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SHORT COMMUNICATION

Allelopathic potential of reproductive organs of exotic weed *Lantana camara*

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ABSTRACT

We studied the allelopathic effects of aqueous extracts of leaf and reproductive organs (flower and fruit) of *L. camara* on seed germination, seedling growth and dry matter production of radish and lettuce. The reproductive organs of *L. camara* were found allelopathic and the effects were concentration-dependent. GC-MS analysis showed that the reproductive and vegetative organs have similar volatile compounds but their contents were different. Fruit extracts were most stimulatory, while flower and leaf extracts had similar stimulatory/ inhibitory effects. Thus the allelopathic effects of *L. camara* reproductive organs were stronger than vegetative organs. Since *L. camara* is perennial, hence, large quantity of flowers and fruits produced more allelochemicals. The allelopathic effects of its reproductive organs makes it more competitive and invasive.

Key words: Alien plants, allelopathy, biological invasion, germination, seedling growth.

INTRODUCTION

Invasion of exotic species is major global problem in natural ecosystems (22). *Lantana camara* (lantana), is of tropical America origin and one of the world's 100 worst invasive alien species (14). It is an ornamental plant, hence, now exists in many forms throughout the world (25). It has become major problem in over 70 countries, causing degradation of pastures and native forests (5,12). It is successful invader due to its allelopathic effects, which drastically reduces the growth of all plant species under its canopy and also reduces the growth of mature trees and shrubs (3,8,17). Allelopathy is an important strategy to increase the competitive ability of invasive weeds (6,7,18) by releasing compounds to inhibit the germination and/or growth, or causes the mortality of another species (26). Its allelopathic effects have been studied on alga *Microcystis aeruginosa* (16), moss *Bryum cellulnr* (4), fern *Cyclosorus dentate* (28), milkweed vine *Morrenia odorata* (1), weed *Eichhornia crassipes* (14,21) and many crops: wheat, corn and soybean (2,15,20,23). It contains allelopathic chemicals such as triterpenes, which are implicated in allelopathic responses (6,10,15,16,18).

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Most of the studies focussed on its rhizosphere soil, root exudate, leaf and shoot leachates or aqueous extracts and not on reproductive organs. This study aimed to determine the effects of aqueous extracts of leaves and reproductive organs (flowers and fruits) of *L. camara* on germination, seedling growth and dry matter production of radish (*Raphanus sativus* var. *acanthiformis*) and lettuce (*Lactuca sativa* var. *capitata*).

MATERIALS AND METHODS

The fresh reproductive organs (flowers and fruits) and vegetative organ (leaves) were collected in October, 2007 from the Baiyun Mountain (23°10′~23°11′N, 113°16′~113°18′E), Guangzhou, South China. The seeds of test species were obtained from Seed Company, Guangzhou.

Bioassay

The fresh leaves and flowers were cut into pieces (< 2cm) and the fruits were meshed to prepare extracts and 1, 5, 10 and 20 g of their fresh materials were soaked in 100 ml distilled water for 24 h at room temperature (20-24^{o} C). The solutions were filtered twice through filter paper and filtrates were designated as 1, 5, 10 and 20% aqueous leaf, flower and fruit extract and were stored below 6^{o} C. The seeds of test species (radish and lettuce) were surface-sterilized in 0.5% potassium permanganate solution and repeatedly rinsed in distilled water. In each Petri dish (9 cm dia.), 30 seeds of radish and lettuce were kept on top of two sheets of germination paper. For each treatment, 5 ml aqueous extract of test concentrations of each organ was added per Petri dish and distilled water was used as control. The Petri dishes were kept in dark room at temperature of 20-24^{o} C. The treatments were replicated three times in complete randomized design. The germinated seeds (when radicle length was about 1-2 mm) (13) were counted every 12h for the first day, then every 24h. Germination was recorded up to 5 days and at the end of the experiment (7th day), the seedling growth (root and shoot length) of test species was recorded.

Pot culture

Seeds of test species were sown in pots (15 cm dia. × 13 cm height) containing sand. Four aqueous extracts concentrations [0(control), 10, 20 and 30%] were prepared for the bioassay. The pots were kept in the greenhouse (24-29^{o} C) and each treatment was replicated five times. Twenty seeds of test species were sown per pot. One week after sowing, seed germination was recorded. The seedlings were thinned to 5 seedlings per pot and watered daily with 20 ml of appropriate extract concentration (flower, fruit and leaf). Four weeks after sowing, the seedlings were harvested and dry matter was recorded.

