Molluscicidal and mosquito larvicidal efficacy of Lantana indica Roxb. leaf extracts

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Received 27 March 2006; Accepted 13 November 2006

Abstract
The aqueous and acetone extracts of Lantana indica Roxb. leaves were governed for 24 to 96 hours to the freshwater snails, Lymnaea acuminata and larvae of Culex quinquefasciatus mosquitoes in order to test their lethality. It was observed that the acetone extract of leaves has potent toxic effect against both the vectors in time and dose dependent manner. There was a significant negative correlation between LC values of the extracts and the exposure period, thus LC50 values decreases from 7.32 mg/l (24 hours) to 1.02 mg/l (96 hours) against L. acuminata and 10.99 mg/l (24 hours) to 5.18 mg/l (96 hours) against C. quinquefasciatus larvae. It is clear from this study that the active moiety extracted from this plant may prove to be of great promise for being used as natural pesticide for vectors control.

Keywords: Freshwater Snail, Lymnaea acuminata, Mosquito, Culex quinquefasciatus, Molluscicide, Larvicide, Lantana indica, Verbenaceae.

IPC code; Int. cl.— A61K 36/85, A01P 7/04, A01P 9/00.

Introduction
The present study was carried out on the fresh water snail, Lymnaea acuminata Lamk and larvae of Culex quinquefasciatus Say mosquito. L. acuminata is an intermediate host of liver flukes Fasciola hepatica Linn. and F. gigantica which cause endemic fascioliasis in cattle and livestock in tropical and sub-tropical countries1 where as C. quinquefasciatus is an urban mosquito vector of human lymphatic filariasis2, a disfiguring malady of people in the tropics and sub-tropics caused by Wuchereria bancrofti3,4. Application of insecticides against mosquitoes has resulted in the development of resistance to organochlorine (Cyclodiene), organophosphate and carbamate insecticides5. Due to the problem of resistance development, it would be difficult to use chemical insecticides continuously for a long time. Use of synthetic pesticides for vectors control also causes environmental hazards by contamination of the aquatic environment6-8. In this context, the safer natural pesticides are widely accepted because natural products, being eco-friendly, easily biodegradable and cost effective, are the focus of attention as an alternative to conventional chemical insecticides. Therefore, there is a need to test phyto-pesticides which are unlikely to show cross-resistance to the chemical insecticides. Plants belonging to genus Lantana Linn. (Family — Verbenaceae) are hairy shrubs, native to tropical America, found wild and cultivated as an ornamental or hedge plant. About 8 species of Lantana occur in India. Different parts of these plants are reported to be used in traditional medicine for the treatment of various human ailments such as ulcers, eczema eruptions, malaria and rheumatism9-11. In the present study leaf extracts of Lantana indica Roxb. were used for determining the larvicidal and molluscicidal activity against larvae of C. quinquefasciatus and snail L. acuminata, respectively.
Materials and Methods

Collection and storage of experimental animals
Freshwater snail, *L. acuminata* (2.6±0.3cm length and 1.2±0.1cm width) were collected from local water bodies of Gorakhpur district and stored for 72 hours in dechlorinated tap water for acclimatization under laboratory condition before experiment. For larvicidal efficacy study, third instar larvae of *C. quinquefasciatus*, obtained from the colony and maintained in the laboratory were used.

Collection and preparation of aqueous extract of leaves
The leaves were collected locally from Botanical Garden of DDU Gorakhpur University, Gorakhpur. They were washed with water, cut into small pieces and crushed in mortar and pestle with distilled water, centrifuged at 1000×g for five minutes and supernatants were used as aqueous extracts for toxicity experiments.

Preparation of acetone extract of leaves
Leaves (2Kg) were washed with water, dried in incubator at 37°C and powdered with the help of a mechanical device. The dried powder (50g) was extracted through Soxhlet apparatus in 200ml of acetone for about 20 hours and after extraction the solvent was evaporated using vacuum pump to obtain the extract in dried form. Dried residue of acetone extract was stored in airtight desiccators and used for experiments.

Toxicity experiments
Toxicity experiments were performed by the method of Singh and Agarwal12. Ten experimental animals were kept in a glass aquaria (26 cm in diameter and 13 cm in height) containing 2 litres of dechlorinated tap water. Snails were exposed for 24 to 96 hours at four different concentrations of aqueous extracts and acetone extracts and 6 aquaria were setup for each dose. Control animals were kept in similar conditions without treatment. Mortality was recorded after every 24 hours up to 96 hours exposure period. In the case of snails, the contraction of the body within the shell and no response to a needle probe were taken as evidence of death.

