植物生态学报 2000, **24**(3) 362～365
Acta Phytoecologica Sinica

紫茎泽兰的化学互感潜力*

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摘 要 广布于澜沧江流域的紫茎泽兰是一入侵性极强的外来杂草。研究表明它对其它植物有化学互感作用,其地上部分的石油醚、乙醇和水提取物对豌豆的种子萌发和幼苗生长均有抑制作用。在 2% 相对浓度下, 紫茎泽兰的石油醚、乙醇和水提取物对豌豆的萌发分别产生 100%、40% 和 50% 的抑制。它的化学互感物质主要集中于石油醚提取物及石油醚提取物吸附于活性炭后的甲醇洗脱物中。从石油醚提取物分离出的第二、第三和第四组分为主要的活性成分, 在 1% 的相对浓度下,它们对豌豆的萌发分别表现出 100%、90% 和 90% 的抑制,且萌发的豌豆无根和芽的生长。经鉴定, 化学互感活性强的石油醚第二组分为 9-酮-泽兰酮。通过研究, 本文提出了紫茎泽兰地上部分之挥发性互感物质的释放以及地上部分和凋落物中的水溶性他感物质的淋溶等植物化学互感途径。

关键词 紫茎泽兰 化学互感 9-酮-泽兰素

ALLELOPATHIC POTENTIAL OF *EUPATORIUM ADENOPHORUM*

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Abstract *Eupatorium adenophorum*, a weed with strong invasibility and exotic to the Lancang River watershed, has an allelopathic effect on other plants. Its petroleum, ethanol and water extracts showed inhibitory effects on the germination and seedling growth of the garden pea (*Pisum sativum*). At the relative concentration of 2%, the petroleum, ethanol and water extracts showed 100%, 40% and 50% inhibition on pea germination respectively. Its allelochemicals are mainly located in petroleum extract and the methanol eluent of the petroleum extract absorbed in activated charcoal. The major active fractions of the petroleum extract are FR2 (identified as 9-Oxo-Ageraphorone), FR3 and FR4. At the relative concentration of 1%, the FR2, FR3 and FR4 showed 100%, 90% and 90% inhibition on the germination of pea respectively. Moreover, germinated peas treated with 1% FR3 and FR4 did not show any shoot and root growth. It is suggested that the allelopathic effect of this plant occurs through the release of volatile allelochemicals from aerial part tissues and the leaching of water soluble allelochemicals from both aerial tissues and litter.

Key words *Eupatorium adenophorum*, Allelopathy, 9-Oxo-Ageraphorone

Special invasibility is the internal basis of some plants for their successful invading and pervading in the degraded ecosystems caused by human disturbance. Originated from Mexico, *Eupatorium adenophorum* Spreng invades, settles down and spreads in the South Asia, Southeast Asia, Australia and the southwest of China by means of its strong invasibility. Up to now, *Eupatorium adenophorum* has spread out and become a vicious weed all over the watershed of Lancang River (Upper reach of Mekong River). The causes of this process and its impact on the biodiversity in the watershed need to be studied systematically. Previous studies indicated that *Eupatorium adenophorum* adopts allelopathic effect as a special invading tool to suppress and eventually exclude other plant species in plant community.

* Received: 1999-05-19 Revised: 1999-10-08
Foundation item: The authors would thank the Chinese Academy of Sciences for financial support on this study, project code: KZ951-A1-104-02-02 of the Ninth Five-Year key programs
The authors are grateful for the valuable help and support of professor XU Zai-Fu as well.

Several papers reported preliminarily the allelopathic effects of the water extract of *Eupatorium adenophorum* (Zhao & Ma, 1989; He & Liu, 1990; Tripathi *et al*., 1981), but the allelochemicals have not been isolated and identified, its allelopathic effect has not been observed systematically and its impact on biodiversity has not been investigated.

1 Materials and methods

1.1 Plant sample collecting and allelochemical extracting

Aerial part of *Eupatorium adenophorum* was collected at Macun, Kunming on February 15, 1995. The plant sample was air dried and ground.

Plant material was extracted in petroleum ether (30～60 ℃) for 24 hours twice at room temperature, then extracted in ethanol and in distilled water at last. Volumes of petroleum ether, ethanol and water extracts were reduced by rotary evaporator.

1.2 Fractionation of plant extracts

Petroleum ether extract was separated by silicon column using n-hexane: ethyl acetate as eluent system. Active compounds were purified by preparatory TLC on silicon-G plates using n-hexane: ethyl acetate (4:1) as developing system.

