Front. Agric. China 2008, 2(4): 446–450
DOI 10.1007/s11703-008-0068-5

RESEARCH ARTICLE

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The allelopathy of *Flaveria bidentis* (L.) Kuntze, an invasive weed species

Abstract To identify the allelopathic effect of *Flaveria bidentis* (L.) Kuntze (*F. bidenti*) on other plants, the effects of different extracts from *F. bidentis* on the growth of several plants were studied by bioassay. Results showed that the water extracts inhibited the growth of corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), cotton (*Gassypium Hirsutum* L.), soybean (*Glycine hispida* L.), peanut (*Arachi shypogaea* L.), rice (*Oryza sativa* L.), crabgrass (*Digitaria sanguinalis* L.) and rigweed (*Amaranthus retroflexus* L.), with the most reactive indexes found in root and stem of cotton at −0.85 and −0.88, respectively, at a concentration of 0.2 g·mL^{-1}. However, the water extracts accelerated the growth of rice. In addition, the reactive indexes of the extracts of petroleum ether chloroform, ethyl acetate, acet and alcohol were higher than that of the water extracts, and that of the acet extracts was the highest. The melting point of the refined acetone extract ranged from 192.5°C to 193.5°C, and its maximum absorbing wavelength was 220 nm. This extract was found to be herbicide-active and played an inhibitory role in the growth of crabgrass and rigweed at concentrations of 1000, 500, 100 and 50 mg·L^{-1}.

Keywords *Flaveria bidentis* (L.) Kuntze, allelopathy, herbicide activity

1 Introduction

Plant allelopathy refers to plant release of chemical substances to the environment, which can impact other plants directly or indirectly (Rice, 1984). Previous researches showed that allelopathy had important roles in weed invasion (Wang et al., 2004), for example, *Ageratina adenophora* (Song et al., 2002), and *Mikania micrantha* (Zhang et al., 2002) can produce allelopathic substances to compete with the surrounding plants. *Flaveria bidentis* (L.) Kuntze, belonging to Flaveriinae-Asteraceae, was found in Hengshui in Hebei Province and in Nankai University for the first time in 2003 (Gao et al., 2004). As of today, researches have been focused on its biological characteristics (Gao et al., 2004; Li et al., 2006), but so far no research on the allelopathy of *F. bidentis* extracts has been published. Powell (1978) and Agnese et al. (1999) reported that secondary metabolites of *F. bidentis* contained sulfated flavonoids. According to Broussalis et al. (1999), its alcohol extract and chloroform extract were effective in killing weevil, and had an evident repellent effect on cotton bollworm. Li et al. (2006) reported that in the regions where *F. bidentis* first invaded, nearly pure communities of *F. bidentis* developed, with the accompanying species only being Chinese Tamarisk (*Tamarix chinensis* Lour.) and Russian thistle (*Salsola collina* Pall.). Therefore, the allelopathy of *F. bidentis* was researched to know the effects of secondary metabolites from *F. bidentis* on the growth of other plants, to search for pesticide-oriented compounds, and to provide a theoretical and practical basis for the integrated control of *F. bidentis*.

Translated from *Journal of Agricultural University of Hebei*, 2007, 30(6): 63–67 [译自: 河北农业大学学报]

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2 Materials and methods

2.1 Experimental materials

F. bidentis (L.) Kuntze from Hengshui Lake in Hebei Province, corn cv. Zhengdan 958, wheat cv. Gaoyou 503, soybean cv. Hefeng 34, cotton cv. Ji 2000, peanut cv. Jihua 4, rice cv. Jingyou 14, crabgrass and rigweed were used in this study.

2.2 Methods

2.2.1 Water extracts from *F. bidentis* (L.) Kuntze stems and leaves

Fresh stems and leaves of the plant were dried at 50°C, then grinded into 50 mesh powders. The powders were

extracted under ultrasonic wave for 10 min after it was soaked in 3-fold water for 24 h. The water extracts were collected through filtration under reduced pressure, and put into X-5 adsorbent resins to remove chlorophyll; the adsorbent resins were eluted with water to get the eluting solvent, which was then concentrated in vacuum and re-dissolved to a concentration of 0.2 $g \cdot mL^{-1}$ with water (100 mL water contained 20 g of dried substance), and stored at 4°C before use.

