CYTOTOXICITY OF LEAF LEACHATE OF *Chromolaena odorata* (L.) King AND Robinson A POSSIBLE EXPLANATION FOR REDUCTION OF BIODIVERSITY IN NEIGHBOURHOOD

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**ABSTRACT**

Leaf leachate of *Chromolaena odorata* (L.) King and Robinson, siam weed, at different concentrations has been noted to impair seed germination, sapling growth, cell division, chromosomal conformity, protein content as well as catalase activity of *Lathyrus sativus* L. and *Lens esculenta* Moench. The findings imply the potential of the weed to check the growth of other species in and around by the allelochemicals of leaf.

**Key Words**: *Chromolaena odorata*, Allelopathy, Leaf leachate, Cytotoxicity, Biodiversity.

**INTRODUCTION**

*Chromolaena odorata* (L.) King and Robinson (syn. *Eupatorium odoratum* L.), a subshrub species of Asteraceae and native of Central and South America has got weedy nature in different parts of world including India.¹ As an invasive species it causes much impairment to the natural biodiversity.

Exotic invasion of many species has been attributed to allelopathic potential of them.² Allelopathy is defined as the chemical interaction between plants either of beneficial or detrimental nature.³⁵ Allelochemicals are known to play important roles in affecting plant diversity in the neighbourhood by causing oxidative damage, lipid peroxidation,⁶⁷ and also adversely affecting the protein content, catalase activity, soluble sugar content etc. of the plants exposed to them.⁸⁹ Reports on the allelopathic role of *Eupatorium odoratum* L. are quite abounding.¹⁰¹² A related species, *E. adenophorum* Spreng. is also known to have such potential.¹³

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The present study has attempted to find out the impact of leaf leachate of the species on the sapling growth of two important pulse species at the level of their growth of seedling, cell division, chromosome morphology, amount of protein of saplings and catalase content under the influence of different concentrations of leaf leachate of the weed species.

**MATERIAL AND METHODS**

Seeds of two species of Leguminosae, *Lathyrus sativus* L. and *Lens esculenta* Moench. were taken as test plants. Leaf leachate was taken of *Chromolaena odorata* (L.) King and Robinson (syn. *Eupatorium odoratum* L.).

Leaf leachate was collected from fallen and senesced leaves of *Chromolaena odorata* (L.) King and Robinson by dipping 200 gm. of macerated leaves in 1 litter of double distilled water for 48 hours. The strained leachate was considered as concentrated form (100%) of it. Water was used because most allelochemicals are water soluble. The leachate was sieved and kept in a refrigerator at 5°C for subsequent experiments. Different concentrations of extract 10%, 25% and 50% have been prepared with distilled water.

Seeds of both species were soaked with leachate of different concentrations for 6 hours and then allowed to germinate in presence of water and were transferred to the sterilized soil thereafter. Control set was made with the seeds soaking and germinating in water. Height of seedlings (cm.) at the age of 15 days was measured as growth performance.

Cytological study was carried out with the root tips of the treated seedlings following the squash technique to determine the cell division efficiency as well as the extent and nature of cell divisional and chromosomal abnormality.

Quantitative estimation of protein was done by crushing 100mg. of 15 days of old seedlings in 10 ml. of 0.1 N NaOH buffer and following the Lowery method. Catalase content of whole seedlings of same age was carried out taking 300 mg. of seedling by crushing with 10 ml. of distilled water and estimating colorimetrically following Snell and Snell.

**RESULTS AND DISCUSSION**

Growth performances of 15 days old seedlings in terms of height of plants and their mitotic index showed a gradual decline in response of increasing concentration of leaf leachate Table 1, while abnormality index increased considerably in both of the species. Similarly the protein content of seedlings and their catalase contents in both species decreased in the treated plants in comparison to control and also a gradual fall was witnessed with the increase of the concentration of leachate Table 2. Regression curves portrayed the relation between different parameters.
Table 1: Impact of leachate of different concentrations on plant height, mitotic index and abnormality index of *Lathyrus sativus* L. and *Lens esculenta* Moench.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Control</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Lathyrus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Height (cm.)</td>
<td><em>0.594</em></td>
<td>12.55</td>
<td>12.12</td>
<td>11.64</td>
<td>11.16</td>
<td>10.84</td>
</tr>
<tr>
<td></td>
<td><em>0.390</em></td>
<td>11.47</td>
<td>10.83</td>
<td>10.80</td>
<td>10.38</td>
<td>10.16</td>
</tr>
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<td></td>
<td><em>1.017</em></td>
<td>31.97</td>
<td>40.71</td>
<td>32.45</td>
<td>28.27</td>
<td>24.60</td>
</tr>
<tr>
<td></td>
<td><em>0.592</em></td>
<td>31.72</td>
<td>45.70</td>
<td>35.58</td>
<td>28.23</td>
<td>27.19</td>
</tr>
<tr>
<td></td>
<td><em>1.315</em></td>
<td>6.43</td>
<td>14.38</td>
<td>15.16</td>
<td>28.51</td>
<td>30.67</td>
</tr>
<tr>
<td></td>
<td><em>1.332</em></td>
<td>6.51</td>
<td>10.39</td>
<td>16.20</td>
<td>28.64</td>
<td>33.04</td>
</tr>
</tbody>
</table>

