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Table: 1, Figs : 2

Allelopathic effects of *Parthenium hysterophorus* L. volatiles and its chemical components

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ABSTRACT

We studied the allelopathic effects of volatiles from *Parthenium hysterophorus* L. on the seedling growth of *Echinochloa crusgalli* (L.) Beauv. and *Digitaria sanguinalis* L. under laboratory and field conditions. The chemical components of volatiles were analysed using headspace method and gas chromatography-mass spectrometry (GC-MS). The seedling growth of *E. crusgalli* and *D. sanguinalis* intercropped with *P. hysterophorus* in the field was significantly inhibited. Fresh *P. hysterophorus* flower enclosed in a transparent box inhibited the seedling growth of *E. crusgalli* and *D. sanguinalis*. The enclosed fresh leaves of *P. hysterophorus* inhibited the seedling growth of *D. sanguinalis*. The volatiles from *P. hysterophorus* flowers contained 17 components. The main component in volatiles was myrcene (56.67%) and the second component was ocimene (26.28%). There were 18 components in the volatiles from *P. hysterophorus* leaves. The main component in fresh leaf was myrcene (28.07%), the second was β-pinene (14.52%). The volatiles contained high content of terpenoids, indicating that *P. hysterophorus* released its allelochemicals through volatilization. The allelopathy of volatiles may play an important role in enhancing the invasiveness of *P. hysterophorus* and may suppress other plant species in vicinity.

Key words: Allelopathy, *Digitaria sanguinalis* L., *E. chinochloa crusgalli* (L.), flower, GC-MS, leaf, *Parthenium hysterophorus* L., volatiles

INTRODUCTION

Parthenium hysterophorus L. was firstly found in mainland China in 1950's. It has invade into the farmland in Guangxi province in 2007 (15,19). It produces large number of pollens during the flowering stage and these pollens induce rhinitis, bronchitis and allergic dermatitis in human beings (14). It is very competitive and inhibits the growth of radish and *Eragrostis tef* (Zucc.) Trotter (3,14,16). *P. hysterophorus* is recognized as a worst weed in the world as it causes severe loss of biodiversity (13). Therefore, it is imperative to investigate its allelopathy, one of the potential invasion mechanisms for its spread.

Allelopathy is an essential part of evolution and plant defence, and strengthens the competitiveness of plants ecosystem (4,8). It plays an important role in weeds growth and many weeds release special chemical substances to exclude other plants' growth and

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existence in their vicinity (7). *P. hysterophorus* has high content of volatiles with unique smell. It is not clear, whether the volatiles play a role in the competition for existence between *P. hysterophorus* and other weed species. *Echinochloa crusgalli* (L.) Beauv. and *Digitaria sanguinalis* L., major weeds in crop fields also occur in *P. hysterophorus* stands in Shandong province of China. Therefore, this study aimed to assess the allelopathic effects of volatiles from *P. hysterophorus* on *E. crusgalli* and *D. sanguinalis* and identify the chemicals present in volatiles.

MATERIALS AND METHODS

P. hysterophorus plants were collected from our University campus during flowering stage on July 11, 2009. *E. chinochloa crusgalli* (L.) Beauv. and *Digitaria sanguinalis* L. seeds were collected from local farms in 2008 and dried in sunlight. These seeds were then incubated under natural conditions in winter season to break their dormancy and were used in the experiments.

Bioassay in field

The bioassay was conducted in stands of 110 cm tall *P. hysterophorus* infested land in our University. Three replicates, each of 15 newly germinated weed seeds of *Digitaria sanguinalis* L. and *E. chinochloa crusgalli* (L.) Beauv.) were placed in Petri dish (9 dia cm) with 5 pieces of filter paper. The Petri dishes were placed on ground beneath the thick growth of *P. hysterophorus*, so that *E. crusgalli* and *D. sanguinalis* seedlings are fully exposed to the volatiles of *P. hysterophorus*. While, the control Petri dishes were placed under the Chinese scholar tree (50 m away from the *P. hysterophorus* stand) ground covered by tree litter and surrounded by 5-beakers around and one beaker in the centre (10). All beakers were filled with water to keep the same illumination and humidity level for control. The Petri dishes were sprayed with water 6-times daily to keep the filter paper moist. The mean temperature during the experiment was 23°C to 28°C. The experiments were repeated thrice in randomized complete design. The growth parameters (root length, shoot length, fresh weight and dry weight) were determined at 7 days.

