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Laboratory studies on mosquito larvicidal efficacy of aqueous & hexane extracts of dried fruit of *Solanum nigrum* Linn.

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***Background & objectives*: Aqueous and organic solvent extracts of plants/plant parts were effective in killing the mosquito larvae. Comparative efficacy of the aqueous and hexane extracts of dried fruit of *Solanum nigrum* was tested against five laboratory colonized strains of mosquito species, namely *Anopheles culicifacies* species A, *An. culicifacies* species C, *An. stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* to assess the possibility for use of these extracts for their control.**

***Methods*: Concentrations of aqueous extract of dried fruit in the range of 62.5 to 2000 ppm and hexane extract of dried fruit in the range of 0.781 to 150 ppm were used in bioassays. The mortality data were subjected to log probit regression analysis to detertmine the median lethal concentrations (LC_{50} and LC_{90}) to kill 50 and 90 per cent of the treated larvae of the respective species.**

***Results*: All the five species registered 100 per cent mortality in larval bioassays at 1000 ppm with aqueous extract and at 100 ppm with hexane extract of dried fruit. In bioassays with aqueous extract *An. culicifacies* species A registered the lowest LC_{50} of 208.5 ppm (range-208.5-359 ppm for different mosquito species) while with hexane extract, *An. stephensi* registered the lowest LC_{50} of 6.25 ppm (6.25-17.63 ppm for different mosquito species). The LC_{50} of aqueous extract was 13-39 fold higher than the values of hexane extract of dried fruit for different species. The calculated LC_{90} for hexane extract of dried fruit for different species was in the range of 43.38-95.28 ppm.**

***Interpretation & conclusion*: Hexane extract showed good mosquito larvicidal efficacy than that of the aqueous extract. The calculated LC_{90} for the extract for different species was below 100 ppm and could be effective for comprehensive control of disease vectors.**

Key words Dried fruit extract - larvicide - mosquito - *Solanum nigrum*

Compounds of plant origin such as rotenone, nicotine, anabasine, methyl anabasine and lupinine were found effective in killing *Culex territans* (Diptera: Culicidae)[1]. Most effective compound of plant origin for the control of adult mosquitoes is pyrethrum extract (mixture of esters of pyrethrins and cinnerins) obtained from the flowers of *Chrysanthemum cinneraefolium* (Family: *Asteraceae*). This extract was first used successfully in vector control operations in South Africa[2,3] and later in India[4-6]. In India pyrethrum extract

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is still used for liquidation of epidemic foci in antimalaria programme[7]. Due to environmental concern on use of synthetic insecticides for vector control and due to existing and further risk of development of widespread insecticide resistance in disease vectors, interest on possible use of environment friendly natural products such as extracts of plant/plant parts increased for vector control. Sukumar *et al*[8] listed 346 species from 276 genera and 99 families which have been tested against mosquitoes for various effects such as toxicity, growth inhibition, ovipositional deterrency and repellency. This list included 5 species from family *Solanaceae* namely *Capsicum frutescens*, *Datura candida, D. stramonium, Lycopersicon lycopersicum*, and *Nicotiana rustica*. Recently larvicidal properties of the aqueous extract of the leaf of *Solanum nigrum* against *Anopheles culicifacies* species A, *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linn.) and larvicidal properties of fruit and root extract of *Solanum xanthocarpum* against *An. stephensi*, *Ae. aegypti* (Linn.) and *Cx. quinquefasciatus* (Say) were reported[9,10]. This study was carried out to evaluate the larvicidal efficacy of the aqueous and hexane extracts of the dried fruit of *S. nigrum* against five important mosquito species, namely *An. culicifacies* species A and *An. culicifacies* C and *An. stephensi* (malaria vectors), *Cx. quinquefasciatus* (filariasis vector) and *Ae. aegypti* (dengue vector).

Material & Methods

Mosquito strains: The study was conducted in the National Institute of Malaria Research, New Delhi. Laboratory colonized mosquito strains namely *An. culicifacies* species A, *An. culicifacies* species C, *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* were used for the studies.

