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BRIEF COMMUNICATION

Allelopathic potential of *Pueraria thunbergiana*

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Abstract

The allelopathic potential of *Pueraria thunbergiana* was investigated under laboratory conditions. The powder of freeze-dried leaves of *P. thunbergiana* inhibited the germination and the growth of roots and shoots of cress, lettuce, timothy and ryegrass. Significant reductions in the germination and growth of roots and shoots were observed as the powder concentration increased in all bioassays. The putative compounds causing the inhibitory effect of the powder were isolated and determined by their spectral data as *cis,trans-* and *trans,trans*-xanthoxin.

Additional key words: allelopathy, germination inhibitor, growth inhibitor, phytotoxicity, weed management.

The perennial legume *Pueraria thunbergiana* is widely used in agriculture systems in tropical regions as a forage or cover crop to reduce soil erosion (Tian *et al.* 1999, Chikoye *et al.* 2001, Schroth *et al.* 2001). The residue of its leaves was found to affect several chemical constitutions including nitrogen in the soil (Vesterager *et al.* 1995, Luna-Orea and Wagger 1996, Schroth *et al.* 2000). However, little knowledge exists on the allelopathic potential of the residue of this plant leaves.

The negative impacts of commercial herbicide use on the environmental systems of the world make necessary to diversify weed management options (Putnam 1988, Weston 1996, Einhellig 1996). It has been observed that many plant species can provide excellent weed suppression after incorporation of their residues into soil (Weston 1996, Narwal 1999, Semidey 1999, Caamal-Maldonado *et al.* 2001, Kato-Noguchi 2001). It was therefore of interest to assess the allelopathic potential of this species under laboratory conditions for possible weed control purpose.

Leaves of *Pueraria thunbergiana* Benth. were washed thoroughly with tap water and rinsed with distilled water. After blotting dry with filter paper (*No. 1*; *Toyo Ltd*, Tokyo), the shoots were freeze-dried and ground to a fine powder using a mortar and pestle.

Four species, cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), timothy (*Phleum pratense* L.), and ryegrass (*Lolium multiflorum* Lam.) were used for bioassay as test plants because of their known germination behaviour.

Leaf powder (0, 3, 10, 30, 100 or 300 mg) of *P. thunbergiana* was mixed with sterilized quartz sand (25 g) in a 9-cm Petri dish and quartz sand was moistened with 10 cm^3 of distilled water according to the method of Shilling *et al.* (1992). The concentration of the powder in the bioassay was 0, 0.3, 1, 3, 10 and 30 g dm^{-3}. Seeds of the test species were sterilized in 25 mM solution of sodium hypochlorite for 15 min and rinsed in distilled water four times. Fifty seeds of each species were separately sown on the quartz sand in the Petri dishes and allowed to germinate in the dark at 25 °C for 36 h (cress, lettuce) or 48 h (ryegrass, timothy). Then the germinated seeds were counted and the percentage germination was calculated by reference to that of control seeds which had been treated with distilled water.

Seeds of the test species after sterilization were allow to germinate on filter paper (*No. 2*; *Toyo Ltd*, Tokyo, Japan) in the dark at 25 °C for 24 h (cress, lettuce) to 36 h (ryegrass, timothy). Then, the 30 germinated seeds of each species were separately arranged on the quartz sand in the Petri dishes which contained leaf powder of *P. thunbergiana* as described above, and incubated in the dark at 25 °C for 48 h. The shoot and root length of the seedlings was then measured with a ruler and the percentage length of seedlings was calculated by reference to the length of control plants treated with distilled water.

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Abbreviation: I_{25} - concentration required for 25 % inhibition in the assay.
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The allelopathic potential of leaf powder of *P. thunbergiana* was tested with seed germination and plant growth of cress, lettuce, timothy, and ryegrass. The powder suppressed the germination at concentrations greater than 1 g dm^{-3} for cress, lettuce and timothy seeds and 3 g dm^{-3} for ryegrass seeds, respectively (Fig. 1*A*). When germination percentage was plotted against the logarithm of the concentrations, the response curves of the test plants were linear between 10 and 50 % inhibition for lettuce and cress, 10 and 40 % inhibition for timothy and 10 and 30 % inhibition for ryegrass. The concentration required for 25 % inhibition in the assay (defined as I_{25}) were 1.82, 2.48, 3.42 and 11.3 g dm^{-3} for lettuce, cress, timothy and ryegrass, respectively. Response curves and I_{25} values indicate that the effectiveness of the powder on lettuce germination was greatest and followed by that on cress, timothy and ryegrass.