Bioassay in a transparent enclosed box

Each of 15 just germinated weed seeds were placed in Petri dish (9 cm dia) lined with 2 pieces of filter papers. The Petri dish was placed in a transparent box (15cm × 20cm), containing 100 g fresh *P. hysterophorus* flowers or leaves, to make full contact of *E. crusgalli* or *D. sanguinalis* seedlings with the volatiles from flowers or leaves. The flowers or leaves in transparent box were changed by fresh ones every 2-days and were sprayed with water to keep the filter papers moist. The Petri dish in transparent box without flowers or leaves was used as control (6). The experiments were repeated three times in a randomized complete design. The growth parameters (root length, shoot length, fresh weight and dry weight) were determined after 7 days.

GC-MS analysis

Six g of fresh leaves or flowers was placed in a sample vial, which was sealed with synthetic rubber septa of PTFE-butyl. Volatiles were extracted by Perkin Elmer

Turbo Matrix 40 Trap headspace sampler. Samples were heated at 45°C for 30 min, and sample needle and transfer line temperatures were set at 70°C and 70°C respectively, and then sample vial was pressed at 15 psi for 5 min (18).

The analysis of volatiles was performed with Shinadzu GCMS-QP2010 apparatus equipped with an Restek Rtx-1 MS capillary column (30m×0. 25mm .i d. 0. 25μm film thickness). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1.06 ml/min. Injector and MS transfer line temperatures were set at 200°C and 200°C respectively. The oven temperature was programmed from 35°C to 180°C at 8 °C/min, then held isothermal for 5 min and finally raised to 230°C at 15°C/min. The MS was operated in the EI mode, in m/z range 45 – 450 (12,17). Compounds were identified by NIST05 mass spectral data base.

Statistical analysis

Data were submitted to analysis of ANOVA with Microsoft Excel 2003. Statistical significance of difference was analyzed by DPS program. The means were compared by Tukey test ($P < 0.05$).

RESULTS

Weeds seedling growth

The volatiles significantly reduced the root length and shoot length of *E. crusgalli* by 23.0% and 24.8% than control, respectively (Figure 1). The shoot length and fresh weight of *D. sanguinalis*, were also inhibited by 20.1% and 19.2%. However, the volatiles did not effect the fresh weight and dry weight of *E. crusgalli* and the root length and dry weight of *D. sanguinalis*. The volatiles from *P. hysterophorus* reduced the seedling growth of *E. crusgalli* and *D. sanguinalis*.

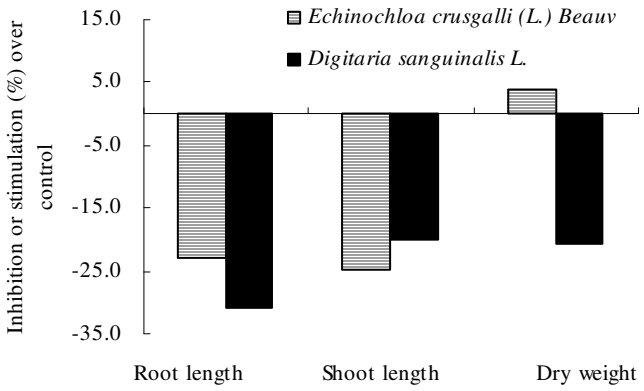
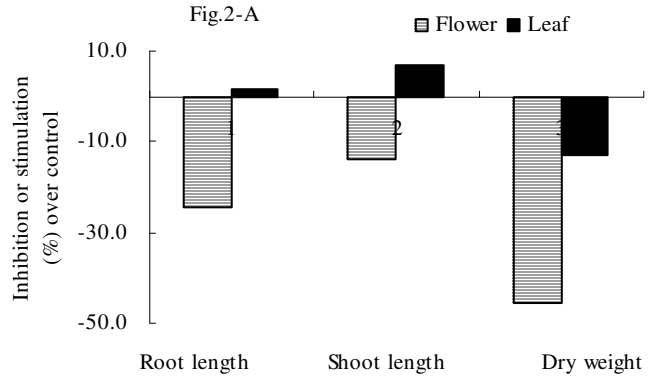


Figure 1. Effects of *P. hysterophorus* L. volatiles on the seedling growth of *Echinochloa crusgalli* (L.) Beauv. and *Digitaria sanguinalis* L. in field.

