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Exploration of larvicidal and adult emergence inhibition activities of *Ricinus communis* seed extract against three potential mosquito vectors in Kolkata, India

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ARTICLE INFO

ABSTRACT

Objective: To determine the larvicidal and adult emergence inhibition activities of castor (*Ricinus communis*) seed extract against three potential mosquito vectors *Anopheles stephensi (An. stephensi)*, *Culex quinquefasciatus (Cx. quinquefasciatus)* and *Aedes albopictus (Ae. albopictus)* in India. **Methods:** The *R. communis* seed extract was tested, employing WHO procedure, against fourth larval instars of the three mosquito species for 24 h and larval mortalities were recorded at various concentrations (2–64 μ g/mL); the 24 h LC_{50} values of the *R. communis* seed extract were determined following Probit analysis. The larval killing, antipupation and adult emergence inhibition rates of the test extract, using a single concentration of 2 μ LC_{50}, were studied at different time periods (24–72 h); the extract toxicity was tested against a fish, *Oreochromis niloticus (O. niloticus)*. **Results:** The *R. communis* seed extract exhibited larvicidal effects with 100 % killing activities at concentrations 32–64 μ g/mL, and with LC_{50} values 7.10, 11.64 and 16.84 μ g/mL for *Cx. quinquefasciatus*, *An. stephensi* and *Ae. albopictus* larvae, respectively. When the larvae were treated with the extract at a single concentration of $2 \times LC_{50}$, significant differences were observed, compared to control groups, in rate of pupation (*P*<0.001) as well as in adult formation (*P*<0.001). **Conclusions:** The present findings suggest that the *R. communis* seed extract provided an excellent potential for controlling *An. stephensi*, *Cx. quinquefasciatus* and *Ae. albopictus* mosquito vectors.

1. Introduction

The most prevalent tropical vector−borne communicable diseases in our part of the globe include malaria, filariasis and dengue haemorrhagic fever, and these are vectored by the mosquito species belonging to the genus *Anopheles*, *Culex* and *Aedes*, respectively. One of the methods to control these diseases is to control the vectors in order to bring interruption in disease transmission, and the control of mosquitoes in larval stage has been the efficient way in integrated vector management[1].

Application of chemical insecticides although highly effective against the target species, vector control is facing a threat due to the development of resistance to such agents resulting in rebounding vectorial capacity[2, 3]. Furthermore,

these synthetic chemicals are responsible for substantial hazards to a variety of non−target organisms and the environment[4]. Hence, more attention has been focused on botanicals, which are ecofriendly and cost effective, and found one of the possible alternatives to synthetic insecticides[5–7]. Many studies on plant extracts against mosquito vectors have been conducted around the world, and their larvicidal, pupicidal, adult emergence inhibition and repellent activities have been reported[5, 8–10].

Elimam *et al* [11] reported larvicidal, adult emergence inhibition and oviposition deterrent effects of *Ricinus communis (R. communis)* leaf aqueous extract against *Anopheles arabiensis (An. arabiensis)* and *Culex quinquefasciatus (Cx. quinquefasciatus)*. The strong toxic activity of *R. communis* leaf aqueous extract against larvae of four mosquito species *Culex pipiens (Cx. pipiens)*, *Aedes caspius (Ae. caspius)*, *Culiseta longiareolata(Cu. longiareolata)* and *Anopheles maculipennis (An. maculipennis)* has been reported [12]. But no report has been documented on larvicidal activity of *R. communis* seed extract against any of the mosquito vectors. The present

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study assesses the killing efficacy of castor *(R. communis)* seed acetone extract against fourth instar larvae of *An. stephensi* (malaria vector), *Cx. quinquefasciatus* (filariasis vector) and *Ae. albopictus* (dengue vector), with respect to antipupation and adult emergence inhibition activities.

2. Materials and methods

2.1. Plant material

The ripe dried fruits from the wild castor plant *(R. communis)* were collected from Palta (suburb Kolkata) of North Chabbispaganas district, West Bengal state (India). The seeds were taken out and shade dried, and ground to fine powder.

