

past, from areas where they have presently disappeared/degraded because of encroachment. Such areas are potential places where regeneration/restoration of mangroves can be taken up. Many areas of the Andaman and Nicobar Islands were devastated by the tsunami of December 2004; however, places having mangrove cover remained unscathed. Other than environmental benefits, the regeneration of mangroves in populated areas would have socio-economic implications and would also provide a buffer to check the wrath of tsunamis¹⁶.

Effect of sub-lethal concentrations of insect growth regulator, lufenuron on larval growth and development of *Aedes aegypti*

S. G. Salokhe^{1,*}, S. N. Mukherjee²,
S. G. Deshpande², V. P. Ghule¹ and J. R. Mathad¹

¹A.M.M., Hadapsar, Pune 411 028, India

²Entomology Laboratory, National Chemical Laboratory, Pune 411 008, India

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The effect of sub-lethal concentrations ($LC_{20} = 0.0002$ and 0.001 ppm, and $LC_{40} = 0.002$ and 0.02 ppm for II and IV instar larvae respectively) of a dispersible concentrate formulation of the insect growth regulator, lufenuron on larval growth and development of *Aedes aegypti* was studied. When II and IV instar larvae were subjected to the above-mentioned sub-lethal concentrations of lufenuron through the culture medium, there was a significant increase in the time taken for pupation (17.2 ± 0.74 and 11.4 ± 0.8 days for II and IV instar LC_{20} -treated larvae respectively, and 19 ± 0.89 and 14.6 ± 1.0 days for II and IV instar LC_{40} -treated larvae respectively). Also, there was increase in the time taken for adult emergence 3.8 ± 0.83 and 5.4 ± 0.83 days from pupation of LC_{40} -treated II and IV instar larvae respectively). There was $28.1 \pm 2.06\%$ and $43.59 \pm 0.87\%$ reduction in pupation in LC_{20} of lufenuron-treated II and IV instar *A. aegypti* larvae respectively. Also, with LC_{20} of lufenuron-treated II and IV instar larvae there was $43.54 \pm 5.12\%$ and $43.59 \pm 0.87\%$ reduction in adult emergence respectively. Further, it was observed that II instar larvae treated with LC_{20} of lufenuron developed into $25.8 \pm 2.08\%$ deformed adults. In LC_{40} -treated II instar larvae there was $33.72 \pm 2.38\%$ reduction in pupation and $63.44 \pm 4.76\%$ reduction in adult emergence. Also, it was observed that there was $54.84 \pm 3.9\%$ and $61.3 \pm 5.2\%$ reduction in pupation and adult emergence respectively, in IV instar larvae treated with LC_{40} of lufenuron. The reduction in pupation of the IV instar larvae treated with LC_{40} of lufenuron was due to failure of the larvae to undergo pupation. These studies are fundamental to the use of lufenuron in *A. aegypti* management.

Keywords: *Aedes aegypti*, larval growth and development, lufenuron, sub-lethal concentration.

AEDES AEGYPTI is the main vector of dengue, yellow fever and chikungunya viruses in many parts of the world affecting millions of people worldwide each year. The only effective vector intervention involves well-organized larval control measures^{1,2}. To control mosquitoes, organophosphate pesticides such as malathion, temephos and pyre-

*For correspondence. (e-mail: shailajasalokhe@yahoo.co.in)

throids such as deltamethrin and cypermethrin are commonly used. Mosquitoes have developed resistance to such pesticides^{3,4}. During the past two decades, considerable progress has been made in the development of natural and synthetic compounds that are capable of interfering with the growth and development process of the target mosquito species.

More recently, insecticides with novel mode of action have been introduced in pest-management programmes. These substances are classified as insect growth regulators (IGRs), which interfere with chitin production leading to moulting disturbances, this resulting in the death of the insects. They are quite selective in their modes of action and potentially act only on target species leading to various abnormalities that impair insect survival⁵. These compounds have lower toxicity against vertebrates compared to conventional insecticides and are safer to use^{6,7}. IGRs have a good potential as environmentally safe and economically important group of chemicals.

