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# Larvicidal properties of cashew nut shell liquid (*Anacardium occidentale* L) on immature stages of two mosquito species

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Mosquito-borne diseases like dengue, malaria, lymphatic filariasis, Japanese encephalitis are major public health problems in India. Effective control of vector borne diseases is possible by early diagnosis and prompt treatment on one hand and effective vector control following integrated approach on the other hand. Intervention measures to restrict the transmission of mosquito-borne diseases by controlling vector population are the main part of vector control. Vector control approach in India, is mostly based on use of natural and synthetic molecules, which have potential to kill the target insect either adult or in aquatic stages. Larvicides of plant origin are currently receiving considerable attention because of their relatively harmless biodegradable properties. Since 1920s more than 2000 plants have been tested for insecticidal properties[1]. Recently, lot of research work is being carried out in India to search for an alternative eco-friendly effective larvicides from botanical origin[2–4].

*Anacardium occidentale* L (*Anacardiaceae)*, a fruit tree grown widely in tropical and sub-tropical areas is cultivated in Africa, south America, India, especially in costal districts of Andhra Pradesh, Orissa and West Bengal for its cashew nuts (Kernel). Recently, Laurens *et al*[5] in France, and Farias *et al*[6] in Brazil noted insecticidal action of cashew nut shell liquid (CNSL) on larvae of *Aedes aegypti* (Diptera: Culicidae) which is safe to mammals. The cashew tree is a native of Brazil and lower Amazons. The cashew has been introduced and is a valuable cash crop in Americas, West Indies, Madagascar and India. Frankel[7] and Tyman & Morris[8] in 1989 described the composition of CNSL which found between the seed coat (pericarp) and the nuts. It is not a triglyceride and contains a high portion of phenolic compound. It is also known as Cardanol or Card phenol. It is a monohydroxyl phenol having a long hydrocarbon chain (C15H27) in a metaposition. As per chemical news (*www.cardanol.net*) cardanol, a oil soluble in resin used as surface coating, paints, lamination, rubber industries and as pesticides. Hence, a study was undertaken to study the larvicidal properties of the extract from CNSL on different species of mosquito larvae in laboratory and field conditions in two selected areas of Andhra Pradesh, India between February and July 2010.

*Preparation of CNSL stock solution:* (i) CNSL : 20% weight; (ii) vegetable soap (50% aqueous solution): 40% by wt; and (iii) alcohol solvent : 40% by wt. The solution was prepared and supplied by courtesy "D" Vaiz Chemicals, Kolkata, India free of cost.

*Preparation of solution for spray:* Three types of dilutions were prepared initially by mixing stock solution with portable water for spraying in breeding places with Knapsack sprayers: (i) 100 ml of stock solution dissolving in 8 L of water (12.5 g a.i. of CSNL); (ii) 100 ml of stock solution dissolved in 5 L of water (20 g a.i. of CNSL); and (iii) 200 ml of stock solution dissolve in 5 L of water (40 g a.i. of CNSL).

Two areas, i.e. part of Rajahmundry town (Corporation area), predominant for breeding of *Ae. aegypti*, and Dwaleswaram (PHC Dwaleswaram) Panchayat

area, within 8 to 10 km from Rajahmundry Municipal Corporation area predominant for breeding of *Anopheles subpictus* (Diptera: Culicidae) were selected for the study.

In Rajahmundry town, out of 68 water holding containers in 88 houses searched for breeding of *Ae. aegypti* mosquitoes, 27 containers in 23 houses were found positive for breeding. House and container indices were noted as 26.1 and 39.7% respectively. Of 27 containers which were positive for *Ae. aegypti* larvae, 22 positive containers containing approximately 1038±5 L of water were selected. Samples of the larvae collected were brought to the laboratory for identification and rearing. Similarly, five breeding sites with approximately 1038±8 L of water volume positive for breeding of *An. subpictus* were selected in Dwaleswaram panchyat area. Samples of mosquito larvae were collected from the breeding places to the laboratory for identification and rearing. Health officials of the study area were requested not to undertake any antilarval activities in the selected areas 15 days before and during the period of study. Special attention was given to note the effect of 38 ppm CNSL on fishes (*Gambusia affinis* and *Poecilia reticulata*), tadpoles (*Bufo* spp), and water bugs (*Heteroptera* spp) both in the field and laboratory conditions. No other species of mosquito larvae were included in the study because of technical reasons.