2.2. Preparation of the extract

The acetone extract of the dried seed powder was prepared following the method of ethanolic extract preparation of neem *(Azadirachta indica)* seed as described earlier[13], with slight modification. Briefly, 50 g of the powder was mixed with 50 mL acetone (SRL, India) in 200 mL capacity bottle and left for 48 h at room temperature. The mixture was stirred manually at 4 h intervals, and filtered through cheese cloth, and then through Whatman No.1 filter paper. The filtrate was allowed to dry at room temperature for 48–72 h in a beaker, and the resultant extract was scratched from the inner surface of the beaker and was stored in air tight glass bottle covered with brown paper at room temperature till use. Finally, the 50% ethanol (in water) was used to obtain a solution of 10 mg/mL of the extract for use in experimental purposes.

2.3. Collection of mosquito larvae

The fourth instar larvae of *An. stephensi, Cx. quinquefasciatus* and *Ae. albopictus* were collected from plastic containers and burnt clay pots of 3 L capacity (filled with pond water that allows the wild strains of female mosquitoes lay eggs) placed in areas with vegetation providing shades for the adults resting positions and breeding activities, near a house at Naihati (suburb Kolkata), India. The larval characters and the adult features were considered for mosquito identification.

2.4. Larval bioassay

Larval bioassays were determined following the WHO protocol[14] at (25±2) ℃ laboratory temperature. Replicates of 25 fourth instar larvae of different mosquitoes were used for bioassays. Six concentrations of the extract, 2, 4, 8, 16, 32 and 64 μ g/mL, were prepared in 100 mL filtered pond water, in separate plastic containers. The control replicate was run simultaneously that included 1 000 μ L of 50 % ethanol mixed in 99 mL of filtered pond water. The percent mortality of larvae for all concentrations was recorded at 24 h. The dose mortality response of the extract with different mosquito species was subjected to log–probit regression analysis[15] in order to determine lethal concentrations that kill 50% of the treated larvae (LC_{50}). The larvae killing, antipupation and adult emergence inhibition rates of *R. communis* seed extract, with a single concentration 2×LC_{50}, were studied at different time periods (24–72 h), following the criteria mentioned above. The positive test control contained 0.2 μ g/mL of 95% dimethoate.

2.5. Toxicity test of the plant extract

To determine the susceptibility of a non–target organism, *Oreochromis niloticus (O. niloticus)*, to the plant extract, the protocol published earlier[16] was followed, with slight modification. Tests on *O. niloticus* were performed as static tests (without renewal of water, for 48 h, 10 individuals in 10 L of water in 25 L capacity glass aquaria). The concentrations of the extract, 16.84, 33.68 and 67.36 μ g/mL) used in the experiment, respectively were the LC_{50}, $2 \times LC_{50}$ and $4 \times LC_{50}$ values to *Ae. albopictus*. The positive test control was with 0.2 μ g/mL of 95% dimethoate under the similar condition as mentioned.

2.6. Statistical analysis

The χ^2 test was used to compare the larval death occurred between the lowest (2 or 4 μ g/mL) and the highest (32 or 64 μ g/mL) concentrations, and the rate of development (pupation and adult emergence) between the control and the treated (with $2 \times LC_{50}$ of the extract) groups. A *P*– value of < 0.05 was considered as significant.

3. Results

The larvicidal activities of different concentrations of *R. communis* seed extract against the test mosquito species are represented in Table 1. The *R. communis* seed extract started to show larvicidal activity at the concentration of 2 μ g/mL, against *An. stephensi* and *Cx. quinquefasciatus*, at 4 μ g/mL against *Ae. albopictus* with 8% larval mortality. The *An. stephensi, Cx. quinquefasciatus* and *Ae. albopictus* mosquito larvae registered 100% mortality with the extract; *Cx. quinquefasciatus* at 32 μ g/mL, and the other two at 64 μ g/mL. One hundred percent killing of the different mosquito larvae occurred with 0.2 μ g/mL dimethoate in between 8 and 15 h of exposure (data not shown).

The LC_{50} values of *R. communis* for the three mosquito species *An. stephensi, Cx. quinquefasciatus* and *Ae. albopictus*, and the regression equations are represented in Figure 1. The LC_{50} for *Cx. quinquefasciatus* was lowest while that for *Ae. albopictus* was highest, in the order *Cx. quinquefasciatus* (7.10 μ g/mL) > *An. stephensi* (11.64 μ g/mL) > *Ae. albopictus* (16.84 μ g/mL), as determined by probit analysis.

