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Allelopathic potential of *Dicranopteris pedata*, *Acacia auriculaeformis*, *Cinnamomum burmannii*, and *Melastoma malabathricum*, for ecological control of *Ipomoea cairica*

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ABSTRACT

In pot culture, we studied the individual and combined effects of aqueous leachates of four common plants [*Dicranopteris pedata* (Houttuyn) Nakaike, *Acacia auriculaeformis* A.Cunn.ex Benth, *Cinnamomum burmannii* (Nees et T.Nees) Blume and *Melastoma malabathricum* L.] in southern China on physiological properties (biomass allocation, photosynthetic characteristics and allelopathy) of *I. cairica* L. The results showed that (i) the extracts of single species and the mixed leachates had allelopathic effects on photosynthetic activities and growth of *I. cairica* L., (ii) the allelopathic effects increased with the increase of leachates concentrations and (iii) the mixed leachates of 4-species at 200 $mg \cdot L^{-1}$ concentration was most inhibitory to the invasive specie *I. cairica*. Our results indicated that the aqueous leachates of these four donor plant species may control the exotic invasive species *I. cairica*.

Key words: *Acacia auriculaeformis*, allelopathy, *Cinnamomum burmannii*, *Dicranopteris pedata*, ecological control, invasion, *Ipomoea cairica*, *Melastoma malabathricum*, pot culture

INTRODUCTION

Alien plants invasion is an important factor of global change, as it can reduce the biodiversity and degrade the ecosystem (4,15,22,25,28). Thus, it has become one of the most unmanageable environmental problem. Furthermore, the import and export trade related introduction of numerous alien and ornamental plants in South China has increased the risk of introduction of invasive alien species, thereby increasing the risk of severely disturbing the ecological landscape structure and natural ecosystems (12,24). Thus, exploring methods for the effective prevention and control of invasive alien plants is an important part of invasion ecology research in this region.

Invasive plants can influence the growth and development of adjacent plants through allelopathy, a process wherein allelochemicals are released into the external environment (19,29). Allelochemicals released from the co-existing native plant may inhibit the invasive plants. For example, Zhao (16) reported that high population densities of various weeds produce substantial allelopathic effects, which severely affected the growth of *Linum usitatissimum L*. Tian *et al*. (33) reported that the allelopathic effects of four aquatic plants communities inhibited the growth of *Microcystis aeruginosa*. Li *et al.* (8) and Yin *et al*. (18) developed a stable arbor-shrub-grass plant community in Dongguan Dalingshan Forest Farm and Shenzhen Neilingding Island and they reported that these

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plant communities effectively prevented the invasion of *Mikania micrantha*. These studies showed that regulating the ecological properties of plant community structure can help in the prevention and control of invasive alien plants.

Ipomoea cairica (Convolvulaceae family), is perennial climbing vine with strong propagation and expansion abilities (32). Its distribution and damage is only next to the invasive plant species *Mikania micrantha* (6,23,24). The invasive alien plant *Ipomoea cairica* has become a serious problem in southern China. Thus, exploring efficient ways for its control is important for the forestry researchers in this region. Using selected allelopathic plants can effectively suppress the weeds growth and reduce herbicide application (30,31). The present, research on *I. cairica* mainly focusses on its allelopathic effects on other plants or vegetation (9,13,14,20,21), however, its ecological control is rarely investigated (11). Based on previous studies (5,18,30,31), we selected four common Donor plants [*Dicranopteris pedata, Acacia auriculaeformis, Cinnamomum burmannii* and *Melastoma malabathricum*] in southern China. This study aimed to explore the allelopathic potential of these four species to control *I. cairica*.

MATERIALS AND METHODS

The *Dicranopteris pedata* (Houttuyn) Nakaike, *Acacia auriculaeformis* A.Cunn.ex Benth, *Cinnamomum burmannii* (Nees et T.Nees) Blume and *Melastoma malabathricum* L. were donor plants and *Ipomoea cairica* (L.) Sweet was the acceptor plant. The leaves of the donor plants were collected from the arboretum of South China Agricultural University (SCAU) and the stems of the *I. cairica* were collected from our University campus.

Aqueous leachates

The aqueous leachates of single species and their mixed leachates were used to treat *I. cairica*. The photosynthesis indexes, biomass, and other physiological properties of *I. cairica* were examined to test the effects of the aqueous leachates for the ecological control of *I. cairica.* The aqueous leachates of the donor spp. of 50 $mg{\cdot}L^{-1}$, 100 $mg{\cdot}L^{-1}$, and 200 $mg{\cdot}L^{-1}$ concentrations were prepared by soaking the 50, 100, 200 mg biomass in one litre distilled water. Two hundred mg fresh leaves of each donor species were weighed and immersed in 1 L deionized water at room temperature for 24 h. All leachates were filtered twice through 0.45-μm filter membrane to remove microorganisms. The leachates were further diluted with distilled water to prepare required concentrations of 50 $mg{\cdot}L^{-1}$ and 100 $mg{\cdot}L^{-1}$. The leachates were then stored in refrigerator at 4 °C for later use.