In laboratory, 25 III or early IV instar laboratory reared larvae of *Ae. aegypti* and *An. subpictus* were exposed to water containing12, 19 and 38 ppm CNSL solution separately. The test was conducted following standard WHO procedure[9]. Mortality rate of each species against each concentration was noted separately after 24 h of exposure. Temperature was maintained as 24±2°C .

In the field conditions, in selected breeding places of Rajahmundry and Dwaleswaram town, four observations were made for each mosquito species. Three concentrations of CNSL were sprayed in marked breeding sites/containers for each species. Eight litres of CNSL solution containing 12.5 g a.i. was sprayed in breeding sites with ~1038 L capacity (12 ppm of CNSL). Simultaneously, 5 L each containing 20 and 40 g of CNSL were sprayed in breeding sites of ~1038 L capacity (19 and 38 ppm of CNSL). For each species of mosquito larvae mixture of vegetable soap and alcohol solvent without CNSL were sprayed in fourth breeding sites as control.

Three concentrations, i.e. 12, 19 and 38 ppm were sprayed in different breeding containers (six containers with 1038 L water capacity for each concentration and four containers with same water collection as control) of *Ae. aegypti* in Rajahmundry town. Four breeding sites (three for experiments and one as control) of *An. subpictus* in Dwaleswaram town.

The larval density was noted as per standard entomological technique, i.e. number of larvae/5 dips on Day 0, just before spray of CNSL fluid. Thereafter, larval density was noted 24 h (one day) after CNSL spray, 5 and 10 days after treatment of CNSL solution in the field.

In laboratory condition, 200 larvae of *Ae. aegypti* and *An. subpictus* were exposed to each of the three concentrations of CNSL separately. Out of 200 larvae of *Ae. aegypti* exposed to 12 ppm CNSL, 197 (98.5%) died in 24 h. Same percent mortality of the same species were noted in 24 h under laboratory condition, when exposed to 19 ppm which corroborates the findings of Laurens *et al*[5]. Out of 200 larvae of *An. subpictus* larvae exposed to 12 ppm CNSL, 102 (51%) died in 24 h of exposure where 90.5 and same percent mortality was noted when *An. subpictus* larvae exposed to 19 and 38 ppm CNSL respectively. $LC_{50}$ and $LC_{90}$ of *An. subpictus* was noted as 12 and 19 ppm respectively. No death was noted in 24 h when 36 *Gambusia affinis,* 21 Tadpoles (*Bufo* spp) and 18 water bugs (*Heteroptera* spp) were exposed to 38 ppm CNSL solution. The present findings thus corroborates the findings of Laurens *et al*[5], Farias *et al*[6] and findings of School of Tropical Medicine, Kolkata (Personal communication—Unpublished data).

Effect of different concentrations of CNSL/ Cardanol/ Card phenol solution on aquatic stages of *Ae. aegypti* and *An. subpictus* in the field conditions is shown in Tables 1 and 2 respectively. An average of 18 larvae of I–II stages, 20 of III–IV stages and eight pupae were noted per five dips, i.e. zero day before CNSL spray @ 12 ppm. A larval density of 7 and 6 per 5 dips of I–II stages larvae respectively were noted after 24 h and on Day 5 of CNSL spray showing 61.1 and 66.7% reduction respectively. No III–IV stages larvae were noted till Day 5 of spray showing same percent death of *Ae. aegypti* larvae in 12 ppm concentration of CNSL. In all, 37.5% pupal death was noted after 24 h and on Day 5 also of CNSL spray @12 ppm. Similarly, all the four stages of larvae and pupae of *Ae. aegypti* were found highly susceptible to 19 ppm CNSL. Increase in larval and pupal density however was noted on Day 10 of spray.

Laurens *et al*[5] in 1997 in France demonstrated antilarval properties of CNSL and calculated $LC_{50}$ in *Ae. aegypti* larvae as 12.6 ppm. Farias *et al*[6] in 2009 made a detail observation on CNSL toxicity on immature stages of *Ae. aegypti* in Brazil and found highly effective on eggs, larvae and pupae, without any mammalian toxicity. Our present observation thus corroborates with the findings of Laurens (France) and Farias (Brazil).

