

## Studies on larvicidal properties of leaf extract of *Solanum nigrum* Linn. (family Solanaceae)

Continued use of synthetic chemical insecticide-based intervention measures for vector control has resulted in lower efficacy of the insecticide in controlling the medically important disease vectors. Malaria contributes to the major disease burden, and operational control failure, namely development of insecticide resistance in malaria vector to the commonly used synthetic chemical insecticides in public health sprays has made the disease control more difficult<sup>1</sup>. In recent years use of environment-friendly and easily biodegradable natural insecticides of plant origin has received renewed importance for disease vector control. Interest in this field has increased more so, as they are least phytotoxic and do not accumulate chemical residues in flora, fauna and soil<sup>2</sup>.

In fact, the first compound that was used extensively against adult mosquitoes was the flower extract of *Chrysanthemum cinerariaefolium* (family Compositae)<sup>3</sup>. This extract was first used in South Africa<sup>4</sup> and India<sup>5,6</sup> with desired results. This extract is still in use in indoor space sprays against adult vector species for liquidation of foci during the epidemics and outbreaks<sup>7</sup>.

Studies on the natural plant products for their efficacy as larvicides during the last decade have indicated them to be possible alternatives to synthetic chemical insecticides<sup>8–15</sup>. However

more concerted efforts have to go into these studies to make these environment-friendly compounds viable for field use and for large-scale vector control operations<sup>10,14</sup>. Sukumar *et al.*<sup>15</sup> reported 99 families, 276 genera and 346 species to have insecticidal properties. These include members of the family Solanaceae comprising 5 species belonging to 4 genera, namely *Capsicum frutescens*, *Datura candida*, *D. stramonium*, *Lycopersicon lycopersicum* and *Nicotiana rustica*.

The present communication deals with the laboratory studies carried out to ascertain the larvicidal properties of *Solanum nigrum* (Figure 1) in three mosquito species, viz. *Anopheles culicifacies* Giles species A, a vector of importance for the transmission of malaria in rural and peri-urban areas (*An. culicifacies* is a complex of five sibling species provisionally designated as species A, B, C, D and E. Of these A, C, D, and E are vectors of malaria<sup>16</sup>), vector of filariasis *Culex quinquefasciatus* Say, and *Aedes aegypti* Linn, a vector of dengue and DHF. This plant is widely distributed in the wild in many parts of India. Taxonomic position of this plant is as follows: Division – Embryophyta; Sub-division – Angiospermae; Class – Dicotyledoneae; Order – Tubeflorae; Sub-order – Solanales; Family – Solanaceae; Genera – *Solanum*. The local name in some important vernacular languages are: Hindi – Makoi, Telugu –

Kachchipandu, Tamil – Munatakali, Gujarati – Piludi, Marathi – Kamuni<sup>17</sup>. This species is reported to have many medicinal properties and is used mainly as antidiysenteric, diuretic, laxative and antispasmodic; ripe fruits are also used in pies and preserved as jams<sup>18</sup>.

For these studies, plants were collected from rural areas of district Agra, Uttar Pradesh and Delhi, in North India. The plant was submitted to National Institute of Science Communication, The Wealth of India Division, CSIR, New Delhi, for taxonomic identification and confirmation of the species.

Fresh leaves were washed with tap water and cleaned thoroughly with a cloth. The leaves were cut into small pieces and immediately ground using a pestle and mortar. The ground material was filtered through muslin cloth and filtrate of the crude leaf extract was stored in a clean brown bottle till further use.

Laboratory-reared I, II and III, IV instar larvae of *An. culicifacies* species A, *Cx. quinquefasciatus* and III, IV instar larvae of *Ae. aegypti* mosquitoes were treated with different per cent concentrations of aqueous solutions of this extract, following the standard WHO larval susceptibility test method<sup>19</sup>. The tests were conducted at room temperature (27–30°C). Concentrations (0.0025–0.3%) of the extract in water were prepared fresh and used for the tests. Solutions of the extract were



**Figure 1.** *Solanum nigrum* Linn. plant (1/8 of the original size).

**Table 1.** Larvicidal activity of different concentrations (0.0025–0.3%) of the crude extract of leaves of *Solanum nigrum* Linn.

Species instar (N)	LC <sub>50</sub> <sup>a</sup> (% in water)	LC <sub>90</sub> <sup>b</sup> (% in water)	χ <sup>2</sup> (df = 6)
<i>An. culicifacies</i> species A			
I/II (2)	0.0167	0.169	87.1
III/IV (6)	0.027	0.176	88.0
<i>Cx. quinquefasciatus</i>			
I/II (2)	0.019	0.185	75.8
III/IV (6)	0.027	0.205	103.5
<i>Ae. aegypti</i>			
III/IV (6)	0.032	0.212	119.8

N, Number of replicates (25 larvae/replicate); a, Lethal concentration to kill 50% of the treated larvae; b, Lethal concentration to kill 90% of the treated larvae; χ<sup>2</sup>, Chi-square for heterogeneity; (df = 6), Six degrees of freedom (df = k–2).

prepared in boiled and cooled tap water. At each of the given concentrations, at least two replicates comprising 25 larvae each were exposed. Results were scored after 24 h of continuous exposure to the test solution and expressed as per cent mortality. The data obtained were subjected to log-Probit regression analysis, to calculate the median lethal concentration (LC<sub>50</sub>) and LC<sub>90</sub> values<sup>20</sup>.

