

pared to those without AC. The cultures containing 0.3% polyvinylpyrrolidone showed no improvement in response.

The ultimate success of *in vitro* propagation lies in the successful establishment of plants in the soil⁹. The regenerated plantlets of *C. orchioides* were transferred to thermocol cups containing moist cocopeat and manure for acclimatization at $25 \pm 2^\circ\text{C}$ for ten days during which elongation and growth of leaves was observed (Figure 1d). Later, these plantlets were transferred to the greenhouse, kept for ten days and then transferred to pots containing autoclaved garden soil. The pots containing the *in vitro* plantlets were finally transferred to the garden plot after one week. The *in vitro*-grown plants exhibited survival rate of 96.27 and 82.57% in polyhouse and field conditions respectively. The high survival rate indicates that this protocol

could be easily adopted for large-scale cultivation of *C. orchioides*.

1. Dhar, M. L., Dhar, M. M., Dhawan, B. N., Mehrotra, B. N. and Ray, C., *Indian J. Exp. Biol.*, 1968, **6**, 232–247.
2. Bishit, B. S. and Nayar, S. L., *J. Sci. Indian Res.*, 1960, **19C**, 25–27.
3. *Dictionary of Chinese Traditional Medicine*, Jiyangsu College of New Medicine, People's Press, Shanghai, 1979, p. 1363.
4. Shah, G. L., *Flora of Gujarat State-II*, Sardar Patel University Press, Vallabh Vidyanagar, 1978.
5. Augustine, C. A. and D'souza, L., *In vitro Cell Dev. Biol.—Plant*, 1997, **33**, 111–113.
6. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
7. Hussey, G., *J. Exp. Biol.*, 1975, **26**, 253–262.
8. George, M. M., Ph D thesis, Sardar Patel University, Vallabh Vidyanagar, 1999.

9. Saxena, S. and Dhavan, V., *Plant Cell Rep.*, 1999, **18**, 438–443.

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Larvicidal properties of a perennial herb *Solanum xanthocarpum* against vectors of malaria and dengue/DHF

The use of different parts of locally available plants and their various products in the control of mosquitoes has been well established¹. The use of chrysanthemum flower heads and tobacco leaf smoke was known to people since ancient times. A number of unsaturated *N*-(2-methyl propyl) amides with larvicidal activities have been reported from the plants of families Compositae, Piperaceae and Rutaceae². Studies on azadirachtin-rich fractions from neem³ and water extracts of de-oiled neem kernel^{4,5} reveal the larvicidal properties of different fractions of these plants. The larvicidal properties of indigenous plants have also been documented in many parts of our country^{6,7}, along with the repellent and anti-juvenile hormone activities^{8,9}. *Solanum xanthocarpum*, the Indian nightshade, commonly known as 'baigan kateli', is found throughout the country, but more abundantly in arid areas. The plant is known to have multiple medicinal properties^{10–12} and the extracts of various parts have been used against agricultural pests as repellent¹³ and contact poison¹⁴, and as molluscicide¹⁵ in public health. However, studies against the pests of public-health importance are

totally lacking. In the present study, the extracts of different parts of the plant have been evaluated against the larvae of important vectors of malaria and dengue, and the findings are summarized here.

For evaluating the larvicidal activity of *S. xanthocarpum*, the crude extracts of fruits and roots from the fresh plants were collected from the fields. For obtaining the fruit extract, the unripe berries of the plant were used. For extracting the fruit extract the pulp of the berries was weighed, blended and finally centrifuged. The supernatant so obtained was used as stock solution, from which the serial dilutions according to requirements, were prepared in distilled water for experimentation. The root extract was also prepared following a similar method. Concentrations between 0.001 and 10.0% were tried initially, but after subsequent experiments the final concentrations of the extracts of different parts were determined to obtain graded-mortalities of tested mosquito species. The concentrations of fruit extract between 0.01 and 0.6% against the larvae of *Anopheles culicifacies*, between 0.005 and 1.0% against *Anopheles stephensi*, and between 0.005 and 1.0% against *Aedes*

aegypti were finally considered for evaluating the efficacy. In case of root extract, concentrations between 0.1 and 6.0% were used against all the three mosquito species.