2.2.2 Chemical reagent extracts from *F. bidentis* stems and leaves

The dried powders were extracted using 3-fold petroleum ether, chloroform, ethyl acetate, acet, and alcohol for 24 h. Five extracts were obtained and dried using N_2, and then they were quantitatively put into mortars, grinded with 5% 1601, and at last re-dissolved to gain a concentration of 0.2 $g \cdot mL^{-1}$ with water (following the same method as in section 2.2.1).

2.2.3 A white crystal isolated from acet extract by crystallization

Acet extracts were dried using N_2 and then washed with petroleum ether, benzene, ethyl acetate, and ether in turn to obtain a yellow substance. The substance was dissolved in acet-methanol 1:1 (V/V). The sample was refluxed in 90°C water for 10 min and filtrated under reduced pressure. The yellow filtrate was put into a flask at room temperature, and filtered after the crystal precipitated to obtain the crystal. Based on the same method mentioned above, pure crystal was finally isolated from the acet extracts.

2.2.4 Determining the physical and chemical properties of the white crystal

The melting point of the white crystal was determined by a micro-melting point apparatus. The wavelength of the white crystal was scanned using an ultraviolet spectrophotometer to measure its maximum absorbing wave length. High performance liquid chromatography was used to determine the purity on a C18 column (2×150 mm), using CH_3OH-H_2O (70:30) as the flowing phase, with a 3 μL injecting amount and 1 $mL \cdot min^{-1}$ flow rate.

2.2.5 Determining the effects of the extracts on the growth of some plants

Qualified seeds of the above mentioned crops were chosen, sterilized with 0.5% H_2O_2 and cultivated at 25°C. Three hundred and sixty grams vermiculite was put into every flowerpot which had diameters of 10 cm and heights of 20 cm, then 10 seeds were planted in each flowerpot and covered with 40 g vermiculite. Afterwards, these plants were treated with 40 mL of the extracts, taking 40 mL-water-treatment as control. All the treatments were replicated three times. The treated plants were then cultivated under the condition of 25°C and a light intensity of 3800 lx with a photoperiodic regime of 16 h. The crops were watered timely and quantitatively depending on their growth status during cultivation. Ten to fifteen days later, the lengths of their stems and roots were measured.

2.2.6 Statistical analysis of allelopathy effects

Based on the method of Williamson and Richardson (1988), the reactive index is defined as $(RI) = 1 - C/T$ ($T \geqslant C$) or $RI = T/C - 1$ ($T < C$), where C and T represent the comparison value and treatment value, respectively. If $RI > 0$, the function is stimulative, while if $RI < 0$, the function is inhibitory. The absolute value is coincident with the effect intensity.

2.2.7 Determining the inhibiting effects of the extracts on the growth of weeds

Based on the method of Zhang et al. (2005), the weeds treated with the extracts were cultivated in suitable conditions for 48 h before inhibition was observed. The grade standard for the inhibiting effect was: Grade 0 when inhibition is the same as that of CK, Grade I when it is lower than 25%, Grade II when it is between 25% and 50%, Grade III when it is between 50% and 75%, Grade IV when it is between 75% and 95%, and Grade V when it is above 95%.

3 Results

3.1 Effects of water extracts on the growth of some plants

The 0.2 $g \cdot mL^{-1}$ water extracts were diluted to 0.1 $mg \cdot mL^{-1}$, 0.05 $mg \cdot mL^{-1}$, and 0.025 $mg \cdot mL^{-1}$ diluents, which were used to treat the plants. Results showed that water extracts at different concentrations accelerated the growth of rice variably, with the highest inhibiting effect at 0.05 $mg \cdot mL^{-1}$. The reactive indexes to roots and stems of rice were 0.12 and 0.18, respectively. However, water extracts differently inhibited the growth of corn, wheat, soybean, peanut, and especially cotton. At the concentration of 0.2 $g \cdot mL^{-1}$, the reactive indexes to roots and stems of cotton were −0.85 and −0.88, respectively, followed by those of soybean and peanut. In addition, the extracts also played different inhibitory roles in the growth of crabgrass and rigweed (Table 1).

