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Allelopathic effects of leaf extract from *Eupatorium adenophorum* on *Alternanthera philoxeroides*

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ABSTRACT

We studied the phytotoxic effects of leaf extract from crofton weed (*Eupatorium adenophorum* Spreng.) on alligator weed (*Alternanthera philoxeroide*s (Mart.) Griseb.). The aqueous leaf extracts from the crofton weed drastically inhibited the root growth of alligator weed at $0.04 g \cdot mL^{-1}$ concentration. There was no root or few roots only at the bottom stem node, when the concentration reached to $0.08\ g \cdot mL^{-1}$. These extracts also changed the physiological and biochemical parameters in treated alligator weed. The chlorophyll content, the root activity and the acetolactate synthase (ALS) activity were significantly decreased. While the superoxide anion (O_2^-), the malondialdehyde (MDA) content, peroxidase (POD) activity and shikimic acid content were significantly increased, these reflect the stressful conditions. Thus, the main allelopathic effects of crofton weed were on the cell structure, cell membrane and the activity of key enzymes in amino acid biosynthesis. Our data also showed that crofton weed may be used in bio-control of alligator weed.

Key words: Allelopathy, alligator weed, *Alternanthera philoxeroides*, biochemical parameters, cell structure, crofton weed, enzymes, *Eupatorium adenophorum*, physiological parameters, phytotoxic effect.

INTRODUCTION

Allelopathy is an important biological phenomenon in exotic plant invasions. Study of this phenomenon may help us to understand, how the plant interactions influence the plant colony and ecosystem functioning. In this biological phenomenon, the allelochemicals synthesized by one plant affects (either inhibitory or stimulatory) the other nearby plants. Invasive exotic plant species displace the native plant species by releasing the phytotoxins to inhibit the growth of native species. The allelochemical triggers a wave of reactive oxygen species (ROS) initiated at the root meristem, which leads to a Ca^{2+} signaling cascade triggering genome-wide changes in gene expression and ultimately, the death of root system (1). Thus the allelopathy of invasive plants has exerted unfavorable effects on the native biodiversity and ecosystem. *Alternanthera philoxeroides*, (alligator weed), is an immersed aquatic plant, originated in South America, but has spread to Australia, China, New Zealand, Thailand and the United States (21). It can grow in variety of habitats, but is usually found in wetland or in water and forms large interwoven mats

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over the water or along shore lines (9). It reduces the water flow and water quality in canals and rivers because the weed mats prevents the light penetration and oxygenation of water. This weed is difficult to control, hence, insects are used for its biological control [e.g. *Agasicles hygrophila* (alligator weed flea beetle) for biocontrol in Australia, China, Thailand, New Zealand and United States]. Besides, the *Amynothrips andersoni* (alligator weed thrips) and *Vogtia malloi* (alligator weed stem borer) have been used in United States (14).

Eupatorium adenophorum Spreng. (crofton weed) is an invasive perennial weed in China (15). Previous researches have showed that *E. adenophorum* has strong allelopathy to other plants, including wheat, maize, pea, chufa and bromegrass (16,19,22). So it is very meaningful to develop crofton weed into herbicides, especially for some weeds difficult to control. The allelopathic chemical affects the membrane permeability, cell division, protein synthesis and respiration. Yang *et al*. (18) reported that allelochemicals of *E. adenophorum* adversely affects the growth and development of rice by changing the cell structure and membrane. Zhang *et al*. (10) found that allelochemicals of *E. adenophorum* caused the membrane lipid peroxidation in *Gynura* and *Macroptilium atropurpureum*. Thus allelochemicals of *E. adenophorum* had significant effects on the membrane permeability of plants, however, the effects of *E. adenophorum* on the key enzymes of amino acid biosynthesis has not been studied.

Alternanthera philoxeroides is important invasive weed in China and very difficult to control (5). This study aimed to assess the allelopathic effects of leaf extracts from crofton weed on alligator weed, chlorophyll content, root activity and acetolactate synthase activity.

MATERIALS AND METHODS

Plant materials

Eupatorium adenophorum leaves were collected from crop field in Xichang city (27.67°N, 102.13°E, mean height above sea level: 1600 m annual rainfall; 1000 mm.), Sichuan Province, China in September 2011. The leaves were dried, grinded and sieved through 100 mesh sieve at room temperature to get powdered material for the experiments. *Alternanthera philoxeroides* plants were collected from the dry land during the vigorous growth period in Yaan city (29.90°N, 102.92°E, mean height above sea level: 1700 m, annual rainfall; 1200 mm.), Sichuan Province, China in May 2012. Then the plants were cut into 6-cm long stem, cuttings with the apical three nodes for experimental use.