1.3 Bioassay

Bioassay method was designed according to Leather & Einhellig(1988). Petri dishes with filter paper saturated by distilled water were used to observe the effects of substances from *Eupatorium adenophorum* on the germination and seedling growth of other plants. The extract of 1 g dry powder sample was dissolved in 1 ml corresponding extracting solvent, put 0.2 ml diluted extract on the filter paper of each dish and 0.2 ml corresponding solvent on the filter paper of the control, let the solvent in the filter paper evaporate thoroughly in the ventilation cabinet, then add 10 ml distilled water into the filter paper to make up 2% treatment of plant extract. Concentration of plant extract in the culture media equals to the percentage of whole plant dry material in the media, it is not the percentage of absolute dry weight of the extract in the media. Concentration of the purified fraction is calculated in the same way. Garden pea (*Pisum sativum*), a crop widely cultivated in the watershed of Lancang River, is chosen to test the allelopathic effect. Each dish was placed 10 seeds of garden pea (*Pisum sativum*) and each treatment has three duplications. Treated dishes were incubated in 25 ℃ temperature and 12 hours lighting per day condition. Number of seeds germinated was observed daily one day after the incubation. The seed that radicle has broken seed capsule is calculated as germinated seed. Length and weight of root and shoot were observed after 6 days incubation.

2 Results and analysis

Table 1 indicates that all the petroleum ether, ethanol and water extracts of the aerial part of *Eupatorium adenophorum* have germination inhibition on garden pea (*Pisum sativum*). At 2% relative concentration, the inhibition rate of petroleum ether extract reached 100%, and those of ethanol and water extracts were 40% and 50% respectively. In addition, germination of garden pea was delayed under the treatment of ethanol and water extracts. After petroleum ether extract has been absorbed completely in activated carbon, the compounds absorbed in the carbon were washed off

Table 1 Effects of the chemical extracts of *Eupatorium adenophorum* on the germination of garden pea (*Pisum sativum*)

Treatment	Germination rate in 2 days	Germination rate in 3 days	Germination rate in 4 days	Germination rate in 5 days	Germination rate in 6 days
Control	100	100	100	100	100
Petroleum extract	0	0	0	0	0
Ethanol extract	0	40	40	50	60
Water extract	10	20	20	50	50
Methanol eluent	0	20	20	20	0[a]
Ether eluent	60	100	100	100	100

Methanol and ether eluents are eluents of petroleum ether extract absorbed in activated carbon. Relative concentration: 2% Temperature: 25 ℃ Lighting: 12 h · d^{-1} Date: 17～22 Feb., 1998 a: All the seedlings died

with methanol and diethyl ether to yield eluents of methanol and diethyl ether. The methanol eluent showed strong inhibition on the germination of garden pea while diethyl ether eluent did not. This implies that there are allelochemicals in the methanol eluent of petroleum ether. Under the treatment of 2% methanol eluent (Relative concentration), the germinated seeds were 20% of the total only and these germinated seeds had no further root and shoot growth and died 4 days later. Length and weight of main root and shoot in addition with number of secondary roots were all zero under the treatment of methanol eluent, while those under the treatment of diethyl ether eluent did not differ significantly from those of the control (Table 2).

That all the three extracts of petroleum ether, ethanol and water showed inhibition on the germination of garden pea implies: 1) *Eupatorium adenophorum* has strong allelopathic effect on other plants; 2) its allelochemicals could have diversified ways to exert allelopathy, such as going into soil to affect other plants by waterfall leaching (As the activity showed in the water extract), or hurt the aerial part, especially leaf of other plant directly by volatile compounds (As the activity showed in the low polarity petroleum ether extract); 3) allelopathy through compounds of low polarity (As the strongest activity showed in petroleum ether extract) could be the major route. Moreover, that the active compounds in petroleum ether extract can be absorbed by activated carbon and released by solvent of large polarity indicates its allelochemicals of low polarity could be absorbed by soil and litter on the soil and then released to affect other plants indirectly.

Table 2 Effects of the eluents of *Eupatorium adenophorum* on seedling growth of garden pea

Treatment	Length of the main root (mm)	Number of secondary roots	Fresh weight of roots (mg)	Length of shoots (mm)	Fresh weight of shoots (mg)	Fresh weight of seedlings (mg)
Control	430	16	515	230	551	1066
Methanol eluent	0	0	0	0	0	0
Ether eluent	400	13	498	205	450	948

Relative concentration: 2% Temperature: 25 ℃ Lighting: 12 h · d^{-1} Date: 17～22 Feb., 1998

Table 3 Effects of the fractions of methanol eluent of *Eupatorium adenophorum* on the germination of garden pea