Chemical analysis

Forty mg aqueous exudates of flowers, fruits and leaves of *L. camara* were collected and put into 10 ml volumetric flasks. After that, 1.5 ml hexane-ether (1:2) and 2 ml methanol were added and fully homogenized, then 2.5 ml of 0.8 mol/l KOH/MeOH were added and fully mixed. Each final volume was made to 10 ml with distilled water after 10 min. The clear supernatant of each mixture was separated, then dehydrated with

Na_2SO_4 and stored in sealed bottles. Samples were analyzed by GC-MS (Finnigan Voyager) equipped with BPX5 column (25 m × 0.22 mm × 0.25 µm). The temperature of injection was set at 220°C and ultra-high-purity helium was used as a carrier gas with a column head pressure of 8 psi. The splitless injection temperature was 220°C and a quadrupole-type mass selective detector with a transfer line temperature at 230°C The ionization potential was 70eV and scan range was 35 to 450 atomic mass units. The oven temperature began with 60°C and kept for 3min, gradually rose to 170°C with 5°C /min, then rose to 270°C with 10°C /min. The components were identified by a peak matching library search using authentic standards and NIST (National Institute of Standard and Technology) and Wiley libraries. The concentrations of chemicals were calculated by area normalization.

RESULTS AND DISCUSSION

Aqueous extracts of reproductive and vegetative organs of *L. camara* influenced the germination rate of radish and lettuce. They were stimulatory at lower concentrations (1%) and inhibitory at higher concentrations (from 5% to 20%). The inhibition increased with increasing concentrations (Fig.1). Radish germinated faster than lettuce. Aqueous leaf extracts were inhibitory to radish but stimulatory to lettuce.

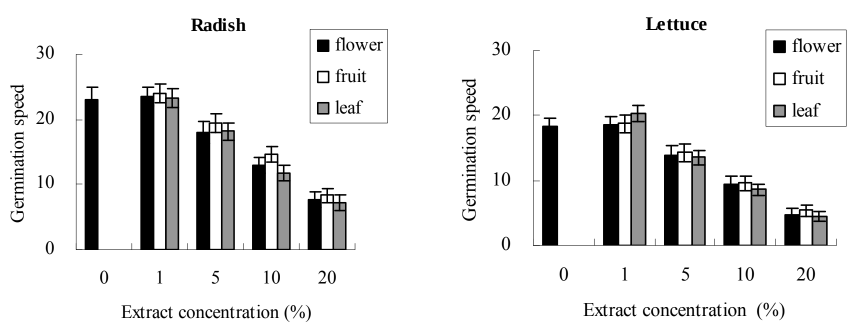

Figure 1. Effects of aqueous extracts of reproductive organs (flower and fruit) and vegetative organ (leaf) of *Lantana camara* on the germination speed of radish and lettuce

The extracts affected seedling growth in a concentration dependent manner. Extracts of flowers and leaves reduced root elongation, with drastic suppression at higher concentrations. Fruit extracts stimulated the root growth at lower concentrations (Fig. 2). For shoot length, except for 5% flower extract, extracts at all lower concentrations (including 5%) promoted the shoot elongation of test species, and fruit extracts had the most significant effect. Extracts of leaves and fruits still increased the shoot length of lettuce. The maximum reduction in shoot length was found with 20% flower extracts (Fig. 3).

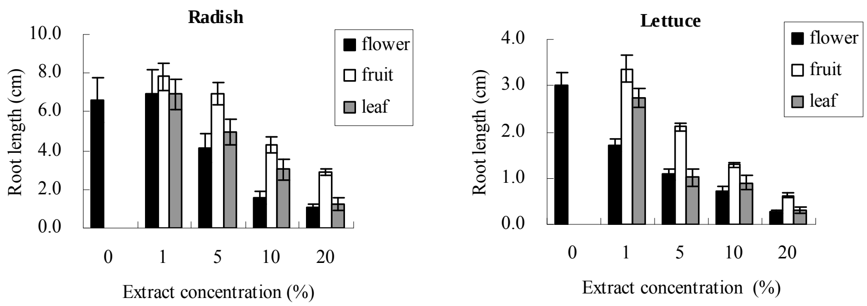

Figure 2. Effects of aqueous extracts of reproductive organs (flower and fruit) and vegetative organ (leaf) of *Lantana camara* on root length of radish and lettuce