**Table 1. Effect of CNSL on immature stages of *Ae. aegypti* (L)**

| Concentration in ppm | Stages of larvae | Day 0 | Day 1 | Day 5 | Day 10 |
|---|---|---|---|---|---|
| 12 | I–II | 18 | 7 (61.1) | 6 (66.7) | 10 (44.4) |
| | III–IV | 20 | 0 (100) | 0 (100) | 4 (80) |
| | Pupae | 8 | 5 (37.5) | 0 (100) | 0 (100) |
| 19 | I–II | 20 | 0 (100) | 0 (100) | 8 (60) |
| | III–IV | 18 | 0 (100) | 0 (100) | 2 (88.9) |
| | Pupae | 6 | 1 (83.3) | 0 (100) | 1 (83.3) |
| Control | I–II | 16 | 14 (12.5) | 18 (–12.5) | 16 (0) |
| | III–IV | 18 | 20 (–11.1) | 25 (–38.9) | 18 (0) |
| | Pupae | 8 | 12 (–50) | 10 (–25) | 10 (–25) |

Figures in parentheses are percentage reduction over Day 0 densities.

**Table 2. Effect of CNSL on immature stages of *An. subpictus***

| Concentration in ppm | Stages of larvae | Day 0 | Day 1 | Day 5 | Day 10 |
|---|---|---|---|---|---|
| 12 | I–II | 40 | 32 (20) | 31 (22.5) | 46 (– 15) |
| | III–IV | 56 | 41 (26.8) | 42 (25) | 38 (32.1) |
| | Pupae | 10 | 6 (40) | 6 (40) | 8 (20) |
| 19 | I–II | 50 | 42 (16) | 38 (24) | 40 (20) |
| | III–IV | 60 | 42 (30) | 32 (46.7) | 30 (50) |
| | Pupae | 8 | 5 (37.5) | 4 (50) | 2 (75) |
| 38 | I–II | 26 | 10 (61.5) | 4 (84.6) | 1 (96.2) |
| | III–IV | 34 | 7 (79.4) | 1 (97.1) | 4 (88.2) |
| | Pupae | 18 | 5 (72.2) | 1 (94.4) | 8 (55.6) |
| Control | I–II | 36 | 38 (– 5.6) | 42 (–16.7) | 42 (–16.7) |
| | III–IV | 40 | 48 (– 20) | 54 (– 35) | 40 (0) |
| | Pupae | 4 | 10 (–150) | 16 (–300) | 18 (– 350) |

Figures in parentheses are percentage reduction over Day 0 densities.

No published data are available on effect of CNSL on immature stages of anopheline mosquitoes. Effect of CNSL on *An. subpictus* larvae is shown in Table 2. As per Table 2, not much significant of effect of CNSL on immature stages in 12 ppm concentration were noted. death rates of 24, 46.7 and 50% of I–II, III–IV instars larvae and pupae respectively were noted on Day 5 of exposure to 19 ppm CNSL. In 38 ppm CNSL, death rate of 84.6% I–II instars, 97.1% III–IV stages of *An. subpictus* larvae was noted on Day 5 of spray and 50% pupal deaths were also noted on Day 5. On immature stages of *An. subpictus* till Day 5 of exposure 38 ppm CNSL was found highly effective. No decreasing effect on breeding pattern was noted in breeding places which was selected as control for both mosquito species. Mode of action of CNSL on immature stages of mosquitoes are not yet known. During the study period no dead fishes, tadpoles, water bugs etc. were noted till Day 10 of spray in study places in the field which corroborates with our laboratory based findings.

Our present observation reveals that *Ae. aegypti* larvae and pupae are highly susceptible to 12 ppm CNSL. *Anopheles subpictus* larvae and pupae, however, were found susceptible to 38 ppm of Cardanol/ CNSL solution in laboratory and in field condition in parts of Andhra Pradesh. Therefore, based on the above preliminary baseline observations, a well-designed laboratory and field based study may be conducted in different eco-systems to note the larvicidal effect of CNSL on different vectors of malaria , dengue and filariasis, as CNSL is cheap, easily available, eco-friendly, non-toxic and biodegradable.

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