The volatiles from flowers reduced the seedling growth of *E. crusgalli* and *D. sanguinalis* (Figure 2). The volatiles from flowers significantly reduced the shoot length and dry weight of *E. crus-galli* by 13.8% and 45.6% compared to control, respectively (Fig 2-A). The root length and fresh weight of *D. sanguinalis* were also inhibited by 70.3% and 22.0% (Fig 2-B). Volatiles from flowers did not effect the root length and fresh weight of *E. crusgalli* and the shoot length and dry weight of *D. sanguinalis*. While the volatiles from leaves had no significant effects on the seedling growth of *E. crusgalli* and shoot length and dry weight of *D. sanguinalis*. However, it significantly reduced the root length and fresh weight of *D. sanguinalis* by 53.6% and 19.3% than control, respectively.



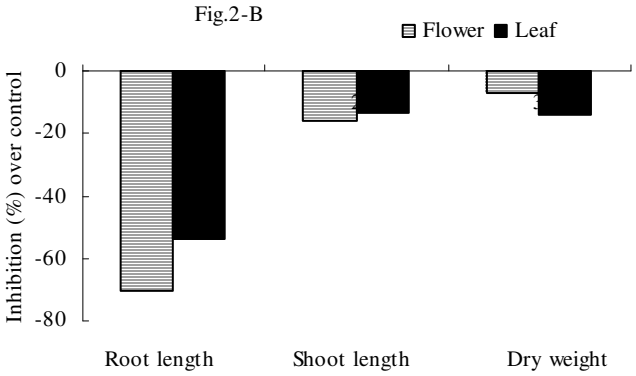


Figure 2. Effects of *P. hysterophorus* L. volatiles on the seedling growth of *Echinochloa crusgalli* (L.) Beauv. (Fig.2-A) and *Digitaria sanguinalis* L. (Fig. 2-B) in transparent box.

Flowers: A total of 17 compounds were identified by GC/MS from the volatiles of *P. hysterophorus* flowers (Table 1). Terpenoids were predominant in volatiles, accounting for 93.62% of the total volatiles composition. The main component was myrcene (42.67% of total volatiles, the second component was ocimene (26.28%) and the other components

were β-pinene (15.74%), alcohol (6.32%), camphene (3.76%), 1-octene (0.07%), 6,6-dimethyl-2-Methylenebicyclo[3.1.1]heptane(1.90%), isobornyl formate(0.06%), 3-thujene (0.26%),3-isopropenyl-5,5-dimethyl-cyclopentene (0.34%), 1,2-Diisopropenylcyclobutane (1.72%), D-Limonene (0.12%), γ-terpinene (0.07%), (-)-Isoledene (0.06%), tricyclene (0.26%), [1a R-(1aα,4α,4aβ,7bα)]-1a,2,3,4,4a, 5,6,7b-octahydro-1,1,4,7-tetramethyl- 1H-Cycloprop [e]azulen (0.20%) and D-germacrene (0.17%).