In the case of mosquito larvae, toxicity of the leaf extract was tested by using the method of WHO13. Different test concentrations were prepared in 500ml of dechlorinated tap water. To each of the beakers containing different test concentrations and the control 10 third instar larvae of *C. quinquefasciatus* were transferred. Mortality was recorded at 24 hours intervals up to 96 hours post-treatment. Six replicates were maintained for each concentration. Dead larvae were removed as soon as possible in order to prevent decomposition, which may cause rapid death of the remaining larvae.

The LC(10, 50, 90) values, upper and lower confidence limits, slope values, ‘t’ ratio, ‘g’ factor and heterogeneity were calculated according to the Probit log method using POLO computer program of Russel et al14.

Results and Discussion
Experimental conditions of water were determined by the method of APHA/WEF15. Atmospheric temperature and water temperature ranged from 30.4-31.6°C and 25.7-27.8°C, respectively, pH of water was 7.3-7.4, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity ranged from 6.7-7.4 ppm, 4.2-6.1ppm and 105.3-108.8 ppm, respectively for the whole experiment.

Behavioural changes
Exposure to both the aqueous and acetone extracts of the plant leaves caused significant behavioural changes in *L. acuminata* and *C. quinquefasciatus* larvae. The snails showed behavioural changes within 20 to 30 minutes of exposure. The initial 1-2 hours was a period of hyperactivity during which the snails moved around rapidly in the aquarium, there after they started crawling and crowding on each other. Muscular twitching also observed and the snails became spirally twisted, followed by complete withdrawal of body inside the shell and death. The most obvious sign of behavioural change in *C. quinquefasciatus* larvae was incapability of rising to the surface or of showing the characteristic diving reaction when water was disturbed. The larvae also showed restlessness, loss of equilibrium and muscular titany; animal became lethargic and finally died. No such behavioural changes and mortality occurred in the control groups indicating that no factor other than plant moiety was responsible for the altered behaviour and mortality. The extract perhaps contains some neurotoxin which might be active against neuromuscular system of the exposed animals.

Dose response relationship
LC50 values, upper and lower
confidence limits, slope value, g-factor, t-ratio and heterogeneity of *L. indica* extracts against snail *L. acuminata* and *C. quinquefasciatus* larvae for the exposure periods ranged from 24 hours to 96 hours are given in Table 1 and 2.

The toxicity of leaf extract against *L. acuminata* was time as well as dose dependent. There was a significant negative correlation between LC values and exposure periods. Thus, the LC50 values of aqueous and acetone extracts against *L. acuminata* decreased from 111.27 mg/l (24 hours) to 31.30 mg/l (96 hours) and from 7.32 mg/l (24 hours) to 1.02 mg/l (96 hours), respectively (Table 1).

The aqueous extract of *L. indica* leaf against *C. quinquefasciatus* larvae was non-toxic up to 200 mg/l while the acetone extract showed time and dose dependent toxic effect. The LC50 values for the exposure period of 24 hours to 96 hours decreased from 10.99 mg/l (24 hours) to 5.18 mg/l (96 hours) (Table 2).

Mortality caused by the leaf extracts showed a significant positive correlation with dosage. The positive correlation between dose and mortality.

### Table 1: Toxicity (LC values) of aqueous and acetone extract of *Lantana indica* leaf against Fresh water Snail, *Lymnaea acuminata* at different time intervals