Mean (S.D.) * LSD
Table 2: Impact of different conc. of leachate on protein and catalase content of seedlings of *Lathyrus sativus* L. and *Lens esculenta* Moench.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>Control</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg/ml)</td>
<td><em>Lathyrus</em></td>
<td>0.61</td>
<td>0.49</td>
<td>0.36</td>
<td>0.35</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>* 0.023</td>
<td>(0.02)</td>
<td>(0.02)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
</tr>
<tr>
<td></td>
<td><em>Lens</em></td>
<td>0.63</td>
<td>0.40</td>
<td>0.32</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>* 0.034</td>
<td>(0.02)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>Catalase gm/unit</td>
<td><em>Lathyrus</em></td>
<td>4.92</td>
<td>4.83</td>
<td>4.58</td>
<td>4.42</td>
<td>4.20</td>
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<tr>
<td></td>
<td>* 0.004</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.02)</td>
<td>(0.01)</td>
<td>(0.10)</td>
</tr>
<tr>
<td></td>
<td><em>Lens</em></td>
<td>4.83</td>
<td>4.75</td>
<td>4.50</td>
<td>4.25</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>* 0.008</td>
<td>(0.01)</td>
<td>(0.02)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.12)</td>
</tr>
</tbody>
</table>

Mean (S.D.) *LSD

Fig. 1: Decoiled chromosomes due to erosion at prophase.
Fig. 2: Clumping of chromosomes at metaphase.

Fig. 3: Clumping of chromosome showing sticky bridge at anaphase.
Different cytological abnormalities in response of leachate were noted to be spindle disturbance, erosion of chromatin content, stickiness of chromatin, clumping of chromosome etc Fig. 1- Fig. 4. However, a general trend showed spindle disturbance instead of distortion of chromosome structure to be more preponderant at lower concentration. Under the influence of higher concentrations the configuration of chromosome worsened due to erosion or stickiness.

Regression analyses Fig. 5 and Fig. 6 show sharp decline in all the cases of plant growth, mitotic index, protein content and catalase activity with the increase of leachate concentration. While the regression line of abnormality percentage is directed upward showing a concomitant increase of cytological abnormality in response of leachate concentration.

The aqueous leachate of leaf at different concentrations has been noted to affect the seedling growth of both of the pulse species in a very similar way. With increasing concentrations of leaf leachate the growth of treated plants declines almost proportionately with minor exceptions, which is reflected in their respective regression curves Fig. 5 and Fig. 6. This finding is very much in congruence with earlier reports on this species and other species too.

Cytological studies show mitotic index in both of the treated species to decline with the increase of leachate concentration.
Fig. 5: Regression curves of Lathyrus sativus L.
Fig. 6: Regression curves of *Lens esculenta* Moench.
This finding is adduced with the experiences of earlier workers in using leaf leachates of different other species. However, the 10% and 25% concentrations of leachate show a higher rate of cell division in comparison to the no application of leachate in both of the treated species. This result might be pointing towards a possible role of a comparatively lower concentration of a leaf leachate of the weed in inducing cell division of plants subjected to that. Such a condition can hardly be expected in nature due to a continuous accumulation of leaves as well as their allelochemicals in the soil of the place the weed grows. Abnormality percentage, however, increases consistently with the concentration of the leachate applied.

Buffer soluble protein contents of whole plants also show a sharp decrease in response of increasing concentration of leachate. Similar impairment of biochemical function by the leachate action has been recorded by earlier workers. Catalase content shows a decline responding to the increase of leachate concentration. Since catalase plays a vital role in sequestering the damaging agent hydrogen peroxide from within cell its decline as an effect of increasing concentrations of leachate indicates impaired health of the suffering plants.

Thus, Chromolaena odorata (L.) King and Robinson affects major life processes of neighbouring plants and thereby exerts a control over their growth surrounding it and leading to the loss of biodiversity.

CONCLUSION
Leaf as a very major contributor of allelochemicals of Chromolaena odorata (L.) King and Robinson, leached out to the soil, proves to be instrumental in the loss of biodiversity of plants in and around the bushes of this species. The influence is noted to be quite wide, affecting many vital life processes like, chromosomal conformity, cell division, protein synthesis, catalase production etc. While in nature the gradual accumulation of these allelochemicals is supposed to cause evasion of biodiversity the promotion of cell division by lower concentration of this leachate may show a prospect of its beneficial use too.

REFERENCES
6. Lara-Nunez A., Romero-Romero T.,


