Relative insecticidal value: An index for identifying neem trees with high insecticidal yield

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Insecticidal property of 38 neem trees, sampled from six locations in Karnataka, was evaluated through laboratory bioassays of the neem seed kernel extract (NSKE) against the second instar larvae of cabbage diamondback moth, Plutella xylostella (L.). The assays revealed a four-fold difference between trees for LC$_{50}$ values generated at 96 h after treatment. This large variation in the insecticidal property of trees, which can be attributed to both qualitative and quantitative differences in neem seed chemicals, has important implications, especially for the identification of trees with high insecticidal yield for propagation. An index, viz. Relative Insecticidal Value (RIV) was developed using the LC$_{50}$ values and the 100 seed weight (test weight). This index can be used to identify elite trees of neem from the point of view of insecticidal yield. Further, in the absence of any information on the quality of seeds obtained from different trees, the farmers can be advised to mix seeds from as many trees as possible for preparing NSKE and thus avoid the possibility of using seeds from trees with poor insecticidal activity.

The insecticidal property of the wonder tree – neem, Azadirachta indica A. Juss. (Meliaceae), has come as a boon for solving many insect pest problems. Neem seeds contain a wide variety of chemicals such as triterpenes, diterpenes and non-terpenol compounds with considerable biological activity. These seed-borne compounds when used against insects, either as purified compounds, crude extracts or vapours, act as growth retardants, antifeedants, repellents, sterilants, antimetabolites, etc. Although several proprietary products based on azadirachtin (triterpene) are available, a fresh aqueous neem seed kernel extract (NSKE) has

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been found superior\textsuperscript{7} and recommended\textsuperscript{8} for pest control. However, the suggestion of using aqueous NSKE is not widely accepted by farmers, largely because it is cumbersome to prepare and its efficacy is highly variable\textsuperscript{9}.

A possible reason for the variable performance of NSKE as a potent insecticide could be the plant to plant variation in the chemical composition\textsuperscript{10,11}. Variation in the content of azadirachtin from neem trees has been documented from different parts of India\textsuperscript{10}, but the relationship between this variation and the overall insecticidal property of neem remains unclear. A knowledge of the variation in the insecticidal property is important for improving the efficacy of NSKE and also to identify trees with high insecticidal activity for propagation. In this study an attempt has been made to quantify tree to tree variation in insecticidal property of neem through bioassay of NSKE against the cabbage diamondback moth, \textit{Plutella xylostella} (L.) and a simple method of identification of ‘elite’ trees has also been suggested.

Seeds were collected from 38 neem trees spread over six locations in Karnataka, viz. Bangalore, Chitradurga, Raichur, Gulbarga, Dharwad and Shimoga during July 1999. The dry weight of seeds and the kernels was recorded for 100 seeds each after drying the seeds for 25 days in shade.

Aqueous extracts from these seeds (NSKE) were used in assays against the second instar larvae of the cabbage diamondback moth. The culture of \textit{P. xylostella} used in the assays had completed more than 20 generations on mustard seedlings in the laboratory. The aqueous extract of nine concentrations ranging from 0.043 to 3.5 per cent (weight to volume) was prepared from neem seeds for each of the 38 trees following the standard procedure\textsuperscript{8}. The extracts also contained 0.05 per cent soap as a sticking agent. These concentrations were decided on the basis of preliminary trials conducted during 1998.

Fully grown mustard leaves approximately 3–4 sq cm were dipped for ten seconds in these extracts and air-dried in shade for one hour. Each leaf that served as a replicate, was placed in a petri dish with its stalk wrapped in moist cotton. Ten freshly moulted II instar larvae were placed on each of these leaves and maintained at room temperature. In each assay (trial) 300 larvae were used in three replications and ten treatments, including the control (distilled water with 0.05 per cent soap).

