

THE EFFECT OF *Phyllanthus virgatus* EXTRACT ON COMMON WEED SPECIES

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ABSTRACT

The potential of allelochemicals from *Phyllanthus virgatus* to inhibit plant germination and growth was tested using 10 common weed species. An aqueous extract of *P. virgatus* (leaf/stem/fruit), was mixed with agar to produce a 5% FW/v substrate concentration. Seeds of *Apium leptophyllum*, *Avena fatua*, *Bidens pilosa*, *Chenopodium album*, *Echinochloa crus-galli*, *Lolium rigidum*, *Parthenium hysterophorus*, *Raphanus raphanistrum*, *Sonchus oleraceus* and *Urochloa panicoides* were placed on the agar and germinated under controlled conditions for 7-16 days. The extract caused a significant reduction in the germination of *A. leptophyllum*, *L. rigidum*, *P. hysterophorus* and *S. Oleraceus* from 100% down to 63.2%, 88.0%, 49.4% and 40.8%, respectively. Shoot growth was significantly reduced by 8.1% to 65.8% in most weed species. Root growth was so severely affected in all species that the survival of seedlings would be unlikely if the extract environment was maintained.

Keywords: Allelochemicals, allelopathy, medicinal plants, natural herbicide.

INTRODUCTION

In the search for better weed management strategies, researchers have been investigating the potential of allelopathic interactions to be developed into a natural herbicide. Many interactions investigated to date have been based on field observations, such as limited growth of plants around particular trees, reduction in crop yields when rotations are not practised and the use of cover crops to reduce weeds. As an alternative approach to identifying allelopathic plants i.e., donor plant selection, this research assesses the potential of medicinal plants. Medicinal plants contain an array of bioactive chemicals, which may have multiple actions.

Phyllanthus sp. are widely distributed in tropical and sub-tropical areas and have long been used in traditional medicine in countries such as India (Calixto *et al.* 1998), particularly for liver disorders and urinary tract infection (Chaudhary and Rao 2002). Studies have shown that the *P. virgatus* extracts have strong antioxidant activity and it has been suggested that this activity helps to scavenge harmful free radicals produced by some diseases and

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medical disorders. The antioxidant activity has been attributed to the phenolic compounds contained in the plants (Kumaran and Karunakaran, 2007). Further work has identified numerous alkaloids, flavonoids, lignans, phenols and terpenes from plants within this genus (Calixto *et al.*, 1998) with five new compounds identified from *P. virgatus* alone, including a norlignan (virgatyne), a tannin (virganin) and three flavonoid sulfonates (Huang *et al.*, 1998).

Phyllanthus sp., like many other traditional medicinal plants have been tested for their pharmacological value, however assessing the potential of bioactive compounds in these plants to inhibit the growth of other plants is rarely reported. In earlier work (Allan and Adkins, 2007), bioassays of a selection of medicinal plants found that aqueous extracts of *P. virgatus* could inhibit the growth of *Lemna aequinoctialis* by up to 91%. To follow on, this work tests whether aqueous extracts of *P. virgatus* can inhibit the germination and growth of common weed species.

MATERIALS AND METHODS

Seed material

Ten common weed species were selected for testing, two summer grasses, two winter grasses and both winter and summer-growing broadleaf weeds. Seeds of *Apium leptophyllum*, *Avena fatua*, *Bidens pilosa*, *Chenopodium album*, *Echinochloa crus-galli*, *Lolium rigidum*, *Parthenium hysterophorus*, *Raphanus raphanistrum*, *Sonchus oleraceus* and *Urochloa panicoides* were stored at the University of Queensland at approximately 15°C and 15-20% RH.

Several methods were used to improve the germination percentage of the seed lots. Seeds of *C. album*, *L. rigidum* and *S. oleraceus* (with the papus removed) were sorted in a New Brunswick General Seed Blower and the lighter unfilled seeds discarded. To overcome physical dormancy, seeds of *A. fatua* were dehusked. Both the pod and seed coat of *R. raphanistrum* were removed as both have been shown to impose dormancy (Young, 2003).

Plant extract

The above ground parts (leaf/stem/fruit) of healthy *P. virgatus* plants were selected from footpaths and public parks in Bardon, Qld.

Leaf, stem and fruit of *P. virgatus* were collected, weighed, loosely washed, patted dry and then chopped into pieces <1cm². The chopped material was then placed in a jar of deionised water to produce a 12.5% Fresh Weight/volume (FW/v) concentrate. The sealed jars were kept in darkness for 48h at 22°C. The concentrate was then filtered through a fine sieve (mesh <1mm).