<table>
<thead>
<tr>
<th>Leaf extracts</th>
<th>Exposure periods (Hours)</th>
<th>Effective dosage</th>
<th>Limits (mg/l)</th>
<th>Slope value</th>
<th>'g' Factor</th>
<th>'t' Ratio</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract of wet leaf</td>
<td>24</td>
<td>LCl =43.50</td>
<td>35.85</td>
<td>49.58</td>
<td>3.14 ± 0.40</td>
<td>0.062</td>
<td>7.85</td>
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<tr>
<td></td>
<td></td>
<td>LC90 =284.64</td>
<td>211.84</td>
<td>461.22</td>
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<tr>
<td></td>
<td></td>
<td>LC50 =111.27</td>
<td>97.62</td>
<td>134.99</td>
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<tr>
<td></td>
<td></td>
<td>LC90 =284.64</td>
<td>211.84</td>
<td>461.22</td>
<td></td>
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<tr>
<td></td>
<td>48</td>
<td>LCl =21.26</td>
<td>13.82</td>
<td>27.47</td>
<td>2.04 ± 0.29</td>
<td>0.077</td>
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<td></td>
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<td>LC90 =79.71</td>
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<td>111.83</td>
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<tr>
<td></td>
<td></td>
<td>LC50 =79.71</td>
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<td>785.56</td>
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<td>LC90 =79.71</td>
<td>249.93</td>
<td>785.56</td>
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<tr>
<td></td>
<td>72</td>
<td>LCl =10.19</td>
<td>5.35</td>
<td>14.88</td>
<td>1.93 ± 0.26</td>
<td>0.074</td>
<td>7.68</td>
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<td></td>
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<td>LC90 =46.80</td>
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<tr>
<td></td>
<td></td>
<td>LC50 =79.71</td>
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<td>366.91</td>
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<td></td>
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<td>LC90 =46.80</td>
<td>156.40</td>
<td>366.91</td>
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<tr>
<td></td>
<td>96</td>
<td>LCl =7.89</td>
<td>4.09</td>
<td>11.73</td>
<td>2.14 ± 0.28</td>
<td>0.065</td>
<td>7.69</td>
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<td></td>
<td></td>
<td>LC90 =31.30</td>
<td>25.02</td>
<td>36.60</td>
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<tr>
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<td>LC50 =79.71</td>
<td>100.07</td>
<td>174.68</td>
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<td></td>
<td>LC90 =31.30</td>
<td>100.07</td>
<td>174.68</td>
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<tr>
<td>Acetone extract of dry leaf</td>
<td>24</td>
<td>LCl =1.86</td>
<td>1.36</td>
<td>2.28</td>
<td>2.15 ± 0.27</td>
<td>0.06</td>
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<td></td>
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<td>LC90 =7.32</td>
<td>6.19</td>
<td>9.29</td>
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<tr>
<td></td>
<td></td>
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<td>18.27</td>
<td>66.16</td>
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<td></td>
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<td>LC90 =79.71</td>
<td>18.27</td>
<td>66.16</td>
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<td>LCl =0.51</td>
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<td>0.78</td>
<td>1.45 ± 0.19</td>
<td>0.06</td>
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<td></td>
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<td>LC90 =3.89</td>
<td>3.23</td>
<td>4.78</td>
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<tr>
<td></td>
<td></td>
<td>LC50 =79.71</td>
<td>19.36</td>
<td>55.13</td>
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<td></td>
<td></td>
<td>LC90 =79.71</td>
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<td>55.13</td>
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<td></td>
<td>72</td>
<td>LCl =0.28</td>
<td>0.16</td>
<td>0.40</td>
<td>1.44 ± 0.19</td>
<td>0.07</td>
<td>7.08</td>
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<td></td>
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<td>LC90 =1.15</td>
<td>0.77</td>
<td>1.49</td>
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<tr>
<td></td>
<td></td>
<td>LC50 =79.71</td>
<td>6.58</td>
<td>14.30</td>
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<tr>
<td></td>
<td></td>
<td>LC90 =79.71</td>
<td>6.58</td>
<td>14.30</td>
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<tr>
<td></td>
<td>96</td>
<td>LCl =0.15</td>
<td>0.05</td>
<td>0.28</td>
<td>2.27 ± 0.23</td>
<td>0.04</td>
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<tr>
<td></td>
<td></td>
<td>LC90 =1.02</td>
<td>0.79</td>
<td>1.24</td>
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<tr>
<td></td>
<td></td>
<td>LC50 =79.71</td>
<td>3.18</td>
<td>4.64</td>
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<td></td>
<td></td>
<td>LC90 =79.71</td>
<td>3.18</td>
<td>4.64</td>
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</tbody>
</table>

LCL= Lower confidence limit; UCL=Upper confidence limit.
in all cases was noted because increased concentration of pesticides in aquarium water resulted in more intake or entry of pesticides as nerve poison in the animal’s body. This trend also depends upon several factors such as, rate of penetration, nature of slope, variability and maximal effects of active moieties. Increased mortality with increase in exposure periods could be affected by several factors, which may be acting separately or conjointly. For example, uptake of active moiety is time dependent, which leads progressive increase in the entrance of the drug and its effects in the snail body.