Preparation of aqueous extract

Dry powder of *E. adenophorum* leaves were extracted by soaking in water for 48h at 20 ^{o}C (100 g per 1000 mL distilled water). The solution was filtered through 2-layers of gauze and two layers of filter paper. Then the filtrate 0.1 g·mL^{-1} concentration (0.1g of dried power per 1mL of distilled water) was stored as stock solution at 4 ^{o}C. The pH and osmotic potential of stock solution were 6.57 and 186 mOsm·kg^{-1} measured with pH meter and osmometer. The stock solution was diluted with distilled water to 5-concentrations (0.005, 0.01, 0.02, 0.04 and 0.08 g·mL^{-1} for use on alligator weed.

Root growth of alligator weed

To obtain uniform plant material without root, the upper 3-nodes of alligator weed stems were cut and planted in culture beds. The culture beds were petri plates, 18 cm dia and 2 cm height; 3-stems cuttings without root were planted in each bed. Culture beds were prepared by using culture substrates sand and soil in ratio of 1:2 filled in petri dishes. No plants nutrients were added. Then, 25 mL aqueous extracts of 5-concentrations (0.005, 0.01, 0.02, 0.04 and 0.08 $g\cdot mL^{-1}$) were added per culture bed as per treatments. Distilled water was used as control and the experiment was repeated thrice. At seven days after the incubation (25℃), the length of stems and roots of alligator weed were measured. The stems of alligator weed were cultivated in 100 culture beds with aqueous extract of *E. adenophorum* leaves at 0.04 $g\cdot mL^{-1}$ concentration and 100 culture beds with water as control. The culture beds were irrigated at 3 days interval with *E. adenophorum* leaf extract (0.04 $g\cdot mL^{-1}$ concentration) or water, respectively. The whole cutting branches were pulled out from the petri plates at 3, 8, 13, 18 and 23 days after irrigation.

Physiological and biochemical parameters

Seven physiological and biochemical parameters of samples [Chlorophyll content, root activity, peroxidase (POD) activity, superoxide anion (O_2^-) references, malondialdehyde (MDA) content, acetolactate synthase (ALS) activity and shikimic acid content] were measured.

The chlorophyll content was determined in 80% acetone extract of 0.1 g leaf tissues as described by Hegedüs *et al*. (6) and expressed using $mg\cdot g^{-1}$ FW. Root activity was measured using the triphenyl tetrazolium chloride (TTC) method (13) and was expressed as the amount of triphenyl formazan (TF) deoxidized by TTC. POD activity was measured by using guaiacol colorimetric methods, which consisted of 1 ml of 40 mM guaiacol solution, 1 ml of Mcllvaine buffer (pH 3.8) and 1 ml of 3mMH_2O_2 solution. Superoxide anion（O_2^-） references was determined by Hydrorylamine Oxidization; Malondialdehyde (MDA) content method and was determined by colorimetric of thiobarbituric acid. The detailed steps were as described in reference (4). Acetolactate synthase (ALS) activity and shikimic acid content was determined as described before (20).

Statistical analyses

Data were analyzed with the software of Data Processing System (DPS) 6.55. Means data were compared by the multiple range tests of Duncan at $P \leq 0.05$.

RESULTS AND DISCUSSION

Root growth of alligator weed

The aerial growth of a plant relies on its root system. If the root growth is stopped or inhibited, the aerial part will more or less be affected. The root growth of alligator weed was inhibited by the leaf extract of *E. adenophorum* at different concentrations (Fig. 1). With the increase in concentration, the inhibitory effects became more and more severe i.e. concentration dependent. Almost no new roots were observed at the concentration of 0.08

g·mL^{-1}. Thus, unknown allelochemicles in *E. adenophorum* leaf extract can significantly inhibits root growth of alligator weed. The allelochemicals are the important part of natural products and they adversely affect the germination and growth of weeds. *E. adenophorum* contains steroids, triterpenes, flavonoids, sesquiterpenoids and phenylpropanoids, among them, some compounds (caffeoylquinic acids, dibutyl phthalate, Euptox A and 2-coumaric acid glucoside) are inhibitory to many plants (8,11,12), hence, we studied the effects of allelochemicals from *E. adenophorum* on *A. philoxeroides* weed. Our results showed that the growth of *A. philoxeroides* was seriously inhibited by aqueous extract from *E. adenophorum* leaves at higher concentration. The main effect was inhibition in root growth. These results will have potential value for control of *A. philoxeroides*.