Table 1. The chemical components of volatiles from *P. hysterophorus* L**.**

Compound	Molecular formula	Molecular weight.	Relative content (%) in volatiles	
			Flower	Leaf
Alcohol	C_2H_6O	46	6.32	1.68
6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane	$C_{10}H_{16}$	136	1.90	10.08
Camphene	$C_{10}H_{10}$	130	3.76	14.01
β-Pinene	$C_{10}H_{16}$	136	15.74	14.52
Tricyclene	$C_{10}H_{16}$	136	0.26	0.70
Myrcene	$C_{10}H_{16}$	136	42.67	28.07
D-Limonene	$C_{10}H_{16}$	136	0.12	0.93
Ocimene	$C_{10}H_{16}$	136	26.28	3.72
γ-Terpinene	$C_{10}H_{16}$	136	0.07	0.40
D-Germacrene	$C_{15}H_{24}$	204	0.17	0.64
3-Thujene	$C_{10}H_{16}$	136	0.26	–
3-Isopropenyl-5,5-dimethyl- cyclopentene	$C_{10}H_{16}$	136	0.34	–
1,2-Diisopropenylcyclobutane	$C_{10}H_{16}$	136	1.72	–
Isobornyl formate	$C_{12}H_{20}O_2$	196	0.06	–
[1aR-(1a α,4α,4a β,7b α)]-1a,2,3,4,4a,5,6,7b- octahydro-1,1,4,7-tetramethyl-,1H-Cycloprop[e] azulene	$C_{15}H_{24}$	204	0.20	–
(-)-Isoledene	$C_{15}H_{24}$	204	0.06	–
1-Octene	C_8H_{16}	112	0.07	
(Z)-Hex-3-en-1-ol	$C_6H_{12}O$	100	–	0.76
1-Phenylheptane	$C_{13}H_{20}$	176	–	1.39
α-Pinene	$C_{10}H_{16}$	136	–	12.44
(4E)-4-Hexenyl acetate	$C_8H_{14}O_2$	142	–	3.89
Sabinene	$C_{10}H_{16}$	136	–	3.02
β-Phellandrene	$C_{10}H_{16}$	136	–	1.51
(R)-1-Methyl-5-(1-methylethenyl)-Cyclohexene	$C_{10}H_{16}$	136	–	1.34
Caryophyllene	$C_{15}H_{24}$	204	–	0.90

Leaves: A total of 18 compounds were identified by GC/MS from volatiles from *P. hysterophorus* leaves (Table 1). Terpenoids were predominant in volatiles, accounting for 98.32% of the total volatiles composition. The main component was myrcene (28.07% of total volatiles), the second component was β-Pinene (14.52%) and the other components were camphene (14.01%), α-pinene (12.44%), alcohol (1.68%), 1-phenylheptane (1.39%), (Z)-hex-3- en-1-ol (0.76%), Germacrene (0.60%), (4E)-4-hexenyl acetate (3.89%), β-phellandrene (1.51%), γ-terpinene (0.40%), D-Limonene (0.93%), ocimene (3.72%), 6,6-

dimethyl-2-methylenebicyclo [3.1.1]heptane (10.08%), caryophyllene (0.90%), (R)-1-methyl-5-(1-methylethenyl)-cyclohexene(1.34%), tricyclene(0.70%) and sabinene (3.02%).

DISCUSSION

We found that volatiles from *P. Hysterophorus* in field (Figure 1) had shown strong inhibitory effects on seedling growth of *E. crusgalli* and *D. sanguinalis*. To determine the true chemical components of volatiles, GC-MS was used to analyze the chemical components of volatiles collected using headspace methods. The volatiles from flowers contain mainly camphene, β-pinene, myrcene, while the ocimene and volatiles from leaves contain mainly camphene, 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane, β-pinene, α-pinene and myrcene. The volatiles from flowers and leaves showed similar chemical composition in terms of terpenoids (Table 1). Thus our results corroborate that terpenoids could be part of bioactive substances in the volatiles of *P. hysterophorus*. However, other minor components in volatiles may also produce herbicidal effects, owing to possible synergistic and antagonistic interactions among the components (9).

Ma *et al.* (11) reported that the volatiles rich in α-pinene, D-limonene and camphene, had strong allelopathic activity. The terpenoids are phytotoxic and are responsible for germination inhibition (1,2,5,17). Therefore, the allelopathic activity of volatiles in this study may be attributed to terpenoids of volatiles. As the toxicity of terpenoids has selectivity to different plants (11), hence, the bioassay results (Table 1) corroborate that volatiles from *P. hysterophorus* flowers or leaves produced a strong or more selective phytotoxic effects against *D. sanguinalis* seedling growth than *E. crusgalli*. On the other hand, the allelopathic effects of volatiles are also related to the composition and the plant species on which they are applied (17). Volatiles from *P. hysterophorus* flowers produced stronger or more selective phytotoxic effects than leaves volatiles against *D. sanguinalis* and *E. crusgalli* seedling growth. The less effectiveness of leaves volatiles than flowers volatiles could due to lower amount of ocimene.

CONCLUSIONS

These results show that volatiles from *P. hysterophorus* have herbicidal effects against *D. sanguinalis* and *E. crusgalli* two important weeds in cultivated areas. However, their herbicidal mechanism was not investigated in this study. Terpenoids as the main volatile compounds can be used in cosmetic industry as fragrance, furthermore, they may be also used as potential bio-herbicides.

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