Observations on the mortality of the larvae were recorded at intervals of 12 h up to 108 h post-treatment. Treated old leaves were replaced with fresh untreated leaves of uniform age and size (area) after 72 h. Median lethal concentrations (LC\textsubscript{50} values) were computed by probit analysis\textsuperscript{12} for all hours of observation. The trend in mean LC\textsubscript{50} values and the CVs followed an exponential decay function with time (Figure 1). On the basis of these results, it was decided that 84 or 96 h post-treatment values will be more reliable and consequently, all analysis and interpretation of data presented here are based on the median lethal concentration (LC\textsubscript{50}) for mortality at 96 h after treatment. However, the LC\textsubscript{50} values for 84 and 96 h post-treatment were strongly correlated ($r = 0.869; n = 38; P < 0.0001$).

The mean LC\textsubscript{30} value for the 38 trees sampled was 0.249 (SD = 0.084) per cent with a coefficient of variation of 33.91 per cent. The values followed a skewed distribution with a range of 0.129 to 0.497 per cent, suggesting a four-fold variation between the trees (Figure 2). Such a large variation in the LC\textsubscript{50} values between the trees strongly suggests significant differences in the chemical composition (quantitative/qualitative) of the seeds from different trees. Quantitative differences in azadirachtin content between trees have been documented earlier\textsuperscript{7}, which is also known to vary geographi-

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{Figure 1. Trend in LC\textsubscript{50} values at different hours of observation after treatment of the II instar larvae of \textit{Plutella xylostella} for the 38 neem trees sampled from six locations in Karnataka. Vertical bars represent one standard deviation.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig2.png}
\caption{Figure 2. Frequency distribution of 38 neem trees sampled from six locations in Karnataka, on the basis of LC\textsubscript{50} values at 96 h after treatment against II instar larvae of \textit{Plutella xylostella}.}
\end{figure}
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cally. However, the extent to which azadirachtin alone might contribute to the differences in LC50 values observed in this study, is not known.

A proximal explanation for the observed variation in LC50 values could be the variation in the age of the trees sampled. This was examined by testing for the relationship between the LC50 values and a widely accepted index of age of perennial trees, the diameter at breast height (DBH). Age was not found to influence the observed LC50 values (Figure 3). Similarly, ecological conditions, specifically the climatic conditions of a given location may also influence the LC50 values and indeed, the mean LC50 values were found to vary with the location (Jayappa, in preparation). Interestingly however, a comparison of trees within each location revealed a two to two and a half-fold difference in LC50 values between trees (Table 1). It is possible that this intra-population variation is largely governed by factors intrinsic to the trees.

The results presented here clearly demonstrate considerable inter- and intra-population variation in the insecticidal property of neem as assessed by bioassays of NSKE (Table 1). Given the extent of variation in insecticidal property, it may be useful to identify trees with low LC50 values (indicating a high insecticidal property). Further, if such trees also have a higher mean kernel weight, they would be preferable both, for use in pest control and large-scale propagation, particularly through vegetative means, such as micropropagation. Indeed, selection of such trees is preferable, since the azadirachtin content of the mother tree and the micropropagated trees is correlated.

The LC50 values were strongly correlated with hundred seed kernel’s weight ($r = 0.44$; $n = 38$; $P < 0.01$). Thus, use of one or both of these parameters to identify the trees with high yield of ‘insecticides’ is not possible. Because, selection of trees on the basis of LC50 values will lead to selection of trees with low seed weight, while a selection based on seed weight will lead to selection of trees with high LC50 values. In order to strike a balance between the two, such that trees with relatively higher seed weights and lower LC50 values were identified, an index, Relative Insecticidal Value (RIV), was developed to characterize the insecticide yielding ability of the neem trees using both these parameters. The index is given as:

$$\text{Relative insecticidal value} = \frac{100 \text{ seed kernels’ weight}}{\text{LC50}}.$$

where LC50 is expressed as per cent concentration on weight (g) to volume (ml) basis and kernel weight in grams. The index, RIV essentially reflects the quantum of ‘insecticide’ obtainable from 100 seeds.