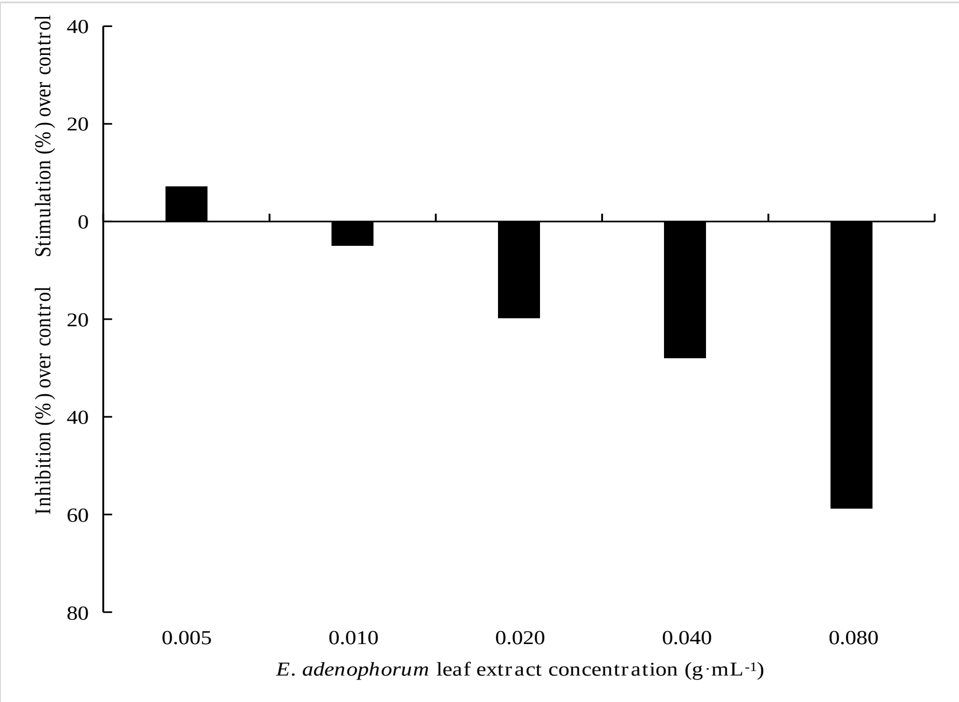

Figure 1. Inhibitory effects of aqueous leaf extract of *E. adenophorum* on growth of *A. philoxeroides* roots at 7 days after application.

Chlorophyll content

Chlorophyll content is the first physiological factor reflecting photosynthesis and the aerial growth of a plant. Because the root growth of alligator weed was significantly inhibited by the *E. adenophorum* leaf extracts at the concentration of as low as 0.02 g·mL^{-1} and the most severe inhibition was observed at the concentration of 0.08 g·mL^{-1}. To justify our data, we performed our experiment at the concentration of 0.04 g·mL^{-1}. The *E. adenophorum* leaf extract at 0.04 g·mL^{-1} inhibited the alligator weed growth. Chlorophyll content of *A. philoxeroides* was lower than that of the control at the indicated days after treatment of the aqueous extract from *E.adenophorum* leaves at the concentration of 0.04 g·mL^{-1}. After 3-days, the inhibition was 10.94% and it reached to 26.51% after 23d. These data suggested that *E. adenophorum* leaf extract inhibited the aerial growth of alligator weed.

Root activity of alligator weed

Root activity reflects the health status of a plant. As shown in Fig. 2, root activity was affected by the treatment of the aqueous extract from *E.adenophorum* leaves at the concentration of 0.04 g·mL^{-1}, and the root activity of alligator weed was steadily and steeply increased from 3 dpi to 18 dpi and remained at higher level in the control experiment. On the contrast, the root activity of alligator weed treated by *E. adenophorum* leaf extract was at higher level than that of control at 3 dpi and 8 dpi. Then significant lower root activity was observed at 13 dpi and 18 dpi. At 23 dpi, root activity was returned to the control level. Such relatively smooth increase of root activity in the treated sample imply that the inhibition of root growth could be due to the lower root activity of alligator weed treated by *E. adenophorum* leaf extract.

