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# Evaluation of allelopathic potential of an aromatic exotic tree, *Melaleuca leucadendron* L.

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**An attempt was made to evaluate the allelopathic potential of an exotic tree species, *Melaleuca leucadendron* L. using mung bean (*Vigna radiata* L.) seeds as responsive bioassay material. This was recorded in terms of the plant extract and plant leachate-induced changes of seed germination behaviour, the levels of DNA and RNA as well as amylase activity in the seed kernels. The results of the present investigation clearly revealed that pretreatment of mung bean seeds with various concentrations [1:1 and 1:2 (w/v)] of *M. leucadendron* bark extract, leaf extract and leaf leachates for 24 h duration, significantly reduced percentage seed germination and increased the $T_{50}$ hours. Levels of DNA and RNA were also significantly reduced with concomitant increase of amylase activity in mung bean seed samples pretreated with the bark extract, leaf extract and leaf leachates of *M. leucadendron*. Tender bark extract and leaf extract showed more inhibitory action on mung bean seed than leaf leachates. Putative allelochemical-induced inhibitory effect, that is, reduction of seed germinability along with stimulation of amylase activity in seeds, being the important allelopathic indices, it can be concluded that *M. leucadendron* can potentially render allelopathic action on the experimental bioassay material.**

**Key words:** Allelopathic potential, *Melaleuca leucadendron*, mung bean, seed germination, DNA and RNA levels, amylase activity.

## INTRODUCTION

The term allelopathy was derived from the Greek words ‘allelon’ meaning ‘of each other’ and ‘pathos’ meaning ‘to suffer’ refers to the chemical influence of one species by another. Although the term allelopathy is most commonly used to describe the chemical interaction between two plants, it has also been used to describe microbe-microbe, plant-microbe, plant-insect or plant-herbivore chemical communication. Allelopathy is defined as “any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems including positive and negative effects” (Torres et al., 1996). In plants, allelochemicals may be present in the leaves, barks, roots, flowers and fruits. Many exotic and invasive plants are threat to ecological diversity throughout the world and the invasive plants are uncommon in their native range but become very abundant in their new habitats (Louda et al., 1990; Callaway, 2002; Dogra et al., 2011). In fact, in some parts of India, the *Melaleuca* species has made a monospecific stand by displacement of the growth of local herbs growing under its canopy.

There are some common indices for assessing allelopathic action of plants or plant parts. These include, among others, germination behaviour and other physio-biochemical

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**Table 1.** Effect of seed treatment with bark extracts, leaf extracts and leaf leachates of *M. leucadendron* on percentage of germination and time (h), to 50% germination ($T_{50}$) of mung bean seeds.

| Treatments | Concentration (W/V) | Germination (%) | $T_{50}$ Hours |
|---|---|---|---|
| Bark extract | 1 : 1 | 32 ± 1.05 | 37.54 ± 0.80 |
| | 1 : 2 | 36 ± 1.48 | 33.38 ± 0.90 |
| Leaf extract | 1 : 1 | 29 ± 1.30 | 40.18 ± 1.34 |
| | 1 : 2 | 31 ± 1.38 | 37.72 ± 0.65 |
| Leaf leachate | 1 : 1 | 44 ± 1.30 | 27.22 ± 2.01 |
| | 1 : 2 | 52 ± 1.64 | 23.12 ± 1.03 |
| Control | 0 | 94 ± 1.58 | 12.72 ± 0.91 |
| LSD (P = 0.05) | | 7.82 | 3.45 |

responses of test species (Dogra et al., 2011). The present investigation is an attempt to evaluate the allelopathic potential of *Melaleuca leucadendra* L. (Myrtaceae), an exotic tree growing abundantly in the coastal belt of Digha in West Bengal state of India. For *prima facie* screening of allelopathic action of this plant, some selected physiological and biochemical parameters were analyzed using mung bean seed as a potential and responsive bioassay material.

## MATERIALS AND METHODS

Fresh, mature and healthy leaves and tender bark (500 g each) of *M. leucadendron* L., collected from the coastal belt of Digha in West Bengal state of India, were thoroughly homogenized separately by mortar and pestles using 300 ml distilled water. The homogenate was strained using fine cloth and then centrifuged at 5000 *g* for 15 min. The supernatant was then made up to 500 ml using distilled water and these were considered 1:1 (W/V) proportion stock solution of leaf extract and tender bark extract, respectively. From these stock solutions another concentration grade of leaf and bark extract in the proportion of 1:2 (W/V) was prepared using distilled water and these were taken as the two concentration graded solutions of the leaf extract and the bark extract.

Another lot of dry leaves (500 g) sample of the *M. leucadendron* was kept immersed in 300 ml distilled water in a 100 ml beaker for 48 h and the leachate was decanted in a separate beaker. The total volume of the leachate was made up to 500 ml using distilled water and this was taken as the 1:1 (W/V) proportion of leaf leachate. From this stock solution another concentration grade in the proportion of 1:2 (W/V) was prepared using distilled water and this was taken as the two concentration graded solutions of leaf leachate. These three concentration grades of each leaf extract, bark extract and leaf leachate were used for possible allelopathic analysis.

Fully viable 200 g mung bean (*Vigna radiata* L.) seeds were surface sterilized with 0.1% $HgCl_2$ solution for 90 s. The seed lots were then separately presoaked in the three concentration grades of the bark extract, leaf extracts and leaf leachate for 24 h.