Lufenuron is one of the most newly introduced synthetic IGRs used for the control of lepidopteran and coleopteran larvae on cotton, maize and vegetables as well as citrus white fly and rust mites on citrus fruits⁸. It is the active ingredient in the veterinary flea control medication programme⁹. It is a benzoylphenylurea compound found to be nontoxic to mammals and other vertebrates at the doses required against insects. Attacking the ability to form chitin may make lufenuron an effective remedy against fungal infections, such as ringworm (a dermatophyte infection and not a worm). Lufenuron is also sold as a crop protection product (pesticide) by M/S Syngenta India Ltd, Pune and has approval in a number of countries for use on a variety of crops, including soybean and maize. The present study was undertaken to examine the growth-regulating activity of sub-lethal concentrations of lufenuron on *A. aegypti* larvae leading to morphogenetic aberrations in the successive developmental stages.

A stock culture of *A. aegypti* was maintained at $25 \pm 2^\circ\text{C}$ and $80 \pm 5\%$ relative humidity. Powdered dog biscuit and brewer's yeast (1 : 1) were provided as food to the larval population. Adult male mosquitoes were provided

with 5% sucrose solution. Adult females were provided with rabbit for blood meal. Each larval stage was maintained in separate containers for easy harvest of the required stages for bioassay.

Dose response of different larval instars was determined by preparing different concentrations of lufenuron in water and releasing II and IV instars of *A. aegypti* larvae into it. Mortality count was taken for 7 days. Five replicates, for each concentration, each replicate with 10 larvae of the stage were prepared. The sub-lethal doses used in the experiments (LC_{20} and LC_{40}) were arrived at by extrapolation from the regression equation obtained by regression analysis¹⁰.

The effect of sub-lethal concentrations (LC_{20} and LC_{40}) of lufenuron on biological parameters (survival and metamorphosis) of *A. aegypti* larvae was examined by releasing II and IV instar larvae, separately in the treated medium. Observations were made daily on mortality of the larvae. Once pupation had begun in any treatment, observations were made everyday for signs of adult emergence. Percentage pupation, time taken for pupation, per cent adult emergence and time taken for adult emergence were recorded. During this period larvae were provided with larval food. Five replicates of 10 larvae each were prepared. Regression analysis was performed to determine dose-dependent effects¹⁰.

LC_{50} of lufenuron through culture medium for II and IV instar larvae of *A. aegypti* was found to be 0.005 and 0.006 ppm respectively, by regression analysis (Figure 1a and b). The sub-lethal concentration of LC_{20} of lufenuron for II and IV instar larvae of *A. aegypti* through culture medium was extrapolated from the regression equation earlier and was found to be 0.0002 and 0.001 ppm respectively. Similarly, the sub-lethal concentration of LC_{40} of lufenuron for II and IV instar larvae of *A. aegypti* through culture medium was found to be 0.002 and 0.02 ppm respectively.

Lufenuron at sub-lethal concentrations (LC_{20} and LC_{40}) significantly affects growth and development of the *A. aegypti* larvae. It was observed that at the sub-lethal concentration LC_{20} of lufenuron, there was delay in pupation

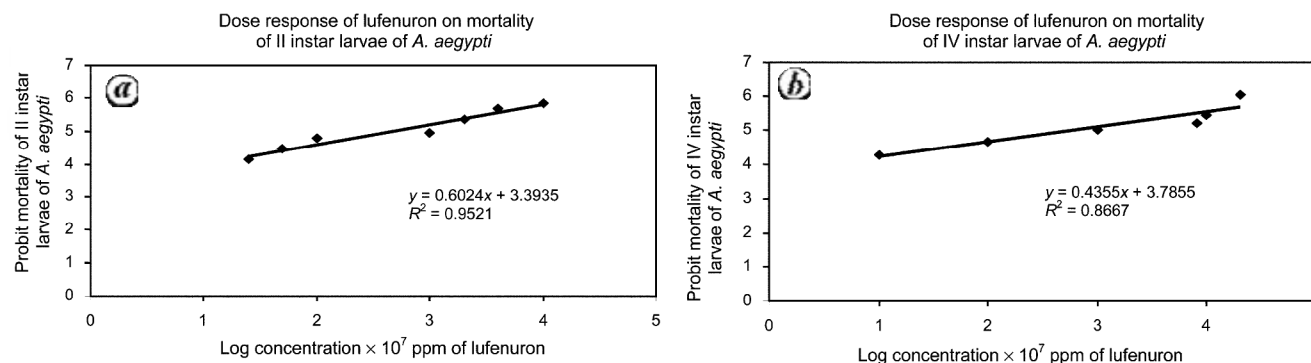


Figure 1. Probit analysis for lufenuron-treated II instar larvae (a) and (b) IV instar larvae of *Aedes aegypti*.