Preparation of extract: Ripe fruits were collected from the wild *S. nigrum* plants from villages in district Agra (Uttar Pradesh) and Delhi State. Fruits were dried in shade and ground to fine powder in an electric grinder. Aqueous extract was prepared by mixing 2 g of dried fruit powder with 1000 ml of water (boiled and cooled tap water) with constant stirring on a magnetic stirrer[11]. The suspension of dried fruit powder in water was left for 2 h, filterd through Whatman No.1 filter paper (M/s Glassil Scientific Industries, Delhi) and the filtrate was stored in amber coloured air tight bottle at room temperature till use. Hexane extract of the seeds was made essentially following the method of Mehra and Hardhar[12], 25 g of the dried fruit powder was mixed with n-Hexane (SRL, India) (10% w/v) in the bottle and left overnight at room temperature. The mixture was stirred for 1 h on magnetic stirrer and filtered through muslin cloth. The residue was remixed with n-hexane (10% w/v) and the above procedure of extraction was repeated. The filtrate was allowed to dry at room temperature for 2-3 days in a beaker. The resultant gummy extract was scratched from the bottom of the beaker and was stored in amber coloured air tight glass bottle at room temperature till use.

Bioassays: Larval bioassays were performed essentially following the standard WHO method[13] in a laboratory maintained at $27^{o} \pm 2^{o}$C. Replicates of 25 late III and early IV instar larvae of different mosquito strains were used for bioassays. Six concentrations of aqueous extract, 62.5, 125, 250, 500, 1000 and 2000 ppm and nine concentrations of hexane extract in acetone, 0.781, 1.562, 3.125, 6.25, 12.5, 25, 50, 100 and 150 ppm were prepared in 250 ml boiled and cooled water. Atleast two control replicates were run simultaneously which included water controls for tests with aqueous extract and acetone controls (1 ml in 249 ml water) for tests with hexane extract in acetone. The number of dead and alive larvae in the replicates was recorded after 24 h and the results were expressed as per cent mortality. Observed mortality in control replicates, if in the range of 5-20 per cent, were corrected with mortalities in test replicates using Abbot's formulae[13]. The dose-mortality response of the respective extracts with different species was subjected to log-probit regression analysis[14] to determine lethal concentrations that kill 50 per cent of the treated larvae (LC_{50}) and 90 per cent of the treated larvae (LC_{90}).

Results & Discussion

All the species registered 100 per cent mortality in bioassays with aqueous extract at 1000 ppm except *Ae. aegypti* (96%). The LC_{50} of *An. culicifacies* species A was the lowest while that of *Ae. aegypti* was highest in the order, *An. culicifacies* species A (208.5 ppm) >*An. stephensi* (242.5 ppm) >*An. culicifacies* species C (251.7 ppm) >*Cx. quinquefasciatus* (337.2 ppm) >*Ae. aegypti* (359 ppm) (Table I). Hexane extract was relatively more effective. With hexane extract, these species registered 100 per cent mortality at 100 ppm except *Ae. aegypti* that showed 100 per cent mortality at 150 ppm. The LC_{50} for different species was in the range of 6.25 to 17.63 ppm in the order *An. stephensi* (6.25 ppm) >*An. culicifacies* species C (9.04 ppm) >*Cx. quinquefasciatus* (12.25 ppm) >*An. culicifacies* species A (15.93 ppm) >*Ae. aegypti* (17.63 ppm) (Table

Table I. Per cent mortality of different mosquito larvae against aqueous extract of dried fruit of *Solanum nigrum* (Linn.)

Mosquito species (R)	Concentration (parts per million)								*P* (df) χ^2	Comparative toxicity* (fold)
	62.5	125	250	500	1000	2000	LC^a_{50} (95% FL)	LC^b_{90} (95% FL)		
An. culicifacies species A (2)	8	32	60	80	100	---	208.5 (169-236)	700.7 (473-827)	> 0.05 (3)	---
An. culicifacies species C (12)	1.6	2.6	28.3	76.6	100	100	251.7 (184-345)	480.5 (343-982)	> 0.05 (4)	1.20
An. stephensi (12)	3.6	8.3	51.3	90.6	100	100	242.5 (193-299)	521.3 (387-780)	> 0.05 (4)	1.16
Cx. quinquefasciatus (12)	2.3	5.3	45	94.6	100	100	337.2 (230-468)	720.5 (459-1052)	> 0.05 (4)	1.61
Ae. aegypti (2)	4	6	24	70	96	100	359.0 (244-496)	931.0 (574-1064)	< 0.05 (4)	1.72