Table 1 Effects of the water extracts from *F. bidentis* on the growth of some kinds of plants

concentration/g·mL^{-1}	corn		wheat		cotton		soybean	
	length of stem	*RI*	length of stem	*RI*	length of stem	*RI*	length of stem	*RI*
0.2	3.46 ± 0.19e	−0.75	3.56 ± 0.23d	−0.67	0.82 ± 0.09d	−0.88	3.25 ± 0.32e	−0.72
0.1	5.62 ± 0.26d	−0.59	5.14 ± 0.52c	−0.53	1.26 ± 0.15d	−0.81	36.38 ± 0.41d	−0.55
0.05	6.14 ± 0.36c	−0.56	8.19 ± 0.35b	−0.25	2.15 ± 0.22c	−0.67	7.62 ± 0.18c	−0.36
0.025	10.89 ± 0.47b	−0.22	9.45 ± 0.26b	−0.14	4.25 ± 0.36b	−0.36	9.83 ± 0.45b	−0.18
CK	13.98 ± 0.67a		10.97 ± 0.89a		6.67 ± 1.69a		11.18 ± 1.23a	
concentration/g·mL^{-1}	rice		crabgrass		rigweed		peanut	
	length of stem	*RI*	length of stem	*RI*	length of stem	*RI*	length of stem	*RI*
0.2	12.05 ± 0.48a	0.14	1.84 ± 0.22c	−0.71	0.95 ± 0.13c	−0.79	1.28 ± 0.17d	−0.85
0.1	12.56 ± 0.72a	0.17	2.75 ± 0.38c	−0.56	1.46 ± 0.25c	−0.67	1.93 ± 0.14d	−0.78
0.05	12.78 ± 0.38a	0.18	3.78 ± 0.42b	−0.39	2.98 ± 0.35b	−0.35	2.87 ± 0.22c	−0.67
0.025	11.95 ± 0.64b	0.13	4.57 ± 0.67b	−0.27	3.17 ± 0.16b	−0.30	6.26 ± 0.25b	−0.28
CK	10.37 ± 1.35b		6.25 ± 0.98a		4.56 ± 0.76a		8.75 ± 0.96a	
concentration/g·mL^{-1}	corn		wheat		cotton		soybean	
	length of root	*RI*	length of root	*RI*	length of root	*RI*	length of root	*RI*
0.2	7.56 ± 0.65e	−0.48	6.34 ± 0.56c	−0.60	1.08 ± 0.09e	−0.85	1.46 ± 0.19d	−0.81
0.1	9.79 ± 0.32d	−0.32	7.15 ± 0.28c	−0.55	1.96 ± 0.15d	−0.72	2.47 ± 0.21c	−0.67
0.05	11.38 ± 0.72b	−0.21	9.27 ± 0.16b	−0.42	3.16 ± 0.18c	−0.56	3.56 ± 0.26b	−0.53
0.025	13.92 ± 0.45a	−0.04	10.19 ± 0.35b	−0.36	5.63 ± 0.71b	−0.22	7.32 ± 0.31a	−0.04
CK	14.48 ± 0.53a		15.94 ± 0.72a		7.24 ± 1.25a		7.64 ± 0.36a	
concentration/g·mL^{-1}	rice		crabgrass		rigweed		peanut	
	length of root	*RI*	length of root	*RI*	length of root	*RI*	length of root	*RI*
0.2	11.95 ± 0.54a	0.01	2.62 ± 0.12b	−0.50	1.02 ± 0.21c	−0.73	1.36 ± 0.15d	−0.79
0.1	12.67 ± 0.35a	0.06	3.12 ± 0.37b	−0.41	1.89 ± 0.42b	−0.49	2.89 ± 0.24d	−0.56
0.05	13.25 ± 0.43b	0.12	4.89 ± 0.25a	−0.07	2.16 ± 0.25b	−0.42	4.25 ± 0.34c	−0.35
0.025	12.16 ± 0.38a	0.02	5.17 ± 0.32a	−0.02	3.24 ± 0.12a	−0.14	5.16 ± 0.47b	−0.21
CK	11.87 ± 0.87a		5.26 ± 1.35a		3.75 ± 0.95a		6.52 ± 1.65a	

Note: The different letters in the same column indicate a statistically significant difference at $P < 0.05$.