More important is the fact that the extract of this plant is much more toxic than synthetic pesticides. The present study demonstrates that the acetone extract of \textit{L. indica} leaves has higher molluscicidal activity than any of the existing synthetic pesticides. Thus, the 24 hour LC$_{50}$ of Aldicarb (30.00ppm) and Famothon (27.00ppm) against \textit{L. acuminata} \cite{1,2,12} is higher than that of \textit{L. indica} (7.32 ppm) which is about 1.6 times stronger than the standard molluscicides Niclosamide (LC$_{50}$ 11.8ppm) \cite{23}.

Euphorbious plants contain a group of diterpene phorbal esters and are inhibitor of AChE \cite{25}. Further, these plants promote the activity of the enzyme protein kinase C, which specifically phosphorylates serine and threonine residue in proteins \cite{26,27}. Since the active site of nervous enzyme acetylcholinesterase contains a serine residue \cite{28}, it is possible that the inhibition of enzyme AChE in the nervous tissue is the main cause of the death of snail and mosquito larvae. This is further supported by the behavioural observation in the present study and above possibility cannot be overruled in present study.

This assumption is reported by several workers, according to Singh and Agarwal\cite{23,29} who reported that the lattices of \textit{Euphorbia royleana} Boiss. and \textit{E. antisypilítica J. Meyran} significantly inhibited the activity of enzyme acetylcholinesterase in the tissues of snail. Tiwari and Singh\cite{30} reported that, the stem-bark and latex of \textit{E. tirucalli} Linn., significantly inhibit the activity of enzyme AChE in tissues of fish \textit{Channa punctatus}. The activity of this neuroenzyme in larvae of \textit{C. quinquefasciatus} treated with \textit{E. royleana} extracts also showed significant \((P<0.05)\) time and dose dependent inhibition in AChE activity \cite{31}.

Table 2 : Toxicity (LC$_{50}$) of aqueous and acetone extract of \textit{Lantana indica} leaf against \textit{Culex quinquefasciatus} larvae at different time intervals

<table>
<thead>
<tr>
<th>Leaf extracts</th>
<th>Exposure periods (Hours)</th>
<th>Effective dosage</th>
<th>Limits (mg/l)</th>
<th>Slope value</th>
<th>'g' Factor</th>
<th>'t' Ratio</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract of wet leaf</td>
<td>Non-toxic up to 200 mg/l</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Acetone extract of dry leaf</td>
<td>24</td>
<td>LC$<em>{10}$=4.50, LC$</em>{50}$=10.99, LC$_{90}$=26.84</td>
<td>3.43, 9.90, 20.10</td>
<td>5.25, 12.99, 45.68</td>
<td>3.30 ± 0.51, 0.09</td>
<td>8.34, 0.13</td>
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</tr>
<tr>
<td></td>
<td>48</td>
<td>LC$<em>{10}$=4.04, LC$</em>{50}$=8.15, LC$_{90}$=16.46</td>
<td>3.28, 7.64, 14.15</td>
<td>4.63, 8.74, 20.80</td>
<td>4.20 ± 0.49, 0.05</td>
<td>8.65, 0.37</td>
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</tr>
<tr>
<td></td>
<td>72</td>
<td>LC$<em>{10}$=3.27, LC$</em>{50}$=6.50, LC$_{90}$=12.95</td>
<td>2.55, 6.00, 11.51</td>
<td>3.84, 6.95, 15.44</td>
<td>4.28 ± 0.49, 0.05</td>
<td>9.08, 0.44</td>
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<tr>
<td></td>
<td>96</td>
<td>LC$<em>{10}$=2.86, LC$</em>{50}$=5.18, LC$_{90}$=9.39</td>
<td>2.20, 4.65, 8.67</td>
<td>3.39, 5.60, 10.49</td>
<td>4.95 ± 0.56, 0.04</td>
<td>9.23, 0.47</td>
<td></td>
</tr>
</tbody>
</table>

LCL=Lower confidence limit; UCL=Upper confidence limit.
Conclusion

As the leaf extracts of *L. indica* were highly toxic at low doses it may eventually prove to be a very useful molluscicide and larvicide. After further purification, these partially purified extracts may become more effective at much lower doses. Hence, the product from this plant would be ecologically sound and culturally more acceptable than a synthetic one.

Acknowledgement

The first author Ms Manisha Srivastava is thankful to University Grants Commission, New Delhi (San. No. F 3-129/2003, SR) for providing financial support for this work.

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13. WHO, Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides, 1981, 81, 807.