Figure 3. Relationship between the insecticidal property measured as LC50 at 96 h after treatment against II instar larvae of Plutella xylostella and the diameter in cm at breast height (1.3 m above ground level) of 38 neem trees sampled from six locations in Karnataka.

RIVs calculated using the LC50 values were independent of the seed kernel weight ($r = 0.137$; $P > 0.3$). In order to identify the neem trees with high insecticidal yield, the RIVs of the 38 trees were plotted against the test weight (weight of 100 seed kernels) and the resultant scatter-plot was divided into four quadrants using the means of the two parameters as cut-off values (Figure 4). Neem trees, which have high RIVs and high test weight occupy the right hand top quadrant and can be termed ‘elite’ trees from the point of view of insecticidal yield, since they best represent the trade-off in investment in seed tissue and defensive chemicals.

This analysis also revealed that the ‘elite’ neem trees were widely distributed and occurred in five of the six locations sampled. Similarly, the low RIV and low seed kernel weight trees were found in four locations. Trees of medium value, i.e. either with low RIV and high kernel weight or with high RIV but with low seed kernel weight were found in all the six locations. Shimoga population was an exception in that all the five trees sampled had low RIVs (Figure 4; Table 1).

Identification of ‘elite’ neem trees with desirable traits, viz. a high ‘insecticidal yield’, can be significantly improved if it is based on the RIV following a bioassay of NSKE against a suitable laboratory insect. However, the LC50 values can vary greatly depending on many factors extrinsic to the insecticidal property of the trees themselves. Therefore, the RIVs which are dependent on the LC50 values, are not likely to be independent of the influence of these factors and consequently are only relative. Nevertheless, RIV serves as an ideal index of measuring the insecticidal potential of neem trees given a uniform set of conditions and the test insect for the assessment of LC50 values.
Table 1. Distribution of 38 neem trees sampled from six locations in Karnataka in the four quadrants of the Euclidean space of RIVs and seed kernel weight and their mean LC$_{50}$ values and RIVs

<table>
<thead>
<tr>
<th>Location</th>
<th>Range in LC$_{50}$ values</th>
<th>Mean LC$_{50}$ value</th>
<th>Mean RIV</th>
<th>Quadrant I</th>
<th>Quadrant II</th>
<th>Quadrant III</th>
<th>Quadrant IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangalore</td>
<td>0.124–0.375</td>
<td>0.222</td>
<td>41.81</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Chitradurga</td>
<td>0.203–0.298</td>
<td>0.248</td>
<td>38.30</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Raichur</td>
<td>0.129–0.291</td>
<td>0.214</td>
<td>48.41</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Gulbarga</td>
<td>0.195–0.236</td>
<td>0.214</td>
<td>42.20</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Dharwad</td>
<td>0.196–0.316</td>
<td>0.242</td>
<td>40.92</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Shimoga</td>
<td>0.291–0.497</td>
<td>0.395</td>
<td>27.64</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.124–0.497</td>
<td>0.249</td>
<td></td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>38</td>
</tr>
</tbody>
</table>

I quadrant, High insecticidal value with low seed kernel weight; II quadrant, High insecticidal value with high seed kernel weight; III quadrant, Low insecticidal value with high seed kernel weight; IV quadrant, Low insecticidal value with low seed kernel weight.

Finally, the variation observed in the insecticidal property of neem between trees has a more important and immediate implication for pest management. According to the present recommendation on the use of NSKE for pest management, farmers are encouraged to make their collections of seeds from the trees and not to procure them from the market. It is very likely that farmers collect these seeds from very few or even a single tree due to the high cost of labour involved in seed collection. Based on this study, a clear recommendation can be made to the farmers to mix the seeds of as many trees as possible, so that the possibility of using the seeds of the trees with low insecticidal property are minimized.

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16. RIV = 100 seed kernels weight/LC50. Therefore RIV = (g)/(g/ml) = ml. Thus RIV is a direct measure of quantity of insecticide in ml obtainable from 100 seeds of neem trees, with an estimated insecticidal property equivalent to LC50 values calculated.

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