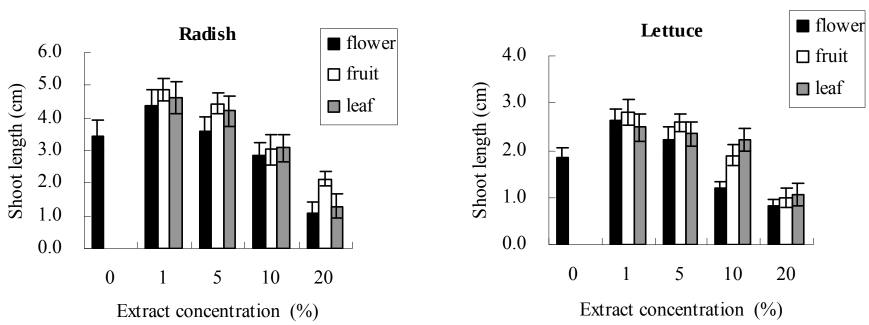

Figure 3. Effects of aqueous extracts of reproductive organs (flower and fruit) and vegetative organ (leaf) of *Lantana camara* on shoot length of radish and lettuce

Germination and dry matter production of radish and lettuce were suppressed by the aqueous extracts of reproductive and vegetative organs of *L. camara* (Table 1). The leaf extract showed the highest inhibitory effect on germination and dry matter production of radish and lettuce, while fruit extract was less active than that of flower and leaf (Table 1).

The allelopathic potential of aqueous extracts from reproductive and vegetative organs of *L. camara* was concentration-dependent. The harmful effects observed may be due to allelochemicals, which inhibit the gibberellin and IAA induced growth (27). Most extracts promoted the germination and seedling growth of test spp at 1% concentration. Similar stimulatpry effects of some perennials occurs on growth and dry weight of annual crops (19).

GC-MS analysis clearly demonstrated the presence of allelopathic compounds in the reproductive organs of *L. camara* (Table 2, Fig. 4). Twenty compounds were identified through GC-MS analysis from the reproductive and vegetative organs of *L. camara* (Table 2). Most of these compounds were alkenes, and the rest were alcohols and alkyls. There were remarkable differences in content of these substances in flowers, fruits and leaves. Some compounds were only found in leaves or reproductive organs (Fig. 4).

Table 1. Effects of aqueous extracts of reproductive organs (flower and fruit) and vegetative organ (leaf) of *Lantana camara* on germination and dry matter production of radish and lettuce in pot culture

Concentration (%)	Flower		Fruit		Leaf	
	Radish	Lettuce	Radish	Lettuce	Radish	Lettuce
Germination (%)						
Control (0)	99.2 ±1.60	87.5±3.13	99.2 ±1.60	87.5±3.13	99.2 ±1.60	87.5±3.13
10	85.3±5.65*	73.2±7.21	91.1±3.34	76.6±3.76	81.6±4.21**	73.6±4.07*
20	76.2±6.76*	64.5±5.67**	79.2±2.43**	69.6±3.93**	73.5±3.17**	59.7±2.78**
30	54.3±4.34**	45.7±3.64**	61.3±5.32**	47.6±5.45**	52.7±3.76**	41.4±3.67**
Dry matter production (g)						
Control (0)	0.965±0.075	0.469±0.040	0.965±0.075	0.469±0.040	0.965±0.075	0.469±0.040
10	0.741±0.081	0.330±0.054	0.773±0.034*	0.349±0.089	0.710±0.074*	0.321±0.020*
20	0.606±0.074**	0.270±0.031**	0.642±0.061*	0.289±0.071	0.553±0.086**	0.248±0.039**
30	0.379±0.061**	0.206±0.064**	0.478±0.085**	0.213±0.097*	0.395±0.051**	0.207±0.027**

All values: means ± standard errors. * $p < 0.05$; ** $p < 0.01$.

Table 2. Identification of volatile components from reproductive organs (flower and fruit) and vegetative organ (leaf) of *Lantana camara* by GC-MS

RT (min)	Formula	Chemical name	Peak Area (%)		
			Flower	Fruit	Leaf
4.93	$C_{10}H_{16}$	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	0.17	0.06	0.07
5.14	$C_{10}H_{16}$	Tricyclo[2.2.1.02,6]heptane, 1,3,3-trimethyl-	0.32	0.18	0.18
6.20	$C_{10}H_{16}$	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	0.75	0.3	0.31
6.36	$C_{10}H_{16}$	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	0.24	0.11	0.18
6.54	$C_8H_{16}O$	1-Octen-3-ol	0.33	0.45	0.43
6.62	$C_{10}H_{16}$	β-Myrcene	0.40	0.22	0.22
7.20	$C_{10}H_{16}$	α-Phellandrene	1.54	0.51	0.95
7.73	$C_{10}H_{14}$	Benzene, 1-methyl-3-(1-methylethyl)-	4.96	1.88	1.68
7.83	$C_{10}H_{16}$	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	0.61	0.24	0.22
7.99	$C_{10}H_{18}O$	Eucalyptol	4.56	5.24	2.34
8.29	$C_{10}H_{16}$	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	0.54	0.27	0.09
8.71	$C_{10}H_{16}$	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	4.51	0.85	1.39
9.98	$C_{10}H_{18}O$	1,6-Octadien-3-ol, 3,7-dimethyl-	0.16	0.52	0.06
16.47	$C_{15}H_{24}$	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1- (1-methylethyl)-, (3R-trans)-	1.31	0.62	1.01
17.64	$C_{15}H_{24}$	Copaene	6.51	6.72	3.43
18.00	$C_{15}H_{24}$	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	3.39	5.6	4.01
18.88	$C_{15}H_{24}$	Caryophyllene	15.12	14.46	24.9
19.06	$C_{15}H_{24}$	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4- (1-methylethylidene)-	8.39	9.7	6.19
21.27	$C_{15}H_{24}$	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1- (1-methylethyl)-, (1S-cis)-	2.9	6.38	1.86
22.32	$C_{15}H_{24}$	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7- (1-methylethenyl)-, [1R-(1α,3aβ,4α,7α)]-	3.82	5.97	3.34