Pot culture

The healthy branches from fresh stems of *I. cairica* were collected and cut as vegetative cuttings (9 cm long). The leaves were removed from the stems, but the shoot buds were retained. The cuttings were placed in plastic pots (20 cm diameter, 25 cm depth). One liter distilled water was applied per pot until the lower stem nodes of cuttings were submerged. The water was irrigated daily. When fibrous roots in the cuttings grew around the lower stem node and reached 3 cm (in 10 days), the cuttings were transferred to plastic pots (20 cm diameter, 25 cm depth) with 700 g soil (field soil from the Ecological Experiment Station, SCAU). The plastic pots with the cuttings (one plant in each pot) were kept in greenhouse for culture. Ten days after transplanting the seedlings/cuttings, the donor plant leachates of various concentrations were applied (100 mL per pot). Clean water was used as control. During the test period (May 2016 to August 2016), each pot was irrigated once every three days.

Parameters studied

After the aqueous leachate treatment, full expanded leaves of uniform sizes were selected from *I. cairica* to determine the photosynthetic indexes. LI-6400 portable photosynthetic apparatus (LI-COR, USA) was used to determine the net photosynthetic rate (P_n), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) of the leaves under a light intensity of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. An open gas system was adopted to determine the photosynthetic rates. In this system, the air flow rate was 0.5 $L \cdot min^{-1}$, the leaf temperature was 25 °C, the relative humidity was 60%, and the CO_2 concentration in the environment (C_a) was 360 $\mu mol \cdot mol^{-1}$. The determination periods with more active photosynthesis were 8:00-11:30 and 14:00-16:30. Three fully expanded healthy leaves were then randomly selected for each repeat and 5-repeats were done per treatment. The measurements were done from the middle of each selected leaf.

For biomass measurements, the parts/organs of each plant were harvested carefully and the stems and leaves were kept separately in envelopes. The roots were washed with clean water to remove the sand and then placed in envelopes. The roots, stems and leaves were oven-dried at 70 °C for 72 h. Their dry weights were obtained. The total biomass, root biomass ratio, stem biomass ratio and leaf biomass ratio were calculated.

Statistical analysis

To test the effects of aqueous leachates and concentrations on photosynthetic index and biomass of *I. cairica*, two-way ANOVA models were run using the five aqueous leachates and three extract concentrations as the factors. All analyses were performed using SPSS 22.0 for Windows.

RESULTS AND DISCUSSION

Photosynthetic indexes

The leachates significantly affected the net photosynthetic rate, stomatal conductance and intercellular CO_2 concentration of *I. cairica* (Table 1) and the source of leachates had significant effects on the stomatal conductance and intercellular CO_2 concentration of *I. cairica*, whereas there were no significant effects on net photosynthsis rate. There were no interaction effects of source of leachates; leachates and its concentration on these three indexes (Table 1).

The leachate decreased the net photosynthetic rate, stomatal conductance and intercellular CO_2 concentration of *I. cairica* with increase in leachates concentration (Fig. 1). Although there were no significant impacts of the five leachates (*D. pedata, A. auriculaeformis, C. burmannii, M. malabathricum* and mixed leachates), but the mixed leachates were most inhibitory to the three photosynthetic indexes, particularly at the highest concentration.

Table 1. ANOVA results of the effects of aqueous leachates on the photosynthetic index of *I. cairica*.

Variable Source		Photo (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)		Cond ($mol \cdot m^{-2} \cdot s^{-1}$)		Ci ($\mu mol \cdot mol^{-1}$)	
	df	F	P	F	P	F	P
Source of leachates (S)	4	1.188	0.335	3.079	**0.030**	3.953	**0.010**
Leachate concentration (C)	2	4.567	**0.018**	7.627	**0.002**	38.144	**<0.001**
S × C	8	0.320	0.952	0.408	0.907	0.879	0.544

Source of leachates was the leachates of *D. pedata, A. auriculaeformis, C. burmannii, M. malabathricum* and the mixed aqueous leachates of the four species. Photo indicates net photosynthetic rate, Cond means stomatal conductance, Ci means intercellular CO_2 concentration. The significant effect at α=0.05 level is shown in bold.