The larvae killing, antipupation and adult emergence inhibition rates for *An. stephensi, Cx. quinquefasciatus* and *Ae. albopictus*, due to the exposure of *R. communis* seed extract (at $2 \times LC_{50}$), are represented in Figure 2. The 20, 16 and 24% larvae were pupated for *An. stephensi, Cx. quinquefasciatus* and *Ae. albopictus*, respectively, in 24–48 h,

Table 1

Percent mortality of *Ae. albopictus*, *Cx. quinquefasciatus* and *An. stephensi* larvae exposed for 24 hours to different concentrations of *R. communis* seed extract.

Mosquito	Number of larval mortality at various concentration (μ g/mL) [n(%)]					
	2	4	8	16	32	64
An. stephensi	2 (8)	3 (12)	7 (28)	12 (48)	19 (76)	25 (100)
Cx. quinquefasciatus	3 (12)	5 (20)	11 (44)	19 (76)	25 (100)	25 (100)
Ae. albopictus	0	2 (8)	5 (20)	11 (44)	16 (64)	25 (100)

and adult emergence was seen for 8, 4 and 8%, respectively, in 48–72 h. The *An. stephensi*, *Cx. quinquefasciatus* and *Ae. albopictus* larvae when remain untreated transformed into pupae for 84, 52 and 56% cases, respectively, in 24–48 h, and emerged into normal adults for 52% of *Cx. quinquefasciatus* and *Ae. albopictus*, and 76% of *An. stephensi*, in 48–72 h (Figure 2).
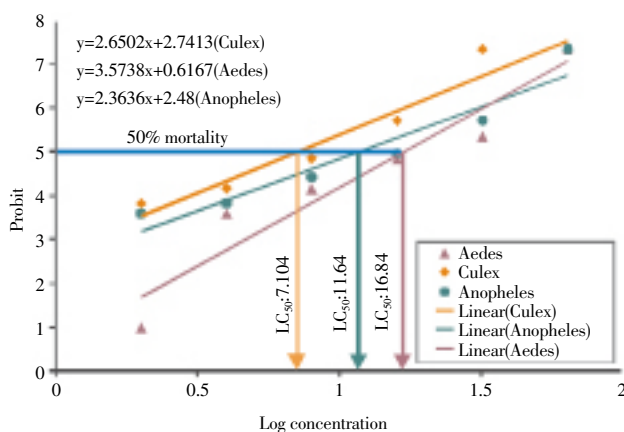

Figure 1. Probit regression lines for response of *Ae. albopictus*, *Cx. quinquefasciatus* and *An. stephensi* larvae to determine the LC$_{50}$ (μ g/mL) values of *R. communis* seed extract; regression equations for the three mosquito species are shown in the figure.
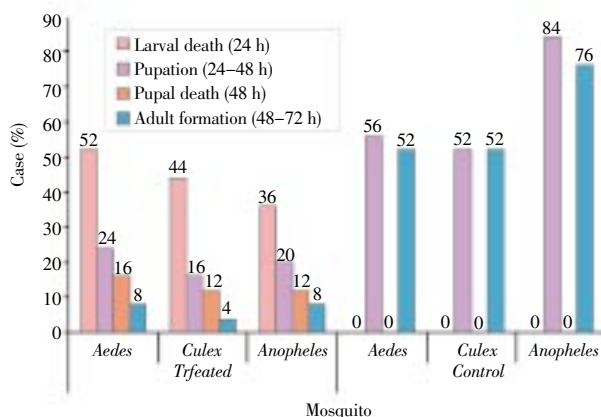

Figure 2. Percent larval mortality, pupation and adult formation of 4th instar larvae of *Ae. albopictus*, *Cx. quinquefasciatus* and *An. stephensi* following exposure to a single concentration (2× LC$_{50}$) of *R. communis* seed extract.

The *R. communis* seed extract was not active against the fish *O. niloticus*, even at the highest concentration used, 67.36 μ g/mL, the 4×LC$_{50}$ value to *Ae. albopictus*, with which the extract had lowest lethality. Dimethoate, a broad

specturm organophosphorous pesticide, killed all 10 (100%) fishes within 15 h of exposure.