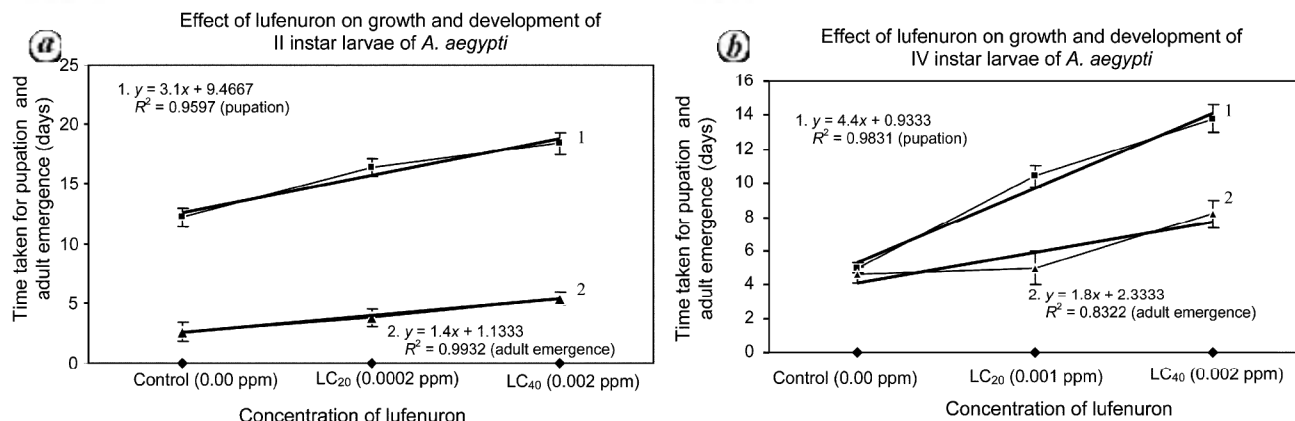


Figure 2. Regression graph of dose-response for lufenuron on growth and development of (a) II instar larvae and (b) IV instar larvae of *A. aegypti*. 1, Trendline for graph and its equation showing dose-response for lufenuron on time taken for pupation. 2, Trendline for graph and its equation showing dose-response for lufenuron on time taken for adult emergence.

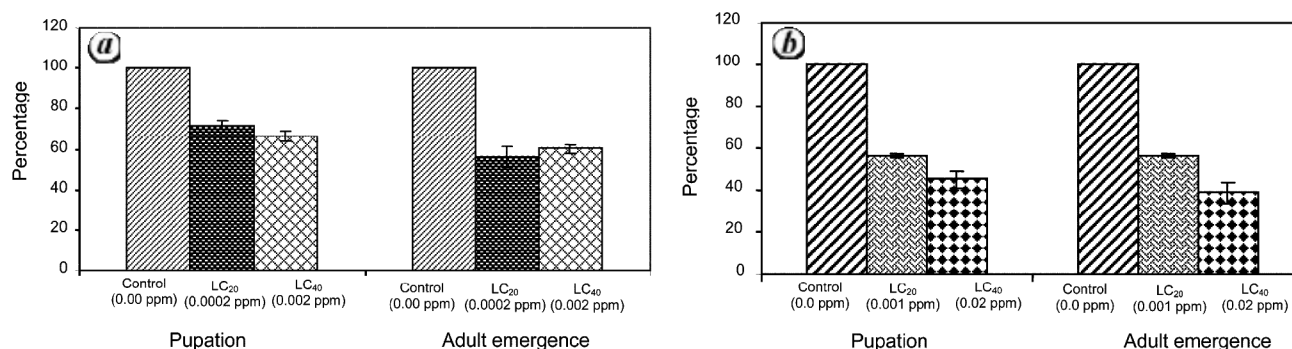


Figure 3. Effect of sub-lethal concentrations of lufenuron on % pupation and % adult emergence of (a) II instar larvae and (b) IV instar larvae of *A. aegypti*.