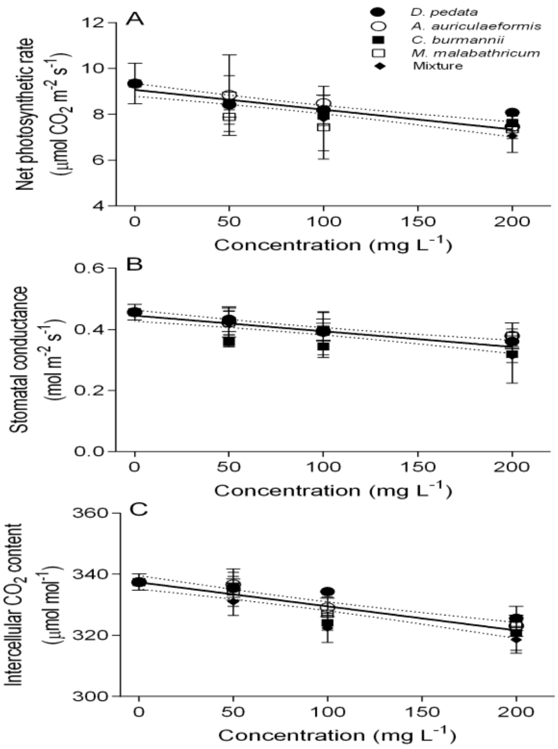

Figure 1. Effects of plant aqueous leachates from *D. pedata, A. auriculaeformis, C. burmannii and M. malabathricum* and mixed aqueous leachates on the net photosynthetic rate (A), stomatal conductance (B) and the intercellular CO_2 concentration (C) of *I. cairica.* Values are means ± SE from three replicates.

The individual leachates significantly inhibited the stomatal conductance of *I. cairica* (Table 1, Figure 1B). At 50 $mg \cdot L^{-1}$ concentration, the aqueous leachates of *D. pedata* was slightly inhibitory (6.52%), while the aqueous leachates of *C. burmannii* was more inhibitory (21.74% inhibition). At the higher concentrations of leachates, similar trend occurred among the five leachates. Overall, the mixed aqueous leachates were more inhibitory than leachates of each single species. The highest concentration (200 $mg \cdot L^{-1}$) of the mixed leachates caused the lowest stomatal conductance (0.31 $mol \cdot m^{-2} \cdot s^{-1}$), suggesting the strongest inhibition (Figure 1B). This indicated that the high concentrations of mixed aqueous leachates of four local plants significantly inhibited the stomatal conductance of *I. cairica* seedlings. In addition, the similar effects occurred on the intercellular CO_2 concentration (Table 1, Figure 1C).

Photosynthesis is the basis for plant growth and development (7,26). The net photosynthetic rate (P_n) determines the substance accumulation in plants and the plant growth rate. The growth rate of a plant determines its superiority in its niche in a certain community (27). The stomatal conductance (G_s) is used to reflect the status of the pores that directly affects the plants using atmospheric CO_2, thus affecting the photosynthetic process of the plants. When affected by the status of the stomata and CO_2 concentration, the intercellular CO_2 concentration (C_i) is reduced under low stomatal conductance because of the unchanged demand for CO_2. Our results showed that the aqueous leachates of several local plants inhibited the net photosynthetic rate, stomatal conductance and intercellular CO_2 concentration and the inhibitory effect increased with the concentration.

Biomass

The leachates showed significant inhibitory effects on the total biomass and biomass allocation of *I. cairica*. The leachates concentrations inhibited the total biomass, the root and leaf biomass allocation (Table 2). The total biomass, stem and leaf biomass showed significant interaction between leachate origin and concentration (Table 2).

Table 2. ANOVA results of the effects of aqueous leachates on biomass and biomass allocation of *I. cairica*.

Variable Source		Total biomass(g)		Root biomass ratio		Stem biomass ratio		Leaf biomass ratio	
	df	F	P	F	P	F	P	F	P
Source of leachates (S)	4	95.395	**<0.001**	66.137	**<0.001**	45.860	**<0.001**	131.391	**<0.001**
Leachate concentration (C)	2	111.830	**<0.001**	3.710	**0.030**	0.463	0.632	7.445	**0.001**
S × C	8	17.511	**<0.001**	1.627	0.135	2.682	**0.013**	6.735	**<0.001**

The bold numbers showed significant influence off actors at α=0.05 significance level.

The increase in concentration of five leachates, except the *A. auriculaeformis* the total biomass decreased. Overall, among the five leachates, the mixed leachates of four species were most inhibitory to the total biomass (Figure 1A). Most of the leachates at the lowest concentration (50 $mg \cdot L^{-1}$) had no significant impacts on the total biomass of *I. cairica*, while all leachates except *A. auriculaeformis* showed inhibitory effects at 100 $mg \cdot L^{-1}$ and 200 $mg \cdot L^{-1}$ (Figure 2A). At the 200 $mg \cdot L^{-1}$ concentration, the mixed aqueous leachates have the strongest inhibitory effect, reduced the total biomass by 31.57% relative to the control. However, no significant difference was observed between the mixed aqueous leachates and *M. malabathricum* leachates (Figure 2A).