The experiments were conducted according to WHO methods^{16,17}. The larvae used in the experiments were laboratory-reared. Tests were conducted during the month of September. In the experiments, the late third and early fourth instar larvae of *An. culicifacies*, *An. stephensi* and *Ae. aegypti* were used. Observations were made after 24 h. In case of individual species, three to four replicates were performed for each concentration. For each species, against a particular extract, six concentrations were used to obtain concentration–mortality data, for determining the lethal concentrations at LC₅₀ and LC₉₀ levels by log-probit analysis¹⁸. Laboratory temperature and relative humidity during the experiments were recorded as $27 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ respectively.

The results of the tests conducted for evaluating the larvicidal efficacy of fruit extract of *S. xanthocarpum* revealed that the extract has larvicidal activity against two tested anopheline species, viz. *An.*

Table 1. Concentration mortality response data of fruit and root extracts of *S. xanthocarpum* against three mosquito vector species

Extract/mosquito species	Concentration (%)	No. exposed	No. dead	Percentage mortality*
Fruit extract				
<i>An. culicifacies</i>	0.01	65	00	00.0
	0.05	70	08	11.4
	0.1	62	26	41.9
	0.2	74	60	81.1
	0.4	52	51	98.1
	0.6	75	75	100.0
<i>An. stephensi</i>	0.005	70	00	00.0
	0.01	64	07	10.9
	0.05	81	37	45.7
	0.1	86	58	67.4
	0.5	80	72	90.0
	1.0	75	75	100.0
<i>Ae. aegypti</i>	0.005	60	00	00.0
	0.01	70	08	11.4
	0.05	66	35	53.0
	0.1	75	50	66.6
	0.5	80	78	97.5
	1.0	70	70	100.0
Root extract				
<i>An. culicifacies</i>	0.1	75	00	00.0
	0.5	115	15	13.0
	1.0	105	43	40.9
	2.0	115	93	80.9
	4.0	125	116	92.8
	6.0	106	106	100.0
<i>An. stephensi</i>	0.1	100	00	00.0
	0.5	140	21	15.0
	1.0	165	74	44.8
	2.0	155	129	83.2
	4.0	145	136	93.8
	6.0	125	125	100.0
<i>Ae. aegypti</i>	0.1	80	00	00.0
	0.5	80	08	10.0
	1.0	80	42	52.5
	2.0	80	58	72.5
	4.0	80	74	92.5
	6.0	80	80	100.0

*No mortality was observed in control.

culicifacies and *An. stephensi*, and one culicine species *Ae. aegypti*. Data on the concentration–mortality response of fruit extract are given in Table 1. The lethal concentrations of fruit extract at LC₅₀ and LC₉₀ levels against *An. culicifacies*, *An. stephensi* and *Ae. aegypti* were determined as 0.112 and 0.258, 0.058 and 0.289 and 0.052 and 0.218% respectively (Table 2).

Tests conducted for evaluating the larvicidal activity of root extract against anopheline and culicine mosquito species revealed that this extract also has larvici-

dal properties, though at higher concentrations in comparison to fruit extract. Data on concentration–mortality response of root extract are given in Table 1. The LC₅₀ and LC₉₀ values against *An. culicifacies*, *An. stephensi* and *Ae. aegypti* were determined as 1.160 and 3.237%, 1.080 and 2.789% and 1.150 and 3.581% respectively (Table 2).

It is clear from the data obtained that fruit extract was 12.5, 9.7 and 16.4 times more toxic than root extract to *An. culicifacies*, *An. stephensi* and *Ae. aegypti* respectively, at LC₉₀ level. However, at

LC₅₀ level, the corresponding values were 10.4, 18.6 and 22.1 respectively. The chi-square test values revealed that none of the tested anopheline species has significant heterogeneity in the test population.