Hundred per cent larval mortality was observed within 24 h of exposure period in *An. culicifacies* species A, *Cx. quinquefasciatus* and *Ae. aegypti* at a concentration of 0.2%. The LC<sub>50</sub> values for I and II instar larvae of *An. culicifacies* species A and *Cx. quinquefasciatus* were 0.0167% and 0.019%, respectively and for III and IV instar larvae it was 0.027% in both the species. For III and IV instar of *Ae. aegypti*, it was 0.032% (Table 1). The LC<sub>90</sub> values for I and II instar larvae of *An. culicifacies* species A, and *Cx. quinquefasciatus* were 0.169% and 0.185%, respectively. The LC<sub>90</sub> values for III and IV instar larvae of the three species were 0.176%, 0.205% and 0.212%, respectively (Table 1). From the LC<sub>50</sub> and LC<sub>90</sub> values, the extract was found to be relatively more toxic to the larvae of *An. culicifacies* species A, followed by *Cx. quinquefasciatus* and *Ae. aegypti*.

Results of this preliminary study with the crude extract of the leaves of this plant have exhibited its toxicity to the three important disease vector species and warrants further investigations. Sukumar *et al.*<sup>15</sup> have stated the existence of variations in the toxicities of phytochemical compounds on target species *vis-à-vis* plant parts from which they are extracted, responses in species and developmental stages of species to the specified extract, solvent of extrac-

tion, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction, etc. Keeping in view the above variations, it will be of importance to study the variations in toxic effects of extracts and also to characterize the active ingredients responsible for the toxicity.

1. Sharma, V. P., *Indian J. Med. Res.*, 1996, **103**, 26–45.
2. Thomas, T. G., Sharma, S. K., Jalees, S. and Rahman, S. J., *J. Basic Appl. Biomed.*, 1994, **2**, 53–55.
3. Bruce-Chwatt and Leonard Jan, *Essential Malariology*, English Language Book Society, Alden Press, Oxford, 1985, II edn, p. 299.
4. De Meillon, B., *Q. Bull. Health Org. L N.*, 1936, **5**, 134–137.
5. Russel, P. F. and Knipe, F. W., *J. Mal. Inst. India*, 1941, **4**, 181–197.
6. Vishwanathan, D. K., *J. Mal. Inst. India*, 1941, **4**, 35–55.
7. Sharma, R. S., Sharma, G. K. and Dhilon, G. P. S., *Epidemiology and Control of Malaria in India 1996*, GOI, MoHFW, National Malaria Eradication Programme (DGHS), Shakun Enterprises, Delhi, 1996, I edn, p. 272.
8. Green, M. M., Singer, J. M., Sutherland, D. J. and Hibben, C. R., *J. Am. Mosq. Control Assoc.*, 1991, **2**, 282–286.
9. Kalyanasundaram, M. and Babu, C. J., *Indian J. Med. Res.*, 1982, **76** (suppl.), 102–106.
10. Kalyanasundaram, M. and Das, P. K., *Indian J. Med. Res.*, 1985, **82**, 19–23.
11. Pathak, Namrata, Mittal, P. K., Singh, O. P., Vidyasagar, D. and Padma Vasudevan, *Int. Pest Control*, 2000, **46**, 53–55.
12. Perich, Michael, J., Carl, Wells, Wolfgang Bertsch and Tredway, Kenneth,

*E., J. Med. Entomol.*, 1994, **31**, 833–837.

13. Vasudevan, P., Kashyap, Suman and Sharma, Satyawati, *Bioresour. Technol.*, 1997, **625**, 29–35.
14. Mehra, Bhavna K. and Haridhar, Pankaj K., *J. Entomol. Res.*, 2000, **24**, 141–146.
15. Sukumar, Kumuda, Perich, Michael, J. and Boobar, Lewis R., *J. Am. Mosq. Control Assoc.*, 1991, **7**, 210–237.
16. Subbarao, Sarala K., Nanda, Nutan and Raghavendra, K., *ICMR Bull.*, 1999, **29**, 75–80.
17. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, PID, New Delhi, 1956, p. 229.
18. *The Useful Plants of India*, PID, CSIR, New Delhi, 1992, p. 581.
19. World Health Organization, Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides, WHO/VBC/81.807, 1981.
20. Finney, D. J., *Probit Analysis*, Cambridge University Press, Cambridge, UK, 1971, III edn, pp. 1–333.

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## Acclimatization of Asiatic hybrid lilies under stress conditions after propagation through tissue culture

Efforts are going on worldwide to boost floriculture industry using biotechnology<sup>1</sup> and the attention is focused on development of new flower colour and novel plant morphology, as these are the main features which determine con-

sumer interest<sup>2</sup>. One of the major constraints of floriculture industry is non-availability of constant supply of quality bloom in all the regions of the country. All floricultural crops are climate-specific and flowers are transported

from one climatic zone to another for sale<sup>3</sup>. Acclimatization of any crop from one climatic zone to another, is normally done through conventional breeding. Work has been initiated at the Floriculture Lab, NBRI, Lucknow to