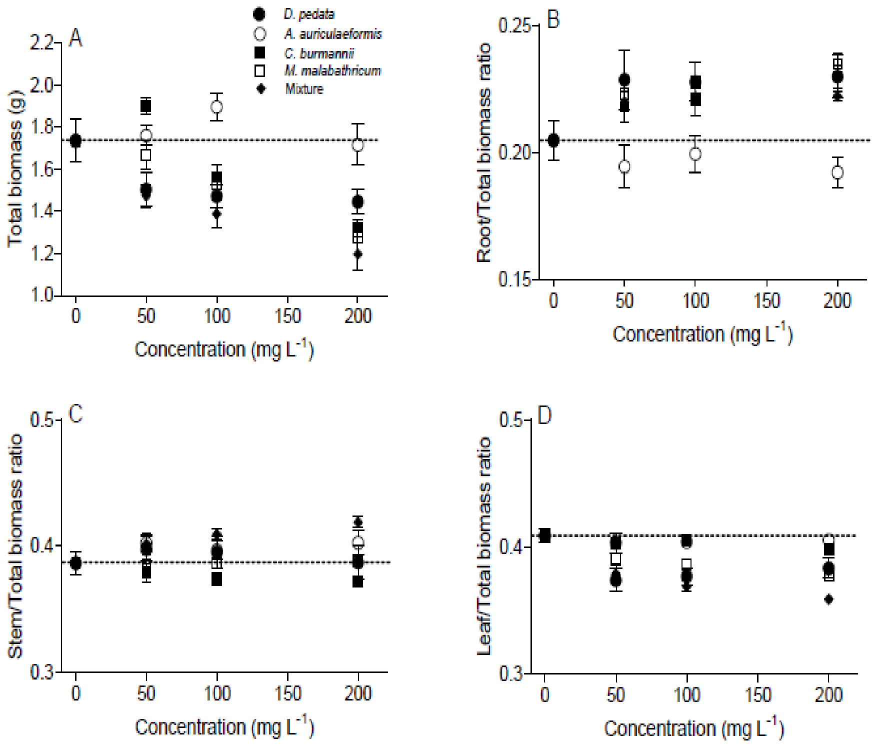

Figure 2. Effects of aqueous leachates from *D. pedata, A. auriculaeformis, C. burmannii and M. malabathricum* and mixed aqueous leachates on the total biomass (A), root biomass ratio (B), stem biomass ratio (C), leaf biomass ratio (D) of *I. cairica.* Values are means ± SE. The dash line represents the control (water). Statistical analysis results are provided in Table 2.

The five leachates and their concentrations had significant effects on the stem and root biomass of *I. cairica* than control (Figure 1B and 1C). The leachates except *A. auriculaeformis* increased the root biomass of *I. cairica*, only a few showed inhibitory effects on root biomass of *I. cairica* relative to control.

The leachates origin/sources and their concentration significantly influenced the leaf biomass ratio of *I. cairica*, and there was significant interaction between leachate origins and leachate concentration (Table 2). The overall leaf biomass decreased when treated with the aqueous leachates (Figure 2D). Among the five leachates, the mixed leachates had the strongest inhibition on the leaf biomass ratio of *I. cairica* across the three concentrations. At 200 $mg{\cdot}L^{-1}$ concentration, the mixed aqueous leachate reduced the leaf biomass ratio by 11.49% relative to the control. No significant difference was observed between the effects of leachate of *D. pedata* and *M. malabathricum*. Moreover, the leachate of *A. auriculaeformis* and *C. burmannii* had no significant effects on leaf biomass ratio relative to the control.

The plant biomass allocation pattern reflects the response of a plant to environmental changes in terms of growth and development (1,3,10). The highest concentration showed the strongest inhibitory effect (2,17). In present study, the aqueous leachate enhanced their inhibitory effects with the increase in their concentration. Among the five leachates, the leachate of *A. auriculaeformis* (Leguminosae family) stimulated the total biomass and reduction in root biomass ratio of *I. cairica* at lower concentration, this may be associated with its N-fixing ability and higher N content in its leaf leachates. Moreover, the mixed leachate showed strongest inhibitory effects on the growth of invasive plant *I. cairica* among the five leachates. Our results indicate that the diversity of aqueous leachates may strengthen the allelopathic potential and resistance to the exotic invasive species *I. cairica*, and the unique trait of a certain species (e.g. N fixing ability of *A. auriculaeformis*) should be considered. This study provides a new insight into the management and control of exotic invasive species. Further study is needed to explore the effects of diversity of allelochemicals from different native plant species on invasive plant species and the possibility of controlling invaders by diverse allelochemicals from multiple plant species.

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