Extract agar was made by dissolving 12.5g of Agar (Sigma) into 600ml of heated deionised water. After the agar was dissolved, 150ml

of cool deionised water was added and the solution allowed to cool. Once at 55°C, the *P. viragatus* aqueous extract was added to the agar and stirred vigorously to make a 5% FW/v concentrate. The extract agar was then poured into clear plastic Petri dishes (90mm diameter) to a depth of approximately 5mm.

A control treatment using 10% agar alone was mixed, the pH measured and poured into Petri dishes in the same manner.

Germination

Seeds were germinated over a 7 to 16 day period in Thermoline Scientific refrigerated incubators (Model 495-1-SD) set on either a summer diurnal regime of 12h (30°C) in light conditions (PPF of 40µmol m⁻² s⁻¹ supplied by 4 Polylux XL F36W/860 daylight 3250 Lm fluorescent tubes) and 12h (20°C) darkness or a winter diurnal regime of 12h (25°C) light conditions (as above) and 12h (10°C) darkness.

The treatment and control consisted of four replicates of 25 seeds per Petri dish. Seeds were recorded as germinated when either the radicle of hypocotyl exceeded 1mm.

Data analysis

The number of germinated seeds were counted over the test period and the treatment germination percentage (T Gm%) was calculated using:

$$T \text{ Gm}\% = [\text{Treatment germinant No.}/\text{Control germinant No.}] \times 100$$

Shoot and root growth was measured and the shoot inhibition percentage (Shoot In%) and root inhibition percentage (Root In%) calculated using the following:

$$\text{Shoot In}\% = 100 - [(\text{Treatment shoot growth}/\text{Control shoot growth}) \times 100]$$

$$\text{Root In}\% = 100 - [(\text{Treatment root growth}/\text{Control root growth}) \times 100]$$

Statistical analysis was performed via a one-way ANOVA and Tukey-Kramer test (P<0.05), using the program Minitab.

RESULTS

Control germination exceeded 80% for all species with the exception of *C. album* (77%) and *R. raphanistrum* (73%). This was thought adequate for treatment comparisons (Table 1).

DISCUSSION

The germination of *A. leptophyllum*, *L. rigidium*, *P. hysterothorus* and *S. oleraceous* was significantly reduced (63.2%, 88.0%, 49.4% and 40.8%, respectively) due to the extract treatment. The effect was seen in both broadleaf and grass weeds and both

summer- and winter-growing weeds. The reduction in germination percentage implies the seeds had become non-viable or that the extract had imposed dormancy. When the non-germinated seeds were taken off the extract agar, after the 7 to 16 day period and placed on plain agar in the same controlled conditions, the seeds failed to germinate, thereby suggesting that the seeds were non-viable.

Table-1. Germination (Gm %), shoot growth inhibition (Shoot In %) and root growth inhibition (Root In %) of *P. virgatus* extract treatments as a percentage of control germination, shoot and root growth of 10 common weeds. Significant difference ($P < 0.05$) is indicated by * before a value.

Weed Species	Gm %	Shoot In %	Root In %
<i>A. fatua</i>	99.0	* 65.8	* 42.2
<i>A. leptophyllum</i>	* 63.2	12.0	* 95.1
<i>B. pilosa</i>	88.4	* 27.3	* 78.2
<i>C. album</i>	87.0	* 25.4	* 89.2
<i>E. crus-galli</i>	106.7	9.0	* 78.4
<i>L. rigidium</i>	* 88.0	* 46.0	* 88.6
<i>P. hysterothorus</i>	* 49.4	* 38.7	* 92.2
<i>R. raphanistrum</i>	98.6	25.0	* 61.2
<i>S. oleraceous</i>	* 40.8	* 53.9	* 91.7
<i>U. pannicoides</i>	99.0	* 8.1	* 62.0

Root growth for all species was significantly affected (up to 95.1%), with much of the root growth consisting of numerous small aerial roots avoiding contact with the extract agar. The lack of root growth would ultimately affect shoot growth and seedling survival.

The extract also significantly affected shoot growth of most weed species. It was not just the small seeded weeds which may have been more drastically affected by the poor root growth, but also the large-seeded weeds such as *A. fatua*, which had a significant reduction (65.8%) in shoot growth.

Aqueous extract of *P. virgatus* at a concentration of 5% FW/v had a significant effect on germination and early growth of several weeds species. Future work will focus on identification and isolation of allelochemicals and glasshouse trials assessing the effect of these compounds on germination and growth of weeds in a soil medium.

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