in seeds treated with different fungi as against control 95.5% and 99.80% in soil and on moist blotter respectively (table 1).

In soil, least per cent germination (59.5) was noticed in seed lot treated with *F. semitectum* and maximum (69.8) was seen in seed lot treated with *F. oxysporum*. Whereas, on moist blotter, least per cent germination (80.1) was seen in seed lot treated with *F. moniliforme* and maximum (93.2) was seen in seed lot treated with *F. oxysporum*.

The pre-emergence death of seedlings was maximum (40.5%) in seed lot treated with *F. semitectum* and least (30.2%) in seed lot treated with *F. oxysporum* in soil, whereas, on moist blotter, maximum (19.9%) was noticed in seed lot treated with *F. moniliforme* and minimum (6.8%) in seed lot treated with *F. oxysporum*. The maximum post-emergence death of seedlings was observed in *F. moniliforme* (100%) and least in *F. oxysporum* (76.6%) in soil, while on moist blotter also *F. moniliforme* caused maximum (86.7%) post-emergence death of seedlings and *F. oxysporum* caused the least (69.2%) death of seedlings.

All the three species of *Fusarium* grew well on both germinated and un-germinated seeds. A thick mycelial coat developed on radicles as well as on plumules; development of necrotic lesion lead to stunted growth, blight and death of seedlings.

This paper forms part of the M.Sc. (Agri.) thesis submitted by the first author to the University of Agricultural Sciences, Bangalore.

25 January 1988; Revised 5 May 1988


**ISOLATION AND PURIFICATION OF A SESQUITERPENE LACTONE FROM THE LEAVES OF PARTHENIUM HYSTEROPHORUS L.—ITS ALLELOPATHIC AND CYTOTOXIC EFFECTS**

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The obnoxious weed *Parthenium hysterophorus* L. causes dermatitis and other forms of allergy. The ingredient responsible for allergy is a sesquiterpene lactone (SL)\(^1\). The weed also suppresses crops and plants around it by virtue of its rapid growth\(^2\) and allelopathic effects\(^3\). To characterize the allelopathic principle, an attempt has been made to extract and purify SL. Cytotoxic and antifungal properties of the SL were also studied.

Oven-dried (50°C) leaf powder (20 g) was repeatedly extracted with acetone (Analar grade) and filtered through four layers of muslin cloth. The filtrate was collected in an evaporating dish and allowed to evaporate. The green residue obtained was then redissolved in acetone in the proportion one part residue to 50 parts acetone and the solution was passed through a silica gel G (300 mesh) column (1 x 6 cm) pre-equilibrated with acetone. The flow rate through the column was adjusted to 4 drops per minute.

The yellowish-brown mass obtained was redissolved in acetone and passed through a sintered-glass filter. A 1 x 8 cm Corning glass column chromatography was prepared. A 1 cm layer of silica gel G (300 mesh) was packed first. This was followed by a 6 cm layer of activated charcoal (E. Merck, Germany) freshly reactivated by heating at 400°C for 1 h in a muffle furnace. Above the activated charcoal, another 1 cm layer of silica gel was packed. The sandwiched charcoal ensured removal of colouring matter. The top of the column was plugged with cotton. The extract was loaded on the column and the flow rate was adjusted to 4 drops per minute. The effluent was collected in an evaporating dish and evaporated at room temperature (28 ± 2°C) till a white crystalline residue was obtained.

The compound was identified from its NMR spectrum (EM 390 90 MHz NMR spectrometer) and by high performance liquid chromatography (HPLC) (Beckman) by the courtesy of Prof. E. Rodriguez, Irvinè, USA.

Allelopathy was investigated by germinating wheat seeds in different concentrations of SL, viz. 0,
250, 500 and 1000 ppm, in petri dishes. Germination percentage and seedling growth were recorded after 72 h of germination. To test for anti-fungal properties, *Aspergillus* spp. were cultured on potato dextrose agar and filter paper disks soaked in various concentrations of SL were used. To test for cytotoxicity *Allium cepa* bulbs were allowed to root in 250, 500 and 1000 ppm of SL, and 24 h after rooting, the roots were excised and squashed in iron acetocarmine on microslides and observed under a light microscope.

Flakes of purified SL and a photomicrograph of the fine crystals of SL are shown in figures 1A and B. The yield of the compound ranged between 1.3 and 2.5% on dry weight basis. The compound is soluble in chloroform, methanol, ethanol, acetone and acetic acid, and insoluble in hexane, benzene, toluene and xylene. The compound has been identi-
fied as parthenin \( \text{C}_{15}\text{H}_{18}\text{O}_{4} \) by studying its NMR spectrum (figure 2) and by using HPLC.

The compound has an inhibitory effect on wheat seed germination (figure 1C). More than 60% reduction in germination percentage, 68% in shoot length, 82% in root length and 81% in biomass (table 1) have been noticed in 1000 ppm SL after 72 h of germination. None of the concentrations used had any stimulatory effects. Aspergillus fungi were not able to grow freely even 3 days after inoculation around filter paper disks soaked in 1000 ppm of SL (figure 1D). However, lower concentrations (250 and 500 ppm) did not show inhibition of fungal growth. Allium cepa roots obtained in 250 and 500 ppm of SL did not show any anomalies, while those obtained in 1000 ppm showed micronuclei, chromosomal breakage, stickiness and lagging fragments (figure 1E–H). It is noteworthy to mention here that roots obtained in higher concentration fail to survive after 96 h. This establishes cytotoxicity.