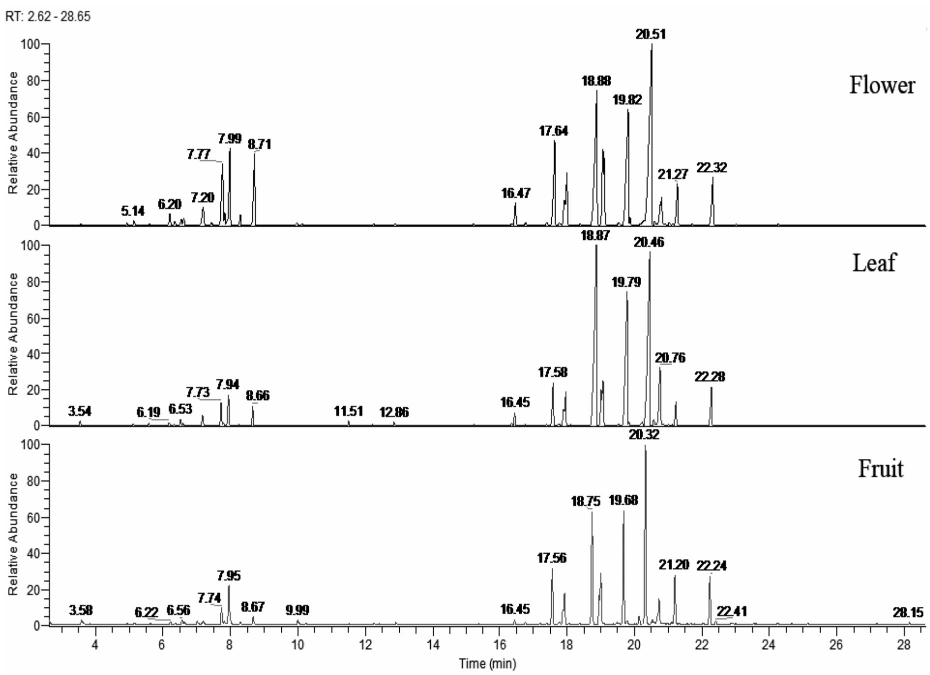

Figure 4. GC-MS comparison of volatile components from the reproductive organs (flower and fruit) and vegetative organ (leaf) of *Lantana camara*

In this work, the aqueous extracts of reproductive and vegetative organs from *L. camara* inhibited the germination, seedling growth and dry matter production of the test species. Extracts of flowers shared stimulatory/inhibitory effects as that of leaves while fruit extracts showed the most significant stimulatory effects, which may be related to the different chemical composition in the aqueous extracts of reproductive and vegetative organs of *L. camara*. *L. camara* can flower all the year when adequate moisture and light are available (9), producing a large amount of fruits and seeds. These reproductive structures may greatly improve the release of allelochemicals, which may contribute to the success of lantana's invasion.

Allelopathic effects in natural systems depend of soil biotic and abiotic factors (19). It is thus not easy to relate results from bioassay studies with processes observed in natural forests and agro-forestry systems (24). So, field studies are needed to characterize the allelopathic effect of reproductive organs of *L. camara*.

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Lantana is widely distributed in South China and forms monodominant communities in pastures and the edge of forests, causing reduction of biodiversity and degradation of ecosystems (10). Some studies showed the resistance of *Lantana* to low temperature and its possible spread in a wide range of the subtropical zone of China (11). Unfortunately, the potential deleterious effects of *L. camara* have not been recognized and it is currently cultivated for ornamental purposes in the subtropical zone. So, it is necessary to develop best-practice guidelines to increase awareness about its damage and take measures for biological control. As lantana's invasion is an international problem, cooperation with other countries is also needed for the successful implementation of management and action plans.

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