Screening of Allelopathy by Bioassay and Isolation of Allelochemicals: New Bioactive Agrochemicals from Natural Products

Yoshiharu Fujii
National Institute for Agro-Environmental Sciences
3-1-3, Kannondai, Tsukuba, Ibaraki 305-8604, Japan

Summary
Exploitation of new assessment methods for allelopathy was conducted, and using these methods, screening of allelopathic plants useful for agriculture was done over ten years for 1200 plant species. A practical application of allelopathic plants for weed control on the field was also conducted using the allelopathic plants screened in the bioassay.

As for the exploitation of new bioassay methods for allelopathy, a specific method for each route of action in allelopathy was established. There are four routes of action in allelopathy. As for the first route, root exudates, a unique mixed planting method in agar medium with donor plants and acceptor plants in the same plant box was established and named "Plant Box Method". As for the second route, leaf leachates, inhibitory effect of leaf litters were evaluated by placing leaves between two layers of agar and named "Sandwich Method". As for the third route, effect through the emission of volatile chemicals, "Dish Pack Method" was established. As for the fourth route, release of allelochemicals to the rhizosphere soil, "Rhizosphere soil method" using rhizosphere soil carefully separated from the surface of the root, was exploited.

By using these methods, about 800 plant species were evaluated over ten years. The most promising plants were as follows; Velvet bean (Mucuna pruriens), Lycoris radiata, Sphenoclea zeylanica, Duranta repens. Using the same bioassay methods, rice cultivars with strong allelopathic characteristics were also evaluated and red rice (old traditional shrine rice) cultivars were found to be strongly allelopathic.

As for the screening of practical allelopathic plants which have weed suppression and vegetation control ability in the field, field trials were conducted and hairy vetch (Vicia villosa) was found to be most promising. Hairy vetch is a leguminous pasture plant, and when sown in the fall could cover the surface of the land almost completely in the spring, and suppress weeds almost completely. After blooming in June, it will eventually die and produce a heavy mulch like straw. Hairy vetch could produce 200 to 400 kg of nitrogen per hectare by nitrogen fixation and serve as a good green manure. By means of these characteristics, it was concluded that hairy vetch is most suitable for weed control under fruit gardens or in abandoned fields. Hairy vetch is now slowly being distributed in Japan.

In order to isolate allelochemicals in action, a new strategy of "Total Activity Method", taking
concentrations of natural chemicals in plants into account, was developed. By means of this strategy, L-3,4-dihydroxyphenylalanine (L-DOPA) was isolated from Velvet bean, lycorine and related alkaloids were isolated from *Lycoris radiata*, rutin, gallic acid and fagomine were isolated from buckwheat, and methyl isothiocyanate was isolated from Spider lily (*Creome spiosa*). From a new invader weed, *Sphenoclea zeylanica*, a series of new cyclic dithiolane compounds were isolated and named Zeylanoxides. From the steam distillation concentrate of cut off mixtures of young tree twigs, 1,2-propanediol was identifies as a plant growth promoting chemical.

As for the allelochemicals of hairy vetch, after 10 years of trials, and careful spectroscopic examination, cyanamide was identified as the main factor of plant growth inhibition. Cyanamide is a well-known compound as a component of artificial nitrogen fertilizer, ‘calcium cyanamide,’ hence our finding is the first to claim that cyanamide exists as a natural chemical in plants.