11.

Bioassay for Phytotoxicity

Phytotoxic compounds including weedicides and herbicides are of importance to the agrochemical industry. The continuous use of synthetic phytotoxic compounds may affect non-target organisms and also cause problems of environmental pollution due to degradation of these compounds. Therefore the search for naturally occurring phytotoxic compounds to replace and/or reduce the use of synthetic hazardous phytotoxic compounds is of importance.

Allelopathy offers a new approach for the discovery of new lead compounds and their use as herbicides and pesticides from plants, fungi and microorganisms. The definition for allelopathy adapted by the International Allelopathy Society in 1966 is "The science that studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agricultural and biological systems". These allelochemicals are bioactive natural products that induce a wide array of biological effects and their understanding could provide great benefits in agriculture and weed management.

Bioassay with Lettuce Seeds

Use surface sterilized (with 10% Clorox) lettuce seeds and rinse with distilled water. This kills fungal spores that can interfere with seed germination. Place sterilized 8 cm filter papers in each 9 cm Petri-dish. If the sample to be tested is water soluble, dissolve the sample (2 mg) in 1 ml of 1% triton X-100 : water (1:9) mixture. If the sample is insoluble in water add 1% ethanol or 1% DMSO or 1% acetone. Dilute the solutions to obtain required final concentrations to be tested (for crude extracts - 2000, 1000, 500 ppm and for pure compounds 500, 250, 100, 50, 25, 10, 5 ppm etc.).

Add each of the test samples in solution (2 ml) to a Petri-dish and spread evenly over the filter paper. Prepare a control by setting up dishes without compounds. To each dish, add 5 lettuce seeds, space evenly on

the filter paper so that they do not touch each other or the sides of the dish. Seal Petri-dishes with Parafilm to ensure close-system model. Incubate Petri-dishes in the dark at room temperature for 5 days. Count the germinated seeds after 1st, 3nd and 5th day. On the 5th day, measure root and shoot length to the nearest mm. If the radicle is >1.5mm in the length, consider that the seed has germinated.

The percentage inhibition of radicle elongation is expressed as:

$$\% \text{ inhibition of radicle elongation} = \frac{\text{Control radicle length} - \text{Treated radicle length}}{\text{Control radicle length}} \times 100$$

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