3.2 Plant allelopathic effect of the chemical reagent extracts on plant growth

It was proved that the water extracts from *F. bidentis* played different inhibitory roles in the growth of seven kinds of plants. The stems and leaves were extracted by using petroleum ether, chloroform, ethyl acetate, acet and alcohol to obtain the corresponding extracts; thereafter, the extracts were diluted into 0.1 g·mL^{-1} and 0.05 mg·mL^{-1} solutions which were then used to test the inhibition effects on corn, wheat, cotton, soybean and peanut. The results showed that the chemical reagent extracts played different inhibitory roles in the growth of plants (Table 2). The petroleum ether extracts accelerated the growth of rice, with nearly the same *RI* as that of the water extracts; chloroform, ethyl acetate, acet and alcohol extracts inhibited the growth of rice, among which the *RI* of the acet extracts was the highest at the concentration of 0.1 g·mL^{-1}. At the same concentration, the inhibiting effect of acet extracts on cotton, wheat, crabgrass and rigweed was the highest. At the experimental concentration, the *RI* of chloroform extracts to cotton was higher than that of the other alcohol extracts, petroleum ether extracts and ethyl acetate extracts. At the concentration of 0.1 g·mL^{-1}, the *RI* of chloroform extracts to wheat was higher than that of any of the other alcohol extracts, petroleum ether extracts or ethyl acetate extracts. At the experimental concentration, the *RI*s of alcohol extracts to crabgrass and rigweed were higher than that of any of the other petroleum ether extracts, chloroform extracts or ethyl acetate extracts. Therefore, these results suggested that among the chemical reagents, the *RI* of acet extracts was the highest, and the inhibitory roles of acet extracts and alcohol extracts on crabgrass and rigweed were higher than that of any of the other extracts.

3.3 Physical and chemical properties of the white crystal and its weeding activity

White crystal was obtained from acet extracts by crystallization, with the melting point at 192.5°C to 193.5°C, and the maximum absorbing wave length at 220 nm. This substance was insoluble in petroleum ether, chloroform, benzene, and ether, but partially soluble in water and ethyl acetate, and completely soluble in acet, methanol, and acetic acid. Therefore, it was judged primarily to be an

Table 2 Allelopathic effect of different chemical extracts from *F. bidentis* on plant growth

extracts	concentration/g·mL^{-1}	corn		wheat		rice		crabgrass		rigweed	
		root	stem	root	stem	root	stem	root	stem	root	stem
petroleum ether extracts	0.10	−0.67d	−0.77c	−0.50b	−0.57d	0.08b	0.19b	−0.41c	−0.44c	−0.57c	−0.64d
	0.05	−0.46b	−0.63b	−0.41b	−0.35b	0.11a	0.29a	−0.23b	−0.27b	−0.34b	−0.43b
chloroform extracts	0.10	−0.83f	−0.88d	−0.58c	−0.64e	−0.09d	−0.03d	−0.22b	−0.28b	−0.37b	−0.63c
	0.05	−0.64d	−0.78c	−0.49b	−0.49c	−0.02c	−0.07d	−0.07a	−0.12a	−0.22a	−0.43b
ethyl acetate extracts	0.10	−0.52c	−0.65b	−0.48b	−0.44c	−0.17e	−0.23e	−0.24b	−0.25b	−0.35b	−0.58c
	0.05	−0. 30a	−0.47a	−0.38a	−0.36b	−0.10d	−0.08d	−0.09a	−0.15a	−0.25a	−0.36a
acet extract	0.10	−0.92g	−0.91e	−0.78d	−0.74f	−0.22f	−0.24e	−0.85g	−0.84f	−0.77e	−0.84f
	0.05	−0.81f	−0.81d	−0.71d	−0.63e	−0.01c	−0.04d	−0.78f	−0.75e	−0.52c	−0.77e
alcohol extracts	0.10	−0.76e	−0.86d	−0.54c	−0.56d	−0.16e	−0.07d	−0.67e	−0.60d	−0.62d	−0.72e
	0.05	−0.63d	−0.69b	−0.46b	−0.46c	−0.11d	−0.01d	−0.53d	−0.56d	−0.38b	−0.56c
water extracts	0.10	−0.73e	−0.82d	−0.45b	−0.45c	0.05b	0.12b	−0.43c	−0.39c	−0.51c	−0.66c
	0.05	−0.55c	−0.69b	−0.33a	−0.17a	0.11a	0.21a	−0.18b	−0.28b	−0.30a	−0.47b