Results of the experiments envisaged larvicidal property in both fruit and root extracts of *S. xanthocarpum*. As the plant is distributed throughout the country and the fruits are available most of the time, the larvicidal properties of this plant species can be well utilized while planning alternate vector control strategies,

Table 2. Log-probit analysis of larvicidal efficacy of fruit and root extracts of *S. xanthocarpum* against different mosquito vector species

Extract/mosquito species	Regression coefficient	Regression equation	Chi-square (df)	LC ₅₀ * with fiducial limits	LC ₉₀ * with fiducial limits
Fruit extract					
<i>An. culicifacies</i>	3.56	$Y = -2.29 + 3.56X$	0.06 (4)	0.112 (0.090–0.141)	0.258 (0.166–0.400)
<i>An. stephensi</i>	1.84	$Y = 1.74 + 1.84X$	0.90 (4)	0.058 (0.041–0.084)	0.289 (0.156–0.535)
<i>Ae. aegypti</i>	2.04	$Y = 1.50 + 2.04X$	1.73 (4)	0.052 (0.036–0.073)	0.218 (0.120–0.397)
Root extract					
<i>An. culicifacies</i>	2.87	$Y = -3.80 + 2.87X$	0.88 (4)	1.160 (0.950–1.417)	3.237 (2.215–4.732)
<i>An. stephensi</i>	3.11	$Y = -4.43 + 3.11X$	0.34 (4)	1.080 (0.915–1.275)	2.789 (2.056–3.784)
<i>Ae. aegypti</i>	2.59	$Y = -2.94 + 2.59X$	3.14 (4)	1.150 (0.893–1.481)	3.581 (2.281–5.621)

*Values of LC₅₀ and LC₉₀ are percentages of fruit and root extracts.

based on integrated vector control measures through community-based approaches. The plant is easily available to the local people and being an ayurvedic herb with multiple medicinal properties^{10–12}, it may be easily acceptable to them, since during application it would neither cause any toxic effect nor any additional economic burden. The study suggests that the active ingredient(s) of the extract responsible for causing mortality in mosquito larvae should be identified and utilized, if possible, in preparing a commercial product/formulation to be used as a mosquito larvicide.

1. Evans, D. A. and Kaleysa Raj, R., *Indian J. Med. Res.*, 1988, **88**, 38–41.
2. Jacobson, M., *Naturally Occurring Insecticides*, Pergamon Press, New York, 1971, pp. 137–145.
3. Raghunath Rao, D., Reuben, R., Gitanjali, Y. and Srimannarayana, R., *Indian J. Med. Res.*, 1988, **88**, 67–70.

4. Singh, R. P., *Neem Newsl.*, 1984, **2**, 16–18.
5. Evans, D. A. and Kaleysa Raj, R., *Indian J. Med. Res.*, 1991, **93**, 324–327.
6. Deshmukh, P. B., Chavan, S. R. and Renapurkar, D. M., *Pesticides*, 1982, **16**, 7–9.
7. Kalyansundaram, M. and Babu, C. J., *Indian J. Med. Res.*, 1982, **76**, 102–106.
8. Hebbalkar, D. S., Hebbalkar, G. D., Sharma, R. N., Joshi, V. S. and Bhat, V. M., *Indian J. Med. Res.*, 1992, **95**, 200–203.
9. Saxena, R. C., Dixit, O. P. and Padam Sukumaran, *Indian J. Med. Res.*, 1992, **95**, 204–206.
10. Govindan, S., Viswanathan, S., Vijayasekaran, V. and Alagappan, R., *J. Ethnopharmacol.*, 1999, **66**, 205–210.
11. Bector, N. P. and Puri, A. S., *J. Assoc. Physicians India*, 1971, **19**, 741–744.
12. Gupta, S. S., Verma, S. C., Singh, C. and Khandelwal, P., *Indian J. Med. Sci.*, 1966, **20**, 554–559.
13. Husain, M. M., *Pak. J. Zool.*, 1995, **27**, 279–280.

14. Pandey, U. K., Verma, G. S. and Pandey, M., *Indian J. Entomol.*, 1980, **42**, 775–776.
15. Wei, F. H., Xu, X. J., Liu, J. B., Dai, Y. H., Dussart, G. and Trigwell, J., *Ann. Trop. Med. Parasitol.*, 2002, **96**, 325–331.
16. WHO, Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides, WHO/VBC/74.583, 1975, pp. 1–5.
17. WHO, Tech. Rep. Ser., 1963, p. 265.
18. Finney, J., *Probit Analysis*, Cambridge Press, London 1972, 2nd edn, p. 295.

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