These studies suggest that SL, identified as parthenin \( \text{C}_{15}\text{H}_{18}\text{O}_{4} \), can cause mutation, and inhibition of seed germination and growth. The compound also has antifungal properties. It can therefore be exploited in agriculture. Critical studies in this direction are in progress.

The authors are grateful to CSIR, New Delhi, for financial assistance to (TMP), and to Prof. E. Rodriguez, Phytochemical Laboratory, California.

### Table 1 Effect of SL on germination and growth of wheat after 72 h of germination

<table>
<thead>
<tr>
<th>SL concentration (ppm)</th>
<th>Germination (%)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Root shoot dry wt. (100 seedlings) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>3.4 ± 0.9</td>
<td>2.86 ± 0.75</td>
<td>1.23 ± 0.15</td>
</tr>
<tr>
<td>250</td>
<td>100</td>
<td>3.6 ± 0.81</td>
<td>2.34 ± 0.61</td>
<td>1.04 ± 0.10</td>
</tr>
<tr>
<td>500</td>
<td>68 ± 2.5</td>
<td>2.4 ± 0.85</td>
<td>1.54 ± 0.67</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>1000</td>
<td>34 ± 1.2</td>
<td>0.6 ± 0.02</td>
<td>0.9 ± 0.1</td>
<td>0.23 ± 0.01</td>
</tr>
</tbody>
</table>

± S.D.
University, Irvine for identifying the compound on NMR and HPLC.

2 January 1988


CHANGES IN DNA CONTENT IN GAMMA-IRRADIATED NUCLEI OF CHARA ZEYLANICA

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Radiation-induced changes in Charophyceae have been studied by many workers regarding cytological, morphological and reproductive behaviour. In the present study changes in the DNA content were measured cytophotometrically in Chara zeylanica var. and f. zeylanica irradiated with gamma-radiation.

C. zeylanica Klein ex Willd. var. and f. zeylanica was cultured in soil water biphasic medium. Gamma-irradiation was given from a $^{60}$Co source at the rate of 2 Kr per min for 2 min. The dose of 4 Kr was chosen because the plant could survive at this dose with varied morphological changes. The irradiated plants were allowed to grow further for 15 days. After 15 days, the nuclei were stained by Feulgen stain following the standard method. Cytophotometric analysis was done in a Reichert Zetopan microspectrophotometer following single wavelength (550 nm) method. The nuclear DNA amount was measured on the basis of optical density in terms of arbitrary units of relative absorbances.

The DNA content of amitotically dividing nuclei in internodal cells increased after irradiation (table 1). The size of the amitotic nuclei also increased 3 to 4 times than the controlled one (1500 $\mu$m$^3$ to 5000 $\mu$m$^3$).

The interphase and mitotic metaphase of antheridial filament cells did not show any remarkable differences in the DNA content between the controlled and the irradiated plants. The antheridial filamentous cells in which DNA content remained unchanged after the irradiation were only able to enter into mitosis; hence, the DNA content of prophase nuclei of control and irradiated plants was similar and normal metaphase started with the same amount of DNA for both the control and the irradiated metaphases. A remarkable differences in the DNA content between the telophase nuclei of control and irradiated plants were noted (table 1) in the antheridial filament cells.

The increased DNA content in vegetative nuclei of the plant body was due to the formation of giant nucleus. Irradiation-induced giant cell formation in algae has been observed by many workers. It has been observed that the giant cell formation in yeast cells after treatment with X-radiation is due to non-formation of septum without hampering the DNA synthesis. In the present case, amitotically dividing nuclei became gigantic, and the increased DNA content implied an endomitotic reduplication of the DNA of the nuclei. Giant cells in Mougeotia are reported to be polyploid. In the case of reproductive cells, i.e., antheridial filamentous cells of Chara, no change in DNA content was observed in interphase and prophase nuclei. In other words, those cells in which DNA content did not undergo any appreciable change due to irradiation were only able to enter into mitosis. A remarkable difference in DNA amount between the telophase nuclei of control (0.36 ± 0.002) and irradiated plants were noted (0.25 ± 0.002). This was probably due to loss of small quantity of DNA during abnormal cell cycle of post-irradiated nucleus.

The authors are grateful to CSIR, New Delhi, for financial support.

### Table 1 DNA content (arbitrary units) of control and irradiated nuclei at different stages of cell division in C. zeylanica

<table>
<thead>
<tr>
<th>Nuclei of different stages</th>
<th>Control</th>
<th>Irradiated (4 Kr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitotic nuclei of internodal cell</td>
<td>4.61 ± 0.006</td>
<td>6.39 ± 0.009</td>
</tr>
<tr>
<td>Prophase of antheridial filament cell</td>
<td>1.01 ± 0.002</td>
<td>1.01 ± 0.002</td>
</tr>
<tr>
<td>Metaphase of antheridial filament cell</td>
<td>1.08 ± 0.004</td>
<td>1.01 ± 0.002</td>
</tr>
<tr>
<td>Telophase of antheridial filament cell</td>
<td>0.36 ± 0.002</td>
<td>0.25 ± 0.002</td>
</tr>
</tbody>
</table>