4. Discussion

Currently, the environmental safety of an insecticide has been considered to be of paramount importance, and the botanical insecticides are found as suitable alternatives to synthetic insecticides, because these are generally pest specific and are relatively harmless to non–target organisms[9]. In the present study, among different mosquito species, variation in larvicidal activity of *R. communis* seed extract were recorded, and the findings, related to the larvicidal activities of various plant extracts reported by the earlier authors corroborate the present study results. Variations in the larvicidal action of essential oil from plants like *Tagetes erecta (T. erecta)* , *Ocimum sanctum(O. sanctum)* , *Mentha piperita(M. piperita)* and *Murraya koenigii (M. koenigii)* for *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were documented[17]. Similar results were also found for the larvicidal activity of *Yucca aloifolia (Y. aloifolia)* crude extract[18] and the toxic effect of *Swartzia madagascariensis (S. madagascariensis)* fruit pod extract[19]. The *R. communis* seed extract, in our study, showed dose–dependent larval mortality, and thus, the significant differences were noticed in between killing at the lowest (2 μ g/mL for *An. stephensi* and *Cx. quinquefasciatus*, 4 μ g/mL for *Ae. albopictus*) and the highest (32 μ g/mL for *Cx. quinquefasciatus*, and 64 μ g/mL for *Ae. albopictus* and *An. stephensi*) concentrations ($P<0.001$).

The larvicidal activity of steam distillated oil extract from the whole plant of *T. errecta*, against larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, with 100% mortality at < 100 ppm has been reported[17]. The *Azadirachta excelsa (A. excelsa)* , *Cleome glaucescens (C. glaucescens)* and *Quercus infectoria (O. infectoria)* extracts caused 100 % mortality of larvae at a concentration 200 μ g/mL after 72 h of treatment[20]. A 100% larval mortality with essential oils of *Ocimum gratissium (O. gratissium)*, *Cymbopogon citrus (C. citrus)*, and *Ageratum conyzoides (A. conyzoides)* was found for *Ae. aegypti* at concentrations of 120, 200 and 300 ppm, respectively[21]. The essential oil of *Ipomoea cairica (I. cairica)* produced 100% larval mortality against *Cx. tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* mosquitoes at concentrations of 100–170 ppm[18]. Similarly, it has been reported that *Anacardium occidentale (A. occidentale)*, *Mammea siamensis(M. siamensis)*, *Phyllanthus pulcher (P. pulcher)*, *Anethum graveolens (A. graveolens)* , *Kaempferia*

galangal (K. galangal) , Cinnamomum porrectum (C. porrectum), Costus speciosus (C. speciosus) , and *Acorus calamus(A. calamus)* possess remarkable larvicidal properties with 100% larval mortality at the concentration of 100 μ g/mL after an exposure of 48 h[6]. In the present communication, the *R. communis* extract caused 100% mortality against *Cx. quinquefasciatus* at 32 μ g/mL, and against *An. stephensi* and *Ae. albopictus* mosquitoes at 64 μ g/mL.

As has been reported earlier, the hexane extract of dried fruit of *Solanum nigrum (S. nigrum)* had LC_{50} values 6.25–17.63 ppm for different mosquito vectors[22]. The toxicological studies on the ethanol extract of *S. nigrum* leaves showed larvicidal activity against *Ae. caspius* and *Cx. pipiens* (LC_{50} 51.29 and 125.89 mg/L within 24 h, and 21.38 and 38.11 mg/L within 48 h, respectively)[23]. The 24 h LC_{50} of aqueous and ethanolic extracts of *Piper nigrum (P. nigrum)* against early fourth larval instars of *Cx. quinquefasciatus* were 63.82 and 29.11 mg/L, respectively, as has been reported earlier[24]. Promsiri *et al* [6] reported that the medicinal plants found promising with larvicidal activity having LC_{50} values of 4.1, 20.2 and 67.4 μ g/mL were *Mammea siamensis (M. siamensis), Anethum graveolens (A. graveolens)* and *Annona muricata (A. muricata)*, respectively. The *R. communis* leaf extract found effective with LC_{50} values of 600, 270, 200 and 1 090 mg/L, against *Cx. pipiens*, *Ae. caspius*, *Culiseta longiareolata (Cu. longiareolata)* and *An. maculipennis*, respectively[12]. The 24 h LC_{50} values of aqueous extracts from leaves of *R. communis* was recorded as 498.88 ppm against fourth instar larvae of *An. arabiensis*, 1 445.44 ppm against fourth larval instars of *Cx. quinquefasciatus* [11]. Herein, in terms of the LC_{50} values, the acetone *R. communis* seed extract had 1.64 and 2.37 folds increased toxicity to *Cx. quinquefasciatus* compared to its toxicity to *An. stephens* and *Ae. albopictus*, respectively, and there was significant difference in toxicity of the extract to *Cx. quinquefasciatus* and to *Ae. albopictus* (*P*<0.001), but no significant difference was found in toxicity of the extract between *Cx. quinquefasciatus* and *Ax. stephensi*. However, in terms of LC_{50} values, the larval susceptibility order of three mosquito species to the *R. communis* seed extract was observed to be *Cx. quinquefasciatus* (7.104 μ g/mL) > *An. stephensi* (11.64 μ g/mL) > *Ae. albopictus* (16.84 μ g/mL). As has been reported earlier, slightly different to the above findings, among the three mosquito species tested, *Cx. quinquefasciatus* was the most sensitive (LC_{50} = 138 ppm) followed by *Ae. aegypti* (LC_{50} = 208.9 ppm) and *An. stephensi* (LC_{50} = 223.9 ppm) to *Millingtonia hortensis (M. hortensis)* leaf extcact[25], and following the same pattern, the essential oil extracted from *Zanthoxylum armatum (Z. armatum)* seed exhibited LC_{50} values 49, 54 and 58 ppm for *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*, respectively.