From the treated seed samples germination behavior (percentage and $T_{50}$ of seed germination), DNA and RNA levels and activity of amylase enzyme in seeds were recorded.

To analyze germination behavior from continuous treatment sets, seven groups of 100 fresh seeds (700 fresh seeds) were transferred to separate Petri dishes containing filter paper moistened with 10 ml each of leaf extract, bark extract or leaf leachates and distilled water for control. Germination data were recorded after 120 h of seed soaking following the International Rules of Seed Testing (ISTA, 1976), DNA and RNA levels were analyzed as per the method described by Cherry (1967) modified by Choudhuri and Chatterjee (1970). Extraction and estimation of the enzyme amylase was done as per the method described by Khan and Faust (1967). For the assay of this enzyme, the blank was taken as zero time control. The activity of this enzyme was expressed as $[(\Delta A \times Tv)/(t \times v)]$, where $\Delta A$ is the absorbance of the sample after incubation minus the absorbance of the zero time control, Tv is the total volume of the filtrate, t is the time (minutes) of incubation with the substrate and v is the volume of the filtrate taken for incubation (Fick and Qualset, 1975).

Statistical analysis of the data was done in terms of least significant difference (LSD) which was calculated at 95% confidence limits and as per the method of Panse and Sukhatme (1967).

## RESULTS AND DISCUSSION

### Effect of treatment on percentage and $T_{50}$ value of seed germination

It is evident from the result (Table 1) that the putative allelochemicals of bark extract, leaf extract and leaf leachates of *M. leucadendron* strongly inhibited the percentage germination of mung bean seeds. Highest inhibitory allelopathic effect was recorded in the case of leaf extract followed by bark extract and lowest in treatment with leaf leachate. The data also reveals that more concentrated extracts were more injurious. Concomitantly, $T_{50}$ value is increased due to the treatment with bark extract, leaf extract and leaf leachate of *M. leucadendron* on mung bean seeds.

Analysis of germination behaviour is considered to be a reliable index for evaluation of allelopathic action (Datta and Chakraborty, 1982; Pati, 2007; Pati and Bhattacharjee, 2008). It is reported that reduced germinability is an important effect of allelopathic action of plants and such action is chiefly exerted by a number of inhibitors of diverse chemical nature (Ghosh and Dutta, 1989). Thus, inhibition of percentage germination and increase of $T_{50}$ value of mung bean seeds with bark extract, leaf extract and leaf

**Table 2.** Effect of seed pretreatment with bark extracts, leaf extracts and leaf leachates of *M. leucadendron* on DNA and RNA levels and amylase activity in kernels of mung bean seeds.

| Treatment | Concentration (W/V) | DNA level (µg/g fresh weight) | RNA level (µg/g fresh weight) | Amylase activity (unit/h/g fresh weight) |
|---|---|---|---|---|
| Bark extract | 1 : 1 | 16.04 ± 0.72 | 86.26 ± 1.00 | 26.44 ± 0.78 |
| | 1 : 2 | 18.64 ± 0.79 | 91.36 ± 0.48 | 24.78 ± 0.56 |
| Leaf extract | 1 : 1 | 15.96 ± 0.89 | 82.64 ± 0.47 | 30.56 ± 0.52 |
| | 1 : 2 | 16.22 ± 0.33 | 87.06 ± 0.69 | 27.16 ± 0.56 |
| Leaf leachate | 1 : 1 | 22.16 ± 0.51 | 118.34 ± 0.73 | 18.30 ± 0.56 |
| | 1 : 2 | 24.84 ± 0.73 | 129.50 ± 1.34 | 15.86 ± 0.68 |
| Control | 0 | 31.25 ± 0.62 | 153.84 ± 1.38 | 11.60 ± 0.50 |
| LSD(P=0.05) | | 2.00 | 2.29 | 2.93 |

leachate of *M. leucadendron* are clear indicative of the allelopathic action of the test material.

## Effect of treatment on changes of DNA and RNA levels and amylase activity in seed kernels

The results of Table 2 show that DNA and RNA levels of the kernel of mung bean seeds decreased significantly with leaf extract, bark extract and leaf leachate of *M. leucadendron* where leaf extract exhibited more significant effect. On the other hand, amylase activity increased in seed kernels irrespective of the treatments with two concentration grades of leaf extract, bark extract and leaf leachate of the selected plant.

Reduction of DNA and RNA levels and increase of amylase activity are suggestive of allelopathic potentiality of the test plant parts. Various inhibitors present in plants having allelopathic property reduce the overall metabolism of plants and anabolic activities of individual plant parts are also reported to be strongly impaired (Datta and Chakraborti, 1982). Reduced plant growth and slowed rate of plant establishment are also convincing evidence of allelopathic action (Ghosh and Dutta, 1989; Pati, 2007; Pati and Bhattacharjee, 2011).

The unreported and preliminary findings of the present investigation point out that the leaves and tender bark of *M. leucadendron* possess some allelochemicals, which efficiently render inhibitory action on the bioassay material. The observation further reemphasized the fact that the experimental exotic plant having growth suppressing property should be treated as a potential threat to plant biodiversity in a natural ecosystem in Indian soil.

Thus, a conclusion can be drawn from this study that the exotic plant *M. leucadendron* is a potential species having allelopathic property. Further work is in progress in our laboratory to identify, by advanced chromatographic technique, the specific allelochemicals which are causal factors for exhibiting allelopathic action.

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