(17.2 ± 0.74 days) and adult emergence (4.8 ± 0.74 days) of the treated II instar larvae compared to that of control larvae (12.2 ± 0.74 days for pupation and 2.6 ± 0.8 days for adult emergence respectively; Figure 2a). Similarly, there was a delay in pupation (11.4 ± 0.8 days) and adult emergence (8.2 ± 0.97 days) in IV instar larvae treated with LC₂₀ of lufenuron (Figure 2b). There was $28.1 \pm 2.06\%$ and $43.59 \pm 0.87\%$ reduction in pupation in LC₂₀ of lufenuron-treated II and IV instar *A. aegypti* larvae respectively (Figure 3a and b). Also, there was $43.54 \pm 5\%$ and $43.59 \pm 4.7\%$ reduction in adult emergence respectively, in II and IV instar larvae treated with LC₂₀ of lufenuron (Figure 3a and b). Further, it was observed that $25.8 \pm 2.08\%$ of the adults emerging from LC₂₀-treated II instar larvae were abnormal. The deformities observed were reduction in wing size and development of five legs instead of six (Figure 4). Such adults failed to fly and remained on the water surface and did not survive for long.

At sub-lethal concentration LC₄₀ of lufenuron pupation of the treated II and IV instar larvae was prolonged to 19 ± 0.89 and 14.6 ± 1.0 days respectively, compared to

the control larvae which required 12.2 ± 0.74 days (Figure 2a and b). Also, time taken for adult emergence from II and IV instar larvae treated with LC₄₀ of lufenuron was found to be prolonged to 3.8 ± 0.83 and 5.4 ± 0.83 days from pupation respectively, compared to the control larvae which required 2.6 ± 0.89 days from pupation (Figure 2a and b). Further it was observed that there was $33.72 \pm 2.3\%$ reduction in pupation and $63.44 \pm 3.9\%$ reduction in adult emergence in LC₄₀-treated II instar larvae (Figure 3a). Similarly, there was $54.84 \pm 4.7\%$ and $61.3 \pm 5.2\%$ reduction in pupation and adult emergence respectively, in LC₄₀-treated IV instar larvae (Figure 3b). Reduction in per cent pupation of LC₄₀-treated IV instar larvae was due to the death of the larvae without attaining pupation during the developmental process.

Sub-lethal concentrations of lufenuron significantly affect the growth and development of *A. aegypti* larvae. Retardation in the growth of the treated II and IV instar *A. aegypti* larvae was reflected by delay in the time taken for pupation and adult emergence. Reduction in per cent pupation and per cent adult emergence was also observed. Similar observations were made in lufenuron-treated I



Figure 4. Abnormal adults emerging from LC₂₀-treated II instar larvae of *A. aegypti* with five legs instead of three pairs, and reduced wings. L, Leg; RW, Reduced wing. About 25.8% of the adults emerging from LC₂₀ treated II instar larvae were abnormal.

and III instars of *Epiphyas postvittana* (Walker)¹¹, lufenuron-treated *Helicoverpa armigera* larvae¹² and flufenoxuron-treated neonates of *Tribolium castaneum* (Herbst)¹³.

At the highest concentration tested (LC₄₀), 50% of the treated larvae were unable to attain the pupal stage and died, compared to that observed in triflumuron-treated *Anopheles* and *Culex* larvae in which 100% inhibition of pupal formation was observed¹⁴.

At lower concentration (LC₂₀) deformed adults were encountered. The legs and wings of such adults were not fully developed. Our findings support those in *Tribolium castaneum* and *Tribolium confusum*¹⁵, in potato tuber moth¹⁶ and in *Lobesia botrana*¹⁷.

Our observations suggest that lufenuron has the potential to reduce the mosquito population and could lead to their control when used judiciously. However, field trials are essential to confirm the results for use of lufenuron as one of the additional tools in the IPM/vector control programme.

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