Treatment	Germination rate in 2 days (%)	Germination rate in 3 days (%)	Germination rate in 4 days (%)	Germination rate in 5 days (%)	Germination rate in 6 days (%)
Control	100	100	100	100	100
FR1	0	50	60	80	70[a]
FR2	0	0	0	0	0
FR3	10	10	10	10	10
FR4	20	70	90	90	90
FR5	60	80	100	100	100
FR6	30	60	60	60	60
FR7	10	10	10	10	10

Relative concentration: 1% Temperature: 25 ℃ Lighting: 12 h · d^{-1} Date: 17～22 May, 1998 a: Some of the seedlings died FR: Fraction

Active compounds in methanol eluent of the petroleum ether extract were separated by silicon gel column chromatography to yield seven fractions.

These fractions were further purified by preparatory TLC. The bioassay of these fractions indicates that: fraction 2 has the strongest activity, its germination inhibition rate reached 100% at 1% relative concentration; fractions 3 and 7 have strong activities, their germination inhibition rates reached 90% and the germinated seeds did not have any root and shoot growth; fractions 1 and 6 have activities too, their germination inhibition rate were 30% and 40% respectively and the germinated seeds have only slight root growth but no shoot growth (Table 3 and Table 4). The active fraction 2 was identified as 9-Oxo-Ageraphorone by EI-MS、^{1}HNMR and ^{13}CNMR (Nuclear

Table 4 Effects of the fractions of methanol eluent of *Eupatorium adenophorum* on the seedling growth of garden pea

Treatment	Length of the main root (mm)	Number of secondary roots	Fresh weight of roots (mg)	Length of shoots (mm)	Fresh weight of shoots (mg)	Fresh weight of seedlings (mg)
Control	425	15	465	225	521	986
FR1	25	0	70	0	0	70
FR2	0	0	0	0	0	0
FR3	0	0	0	0	0	0
FR4	55	0	190	0	0	190
FR5	330	0	436	155	351	787
FR6	50	0	190	0	0	190
FR7	0	0	0	0	0	0

Relative concentration: 1% Temperature: 25 ℃ Lighting: 12 h · d^{-1} Date: 17～22 May, 1998 FR: 同表3 See Table 3

Magnetic Resonance). Structure of other active fractions needs further spectral and chemical study.

3 Discussion and conclusion

The study indicates that *Eupatorium adenophorum* has strong inhibition on the germination and seedling growth of garden pea through allelopathy. In addition with previous studies on its allelopathic effect on several other plants (He & Liu, 1990; Tripathi *et al.*, 1981), it could be concluded that allelopathic effect is one of the mechanisms which *Eupatorium adenophorum* adopted to successfully invade the degraded ecosystem and drive other plant species out of the system. *Eupatorium adenophorum* may reduce the biodiversity of the ecosystem by the tool of allelopathy. It is a plant not only affecting other plants in the natural ecosystem but the major upland crops in the watershed of Lancang River as well. In order to maintain the local biodiversity of Lancang River watershed and protect crops, *Eupatorium adenophorum* needs to be controlled.

Plant has five ways to exert allelopathic effect on other plant generally. Firstly its aerial part could release volatile compounds to hurt plants surrounding it directly or these volatile allelochemicals go inside the body of other plants to affect their physiological processes through stoma and lenticel. Secondly the volatile allelochemicals could be absorbed by the soil then to affect other plants indirectly. The third way is that its allelochemicals in the litter and waterfall leachings of aerial part come downwards to the soil, then are absorbed by other plants. This is another indirect way. Fourthly the allelochemicals in the feces of herbivores which feed on the plant come into the soil to affect other plants. This is an indirect way too. Probably the last way is that allelochemicals come into the soil through root excretion to affect other plants. Most of the previous studies on allelopathy of plants paid attention to the last route of allelopathy only. Up to now, the allelopathy ways of the first and second ones have been seldom refered.

That all the three extracts of petroleum ether, ethanol and water showed inhibition on the germination of garden pea implies that allelochemicals of *Eupatorium adenophorum* are diversified and it has various ways to exert allelopathy. The study suggested that allelopathy through compounds of low polarity (As the strongest activity showed in petroleum ether extract) could be the major allelopathy route of *Eupatorium adenophorum*. Furthermore, the study implies that its allelochemicals of low polarity could be absorbed by soil and the litter on the soil and then released to affect other plants indirectly.

Through follow-up bioassay, spectral and chemical studies, the most active allelochemical in the petroleum extract of the aerial part of *Eupatorium adenophorum* has been identified 9-Oxo-Ageraphorone which is a cadinene derivative. The action mechanism of this chemical and the structure of other allelochemicals of the plant need further study.

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