(R), No. of replicates @ 25 larvae/replicate at each concentration; $^aLC_{50}$, concentration for killing 50 per cent of the treated larvae; $^bLC_{90}$, concentration for killing 90 per cent of the treated larvae; FL, Fiducial limit; *Comparative toxicity of species with reference to LC_{50} of *An. culicifacies* species A

Table II. Per cent mortality of mosquito larvae against hexane extract of dried fruit of *Solanum nigrum*

Mosquito species (R)	Concentration (parts per million)											*P* (df) χ^2	Comparative toxicity#* (fold)
	0.781	1.562	3.125	6.25	12.50	25	50	100	150	LC^a_{50} (95% FL)	LC^b_{90} (95% FL)		
An. culicifacies species A (4)	5	10	13	20	26	58	78	100	100	15.93 (10-26)	95.28 (51-281)	<0.05 (7)	2.54
An. culicifacies species C (2)	6	10	20	36	60	70	90	100	100	9.04 (7-11)	48.92 (36-70)	<0.05 (7)	1.44
An. stephensi (4)	10	22	32	50	58	78	92	100	100	6.25 (5-7)	43.38 (34-56)	<0.05 (7)	---
Cx. quinquefasciatus (4)	2	6	11	30	50	64	86	100	100	12.25 (11-14)	56.24 (46-71)	>0.05 (7)	1.95
Ae. aegypti (2)	---	---	---	---	40	62	80	96	100	17.63 (13-22)	65.22 (51-94)	<0.05 (7)	2.81

(R), No. of replicates @ 25 larvae/replicate at each concentration; $^aLC_{50}$, concentration for killing 50 per cent of the treated larvae; $^bLC_{90}$, concentration for killing 90 per cent of the treated larvae; FL, Fiducial limit; *Comparative toxicity of species with reference to LC_{50} of *An. stephensi* species A

II). No mortalities were recorded in respective control replicates.

Present study indicated variations in larvicidal efficacy of the extracts in different mosquito species. Minjas and Sarda[11] reported variations in toxicological efficacy with three mosquito species to the crude aqueous extract of fruit pods of *Swartzia madagascariensis* to which *Cx. quinquefasciatus* was completely susceptible while *An. gambiae* was relatively more susceptible to the extract than *Ae. aegypti*[11]. Similar observations were made by Sujatha *et al*[15] with petroleum ether extract of six plants *Acorus calamus*, *Ageratum conyzoides*, *Annona squamosa*, *Bambusa arundanasia*, *Madhuca longifolia* and *Citrus medica* against three species of mosquitoes, *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*. Pathak *et al*[16] also reported variations in larvicidal efficacy of essential oil extracts from four plants *Tagetes erecta*, *Ocimum sanctum*, *Mentha piperita* and *Murraya koenigii* against three species of mosquitoes, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Thomas *et al*[17] found variations in three species, *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, of which the former two species were found equitoxic to the crude extract of *Yucca aloifolia*.

In the present study the larvicidal efficacy of hexane extract of dried fruits of *S. nigrum* was found to be higher than the aqueous extract. The LC_{50} values indicated 13-39 fold (LC_{50} aqueous extract of seed/ LC_{50} hexane extract of seed) enhanced toxicity of hexane extract compared to aqueous extract. It was respectively 13 fold against *An. culicifacies* species A, 20 fold against *Ae. aegypti*, 28 fold against *An.*

culicifacies species C and *Cx. quinquefasciatus* and 39 fold against *An. stephensi*. Hexane extract was found comprehensively effective against five mosquito species of three genera and the observed LC_{50} value was <20 ppm and LC_{90} values <100 ppm. In conclusion, our findings showed that the hexane extract of the dried fruit of *S. nigrum* was effective for larval control of the species tested. The feasilility of its use in field, however, needs extensive field trials.

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