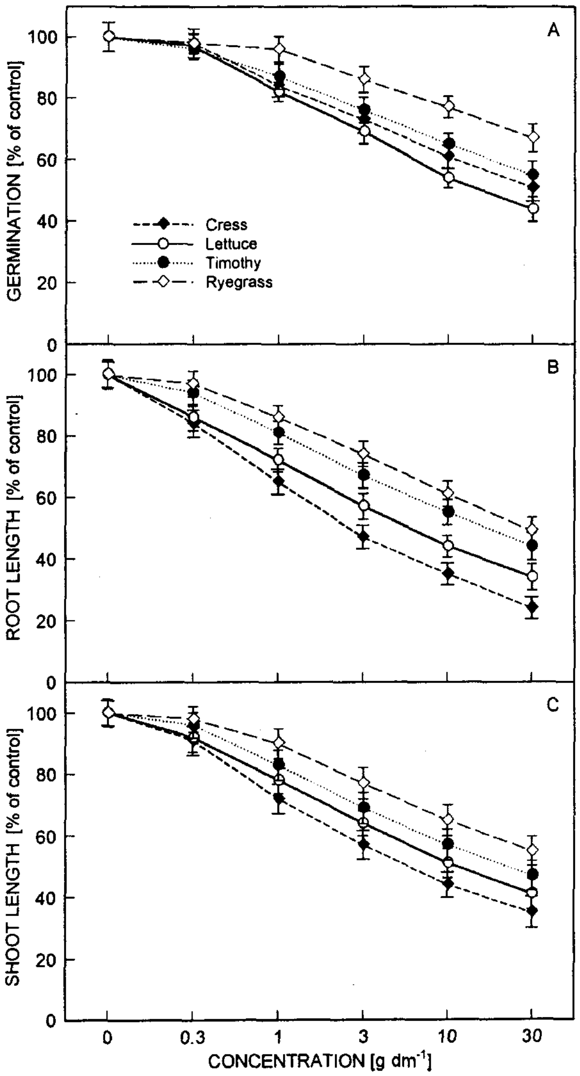

Fig. 1. Effects of leaf powder of *P. thunbergia* on germination (*A*), and root (*B*) and shoot (*C*) growth of test plants. Germination rates of control cress, lettuce, timothy and ryegrass seeds were 95 ± 4, 89 ± 5, 71 ± 8 and 73 ± 6 %, respectively. Root lengths of control cress, lettuce, timothy and ryegrass plants were 24.1 ± 2.1, 19.5 ± 1.9, 22.9 ± 2.2 and 28.7 ± 2.4 mm, respectively. Shoot lengths of control cress, lettuce, timothy and ryegrass plants were 14.2 ± 1.3, 12.7 ± 1.6, 11.5 ± 1.1 and 18.4 ± 1.8 mm, respectively. Means ± SE from three replicate experiments with 50 seeds each for germination and from five replicate experiments with 30 plants each for root and shoot growth are shown.

The powder suppressed the root growth at concentrations greater than 0.3 g dm^{-3} for cress, lettuce seedlings and 1 g dm^{-3} for timothy and ryegrass seedlings, respectively (Fig. 1*B*), and the shoot growth at concentrations greater than 1 g dm^{-3} for cress, lettuce and timothy seedlings and 3 g dm^{-3} for ryegrass seedlings, respectively (Fig. 1*C*). I_{25} values for root growth were 0.56, 0.79, 1.63 and 2.91 g dm^{-3}, while I_{25} values for shoot growth were 0.78, 1.27, 1.99 and 3.54 g dm^{-3} for cress, lettuce, timothy and ryegrass, respectively. Thus, the effectiveness of the powder on the root and shoot growth of cress seedlings was greatest and followed by that of lettuce, timothy and ryegrass seedlings. In the same test plants, the effectiveness of the powder was greatest on the root growth, and followed by on the shoot growth and germination (Fig. 1). Compared with the results of Shilling *et al.* (1992) on allelopathic potential of celery, the effectiveness of the leaf powder of *P. thunbergiana* on lettuce growth was comparable to or better than that of celery residues. In all bioassay, significant reductions in the germination and growth of the roots and shoots were observed as the powder concentration increased. Such rate-dependent responses of the test plants suggest that the powder of *P. thunbergiana* might contain allelochemical(s) (Chung and Miller 1995, Babu and Kandasamy 1997, Kato-Noguchi 2000).

Leaf powder (20 kg fresh mass equivalent) of *P. thunbergiana* was extracted and purified by several chromatographic fractionations as described by Kato-Noguchi (1992), and finally two active compounds, inhibitor α (0.6 mg) and inhibitor β (0.8 mg) were isolated. Inhibitor α and β had UV spectra with peaks of absorbance in methanol at 282 and 284 nm, respectively. High-resolution mass spectroscopy yielded the following data (relative intensity, element composition): M^+, 250.1563 (13, $C_{15}H_{22}O_3$); 168.1157 (38, $C_{10}H_{16}O_2$) and 149.0965 (100, $C_{10}H_{13}O_1$) for α; and M^+; 250.1573 (18, $C_{15}H_{22}O_3$), 168.1162 (41, $C_{10}H_{16}O_2$) and 149.0971 (100, $C_{10}H_{13}O_1$) for β, respectively. These data indicates that inhibitors α and β, respectively, was either *cis, trans*-xanthoxin or *trans,trans*-xanthoxin (Burden and Taylor 1970, Taylor and Burden 1972). Xanthoxin was found in several plant species as a growth inhibitor (*e.g.* Firn *et al.* 1972, Taylor and Burden 1972), however, there has been no record the presence of xanthoxin in *P. thunbergiana*.

Controlling weeds through allelopathy is one strategy to reduce herbicide dependency, although synthetic chemical herbicides may continue to be a key component in most integrated weed management systems (Putnam

1988, Einhellig 1996, Seigler 1996, Duke *et al.* 2000). In the present research, powder of *P. thunbergiana* was found to be able to work as weed inhibiting agents, which might reduce application of the commercial herbicide in a variety of agricultural settings. The putative compounds causing the inhibitory effect of the powder were isolated and determined by their spectral data as *cis,trans-* and *trans,trans-*xanthoxin.

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