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INDIGENOUS PLANT OILS AS LARVICIDAL AGENT AGAINST *ANOPHELES STEPHENSI* MOSQUITOES

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Mosquitoes serve as vectors of several diseases causing serious health problems to human beings. Although eradication of these vectors was considered possible by the use of chemical insecticides, development of insecticide resistance initiated a search for alternative control measures[1]. Biologically active plant extracts are therefore studied for their efficacy to kill larvae of different mosquitoes[2–6]. Supavarn *et al*[2] reported that of the 36 plant samples studied, five of them killed all the larvae of *Aedes aegypti* within 7 days at a concentration of 1000 ppm. Joshi *et al*[3] found natural and synthetic garlic to be an effective larvicide. The present study was carried out to determine the larvicidal activity of oils from 10 plants of 8 genera viz *Cedrus deodara*, *Cymbopogon nardus*, *C. flexuosus*, *C. martini*, *Lavandula officinalis*, *Mentha arvensis*, *Ricinus communis*, *Eucalyptus globulus*, *Eugenia caryophyllus* and *Melia azadirachta* against laboratory colonized fourth instar larvae of malaria vector *Anopheles stephensi*.

The larvae of *A. stepheni*, used in this study were reared in CDRI insectary at a temperature of 26±2°C and a relative humidity of 75±5%. The fourth instar larvae were taken to study the larvicidal activity of different plant oils.

Different parts of the plants were cut into pieces and oil was obtained by steam distillation and then purified to get rectified oil. It was diluted thrice in acetone and added to 150 ml of dechlorinated tapwater to obtain the desired concentration.

All bioassays were performed according to the standard method[7]. Fifty larvae were tested at each dilution—25 larvae in one crystallizing dish (Borosil, 100×50 mm) in 150 ml of dechlorinated tapwater. All oils were tested at 9 different dilutions viz 25, 50, 75, 100, 125, 150, 175, 200 and 250 ppm. A small aliquot of yeast powder was supplied for nutrition. With each experiment, a set of control was included with 5 ml acetone. Larvae mortality was recorded after 24 hr. Toxic activity was reported as LC_{50}, that is ppm of oil that killed 50% larvae in 24 hr.

The results of larvicidal activity of different plant oils against fourth instar larvae of *A. stephensi* are presented in table 1. The maximum activity was observed in the case of *C. deodara* which caused 50% mortality at dose of 63.2 ppm. To our knowledge no reports are available regarding insecticidal activity of *C. deodara* except one report which showed it to be toxic for adult *A. stephensi* mosquitoes[8]. Oils from *C. nardus*, *C. flexuosus*, *C. martini*, *L. officinalis*, *M. arvensis*, *R. communis*, *E. globulus*, *E. caryophyllus* and *M. azadirachta* showed LC_{50} values of 105.4, 91.4, 100.0, 83.6, 83.8, 113.0, 98.5, 96.5 and 88.5 ppm, respectively. Earlier reports[2,5] showed a need for very high concentration of plant extracts for achieving significant mortality of mosquitoes larvae, while oils used in the present study resulted in 100% mortality at dose of 250 ppm in all the cases. The most effective *C. deodara* oil caused complete mortality even at a concentration of 175 ppm.

The present study indicates the efficacy of several plant oils as larvicidal agents and their possible use in the biological control of *A. stephensi*, an important vector for several tropical parasitic diseases. Combined with conventional chemical larvicides

Table 1 *Larvicidal activity of different plant oils against fourth instar larvae of Anopheles stephensi*

Name of plant	Percentage mortality (ppm) 25	50	75	100	125	150	175	200	250	Control	LC_{50} Values ppm
C. deodara	16	32	56	64	72	90	100	100	100	0	63.2
C. nardus	2	10	20	48	62	80	90	94	100	0	105.4
C. flexuosus	4	16	36	56	66	82	92	100	100	0	91.4
C. martini	3	12	28	50	62	78	94	96	100	0	100.0
L. officinalis	10	20	42	58	70	88	90	100	100	0	83.6
M. arvensis	8	22	42	60	74	90	98	100	100	0	83.8
R. communis	7	18	31	47	58	69	86	93	100	0	113.0
E. globulus	11	24	35	51	62	72	88	99	100	0	98.5
E. caryophyllus	10	31	40	51	70	78	97	100	100	0	96.5
M. azadirachta	21	93	41	54	70	81	97	100	100	0	88.5

these oils may provide an extremely effective vector control measure at a rather low cost.

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IN VITRO REGENERATION FROM CALLUS CULTURES OF *SCILLA INDICA* (ROXB.) BAKER

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SCILLA INDICA (Roxb.) Baker of Liliaceae is cultivated in India for its commercial products[1,2]. The ornamental leaves add to its horticultural value.

S. indica responds readily to artificial media unlike most monocots[3,4] and is charaterized by a slow rate of natural propagation. The objective of this work was to explore the possibility of rapid propagation through *in vitro* regeneration from callus cultures, if possible, without resorting to the long process of embryo development. The effects of two auxins on the callus and the chromosome analysis of the callus and regenerated plants have also been worked out.

Diploid plants of *S. indica* ($2n=30$) were collected from Pune and Mahabaleshwar and replanted in the college garden. Young leaf-tips and scale-leaves of sprouting bulbs were sterilized in 0.1% $HgCl_2$ solution for 15 and 25 min respectively. Explants of about 1.5 × 1 cm were excised out from them aseptically, and callus cultures were initiated on the basal medium of Murashige and Skoog[5](MS) with an addition of 2 mg/l 2,4-D and 15% (V/V) coconut milk. The pH of the medium was adjusted to 5.6, solidified by 0.5% Agar and cultures were incubated at a temperature of 25 ± 1°C for 16/8 hr light/dark period.

For cytological investigation, callus pieces were fixed in Carnoy's fluid and stained in 2% aceto-orcein : (N) HCL (9:1). Young root-tips and shoot-tips from regenerated plants were also similarly fixed and stained.

Calli were initiated on the surface of the explants after 30 days of growth in the medium containing 2,4-D. The callus was subcultured onto fresh medium after every 30 days.

Regeneration of shoots and roots was observed when a 60-day-old callus was transferred to MS medium devoid of 2,4-D but containing 15% coconut milk and 2 mg/l NAA (figure 1). The regenerat-