394.0)
Cx. quinque-		24	Y=1.98x+0.77	1.19	137.4±1.14(105.9-178.2)	608.5±1.34(342.5-1081.2)
fasciatus		48	Y=2.51x-0.02	1.47	100.5±1.12(80.6-125.4)	325.8±1.26(205.8-515.6)

Values are mean of replicates ±SE

their insecticidal action unlike the conventional synthetic insecticides. Organic solvent extracts of some plants and their oil cakes have also been reported to be quite effective as compared to aqueous extracts against larvae of *An. stephensi, Ae. aegypti,* and *Cx. quinquefasciatus* (Maurya *et al*., 2008; Shanmugasundaram *et al*., 2008; Srivastava *et al*., 2008).

The LC_{50} values of *T. terrestris* were 70.8, 103.4, 268.2 methanol; 74.0, 120.5, 132.0 for acetone and 73.8, 113.5, 137.4 mg l^{-1} for petroleum ether for the fruits (Table 2) while the corresponding values for the leaves were 124.3, 196.8, 246.5 for methanol; 163.4, 196.9, 224.3 for acetone and 135.8, 176.08, 185.9 mg l^{-1} for petroleum ether for the three mosquito species respectively (Table 3). The results showed that the fruits of *T. terrestris* were more potent in their larvicidal efficacy as compared to the leaves of this plant species which revealed that active larvicidal component may be present more abundantly in the fruits as compared to the leaf of this plant species. Singh *et al*. (2008) also determined the larvicidal efficacy of acetone extracts of leaves and seeds of *T. terrestris* and found that seed extracts were much more effective (24hr LC_{50}; 100, 72, 91 and 91 mg l^{-1}) than the leaf extract (24hr LC_{50}; 117, 124, 168 and 185 mg l^{-1}) for *An. culicifacies*, *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti,* respectively. El-Sheikh *et al*. (2012) also determined the efficacy of the methanolic extracts of leaves and seeds of *T. terrestris* against 3rd instar larvae and adults of *An. arabiensis* under laboratory conditions. The seeds extract showed high insecticidal activity (LC_{50} -36.5) as compared to the leaf extract (LC_{50} -123.5 mg l^{-1}). All extracts exhibited remarkable effects on the fecundity, fertility and sterility index of adult females resulting from treated larvae, but the seeds extract was more effective than leaf extract. These results are in accordance with the our present findings which warrants that fruits and seeds of *Tribulus* may be explored for their active insecticidal components for the control of these disease vectors. Bansal *et al*. (2011, 2012) also showed that methanol fruit and seed extracts of *Withania somnifera* and *Tephrosea purpurea* were very much effective as compared to the leaves extracts of *Prosopis juliflora* and *Solanum xanthocarpum* showing presence of active insecticidal component in fruits.

The observations on 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 of great importance 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 botanical insecticides (Berenbaum, 1989). Many plants from different families seem to possess the most promising botanicals for insect vector control (Jacobson, 1989) which are much economical and environmental friendly (Jang *et al*., 2002, Sivagnaname and Kalyanasundaram, 2004). These phytochemicals like alkaloids, phenolics and terpenoids exist in plants (Wink, 1993) which may jointly or independently contribute

Table 3 : Comparative efficacy of leaves of *Tribulus terrestris* against larvae of major mosquito vectors in different organic solvents

Mosquito species	Solvent	Exposure Time (hrs)	Regression equation	Chi Square (χ^2)	LC_{50} Fiducial limits (ppm)	LC_{90} Fiducial limits (ppm)
An. stephensi	Methanol	24	Y=2.00x-0.82	0.04	124.3±1.15(94.4-163.7)	544.5±1.40(283.1-1047.1)
		48	Y=2.04x+0.97	0.15	94.1±1.16(70.6-125.6)	398.4±1.40(205.6-772.0)
Ae. aegypti		24	Y=2.27x-0.21	0.14	196.8±1.15(149.8-258.7)	721.1±1.35(401.8-1294.2)
		48	Y=2.34x+0.06	0.17	129.3±1.15(98.6-169.7)	455.7±1.37(244.7-848.7)
Cx. quinque-		24	Y=2.19x-0.25	1.86	246.5±1.13(195.0-311.6)	944.4±1.31(553.2-1612.2)
fasciatus		48	Y=2.05x+0.36	1.84	183.9±1.14(143.4-235.9)	775.6±1.37(418.6-1436.7)
An. stephensi	Acetone	24	Y=1.67x+1.33	0.26	163.4±1.19(116.7-228.9)	968.4±1.54(417.6-2245.6)
		48	Y=1.90x+1.20	0.26	100.1±1.19(71.8-139.7)	473.4±1.46(224.2-999.7)
Ae. aegypti		24	Y=1.98x+0.46	0.40	196.9±1.16(146.1-265.4)	873.3±1.41(442.8-1722.2)
		48	Y=1.93x+0.89	0.19	134.9±1.18(98.2-185.3)	622.0±1.49(286.1-1352.1)
Cx. quinque-		24	Y=1.83x+0.71	0.28	224.3±1.16(168.0-299.6)	1127.3±1.43(559.4-2272.0)
fasciatus		48	Y=1.86x+0.95	0.24	151.6±1.16(114.0-201.6)	741.9±1.43(365.7-1505.0)
An. stephensi	Petroleum ether	24	Y=2.02x+0.70	0.24	135.8±1.17(100.5-183.7)	586.4±1.43(289.7-1187.0)
		48	Y=2.26x+0.45	0.24	103.5±1.15(78.1-137.2)	381.5±1.38(203.0-716.8)
Ae. aegypti		24	Y=2.05x+0.39	1.02	176.08±1.17(130.5-237.6)	739.1±1.39(386.2-1414.6)
		48	Y=2.15x+0.54	0.09	119.8±1.16(89.5-160.4)	473.3±1.40(244.2-917.4)
Cx. quinque-		24	Y=2.53x-0.73	1.08	185.9±1.13(146.8-235.3)	596.7±1.29(364.0-978.3)
fasciatus		48	Y=2.35x+0.07	0.52	124.8±1.14(97.0-160.6)	437.2±1.33(251.3-760.5)

Values are mean of replicates ± SE

to the generation of larvicidal activities of mosquitoes (Hostettmann and Potterat (1997).

The results with seeds of *C. viscosa* suggests that methanol and acetone extracts were a little bit more effective than the petroleum ether extracts while the efficacy of fruit extracts of *T. terrestris* was more as compared to leaves extracts in all the three solvents tested. Larvae of *An. stephensi* were found more sensitive to both the fruit and leaves extracts of *T. terrestris* followed by the larvae of *Ae. aegypti* and *Cx. quinquefasciatus.* Extracts from the seeds of *C. viscosa* were found less effective as compared with the fruit extracts of *T. terrestris* indicating that active larvicidal principle may be present in the fruits of this plant species. The study suggests that chemical composition of extracts from different parts of the same or different plants may be different and requires thorough exploration of the larvicidal potential present therein. The present results would be of great importance while formulating the integrated control strategy, for vectors of malaria, dengue and lymphatic filariasis in this semi-arid region, based on alternative plant based insecticides.

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