In the present study, the live larval forms (after 24 h) on approaching 40 h exposure to the extract ($2 \times LC_{50}$) either died or transformed into pupae, which at 48 h exposure were killed mostly, and the remaining though transformed into adults through moulting did not get life. Similar observations were obtained with the extracts of other plants against different mosquito species in earlier studies. The alkaloids

from *Annona squamosa (A. squamosa)* were found to induce morphological abnormalities such as larval–pupal intermediate and half–emerged adults in *An. stephensi* [27]. Shalaby *et al* [28] using peel oils of lemon, grapefruit and naval orange against *Cx. pipiens* observed adults with paralyzed legs which were not able to survive. Varying degrees of morphogenetic abnormalities in immature and adult stages of *Cx. pipiens* have been recorded when larvae were treated with the *Azadirachta indica (A. indica)* extract[29]. Khater and Shalaby[10] reported 100% adult emergence inhibition of *Cx. pipiens* larvae following treatment with 125 ppm of fenugreek (*Trigonella foenum–grecum*) and earth almond (*Cyperus esculentus*) oils, and similar observation was recorded after exposure to 500 ppm of mustard (*Brassica compestris*) , olibanum (*Boswellia serrata*) and rocket (*Eruca sativa*) oil. In presence of the *R. communis* seed extract ($2 \times LC_{50}$), as recorded in the present communication, significant differences were found in killing between *Ae. albopictus* and *An. stephensi* larvae (*P*<0.01), in pupation between *Cx. quinquefasciatus* and *Ae. albopictus* (*P* <0.05), and in adult formation between *Cx. quinquefasciatus* and *Ae. albopictus*, and *A. stephensi* (*P* <0.05); also, the significant differences were observed in rate of pupation (*P* <0.001) and in adult formation (*P* <0.001), in between control and treated groups.

The *R. communis* seed extract, in the present communication, demonstrated no toxicity to fish, *O. niloticus*, and has been found to possess excellent larvicidal and adult emergence inhibition activity against *An. stephensi, Cx. quinquefasciatus* and *Ae. albopictus* mosquitoes; such activity may be due to the synergistic activity of the mixture of bioactive constituents present in the extract. A similar observation has been reported that the neoprocurcumenol, isolated from rhizomes of *Curcuma aromatica* exhibited less efficacy (LC_{50} = 13.69 ppm) than the parent petroleum ether extract (LC_{50} = 11.42 ppm)[30]. The flavonoids from *R. communis* leaves showed potential insecticidal, ovicidal and oviposition deterrent activities against *Callosobruchus chinensis (Coleoptera: Bruchidae)*[31]. Thus, further investigations are required in order to elucidate such activity against a wide range of mosquitoes vectoring diseases in different geographical areas, and also the active ingredients of the extract responsible for larvicidal and adult emergence inhibition activity in mosquitoes should be identified and utilized in preparing a commercial formulation to be used as mosquitocidal, alternative to the chemical agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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