Note: The different letters in the same column indicate significant difference at $P < 0.05$.

alkaline substance. After the physical and chemical properties of the white crystal were tested, HPLC method was applied to determine its purity and provide a basis for isolating and analyzing this crystal. The analytical conditions depicted in section 2.2.4 were suitable for the analysis of this substance (Fig. 1). According to the analysis of the peak area and height, the purity of the substance was above 99.0%. The compound was diluted to different concentrations with methanol, and the herbicide activities to crabgrass and rigweed were tested through stem treatment. Results showed that at the concentrations of 1000, 500, 100 and 50 mg·L^{-1}, the inhibitory effects of the diluents were Grade V, Grade IV, Grade III on the growth of crabgrass (Fig. 2), and Grade V, Grade IV, Grade III, Grade II on the growth of rigweed.

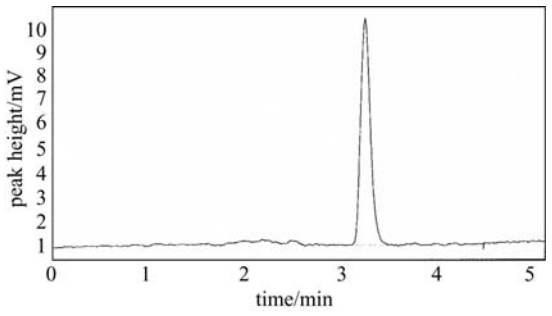

Fig. 1 Chromatogram of white crystallization from acet extracts

4 Discussion

Plant allelochemicals are substances which enter the environment naturally (Kong, 1998). In order to investigate whether the stem and leaf of *F. bidentis* can produce allelochemicals, *F. bidentis* was first extracted with water in our experiment. The results showed that at low concentrations, the water extracts had allelopathic effects on the growth of corn, wheat, cotton, peanut and soybean, but accelerated the growth of rice, which confirmed the allelopathy of *F. bidentis* in competition with other plants. As the test plant species in the experiment were limited, further research is needed to find other plants insensitive to *F. bidentis* to provide feasible projects for rational crop rotation and proper arrangement.

In order to obtain more allelopathic substances, the stems and leaves of *F. bidentis* were extracted with chemical reagents, among which acet extracts had the highest

Fig. 2 Inhibitory effect of white crystallization on crabgrass at the concentration of 500 mg·L^{-1}
Note: The left pot is the CK and the right pot is the treated plant.

inhibitory effect on the growth of the tested materials, including rice. It was likely that acet could extract allelochemical substances or perhaps other active constituents form *F. bidentis* more efficiently. The results also showed more white crystals isolated from acet extracts than from water extracts during quantitative extraction from *F. bidentis*. A constituent with herbicide activity was isolated from the acet extracts, viewed as an alkaline substance, which needs further study. Some researches have indicated that alkaloids from fronds have herbicide activities. (Hao et al., 2006; Yao et al., 2006)

Additionally, the allelopathic substances from *F. bidentis* roots need to be studied in the future. Meanwhile, further chemical information on the constituents responsible for herbicide activity needs to be investigated to lay a good foundation for their exploitation and utilization.

Acknowledgements This research was supported by the Key Programs for Science and Technology of Hebei Province, China (No. 08220301D) and the Natural Science Foundation of Hebei Province, China (No. C2007000464).

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