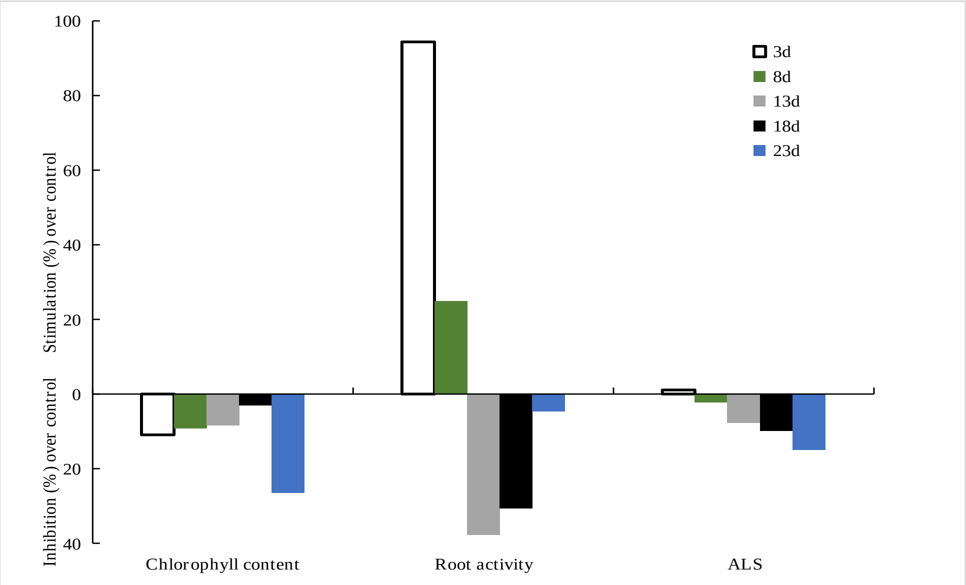

Figure 2. Physiological effects of the aqueous extract from *E.adenophorum* leaves on *A. philoxeroides.*

Acetolactate synthase activity of alligator weed

Acetolactate synthase (ALS) is a critical enzyme in amino acid synthesis and it directly affects the biosynthesis of valine, isoleucine and leucine (2). Its activity is a hall marker to reflect the health status of a plant. Because both the root and aerial growth of alligator were inhibited by E. *adenophorum* leaf extract, we assumed that ALS activity should be affected too. To this end, we measured the ALS activity of alligator weed at five time points at 3, 8, 13, 18, and 23 dpi after treatment with aqueous extract from E. *adenophorum* leaves at the concentration of 0.04 $g \cdot mL^{-1}$. The ALS activity in the samples treated by E. *adenophorum* leaf extraction was steeply decreased and the inhibition (%) over control was 2.15%, 7.75%, 9.89%, 14.98% respectively at 8, 13, 18, 23 dpi (Fig. 2). Thus the amino acid synthesis in alligator weed was significantly decreased by the E. *adenophorum* leaf extract.

The ALS inhibitors play important roles in controlling the weed at low dose (3). However, they may cause resistance of weed owing to their single action mechanism (17). The allelochemicals of E. *adenophorum* can affect not only the acetolactate synthase, but also other targets such as membrane. So it may be valuable to use the allelochemicals of E. *adenophorum* as potential botanical herbicide to control weeds, but the chemical compounds, whose target is ALS, are still unknown and need further studies.

Peroxidase activity of alligator weed

When a plant is in stressful environments, such as drought, too high or too low temperature, and pathogen infection, the activity of certain oxidoreductases will increase so as to buffer such stresses. Peroxidase (POD) is one typical oxidoreductase. To understand whether the treatment of E. *adenophorum* leaf extract generated a stressful environment for alligator weed, we measured the activity of POD according to the method of the colorimetric of guaiacol (4). The POD activitie of alligator weed was increased at 3, 13, 18 and 23 dpi, after treatment with aqueous extract from E. *adenophorum* leaves at 0.04 $g \cdot mL^{-1}$ concentration than control and was decreased at 8 dpi. The inhibition over control was 1.81% at 8 dpi, which indicated that there was no significant difference between the treatment and the control (Fig. 3). These data suggests the existence of stressful environment in alligator plant after application of E. *adenophorum* leaf extract.

Superoxide anion in alligator weed

Superoxide is biologically quite toxic, usually generated during severe stressful conditions. To understand whether the treatment of *E. adenophorum* leaf extract generated a stressful environment for alligator weed, we measured the rate of superoxide anion in alligator weed. The results showed that the rate of superoxide anion in alligator weed treated by *E. adenophorum* leaf extract was significantly higher in the tested five time points after treatment with aqueous extract from *E. adenophorum* leaves at the concentration of 0.04 $g \cdot mL^{-1}$ than that of the control (Fig. 3). From 3 dpi to 23 dpi, the rates of superoxide anion treatment were higher than control and the stimulation over control was 21.67%, 80.10%, 113.16%, 85.08%, 47.12% respectively. The higher accumulation of free oxygen radical in alligator weed treated by *E. adenophorum* leaf extract is consistent with the higher POD activity. These data imply that alligator weed is generates a stressful growth condition after the application of *E. adenophorum* leaf extract.

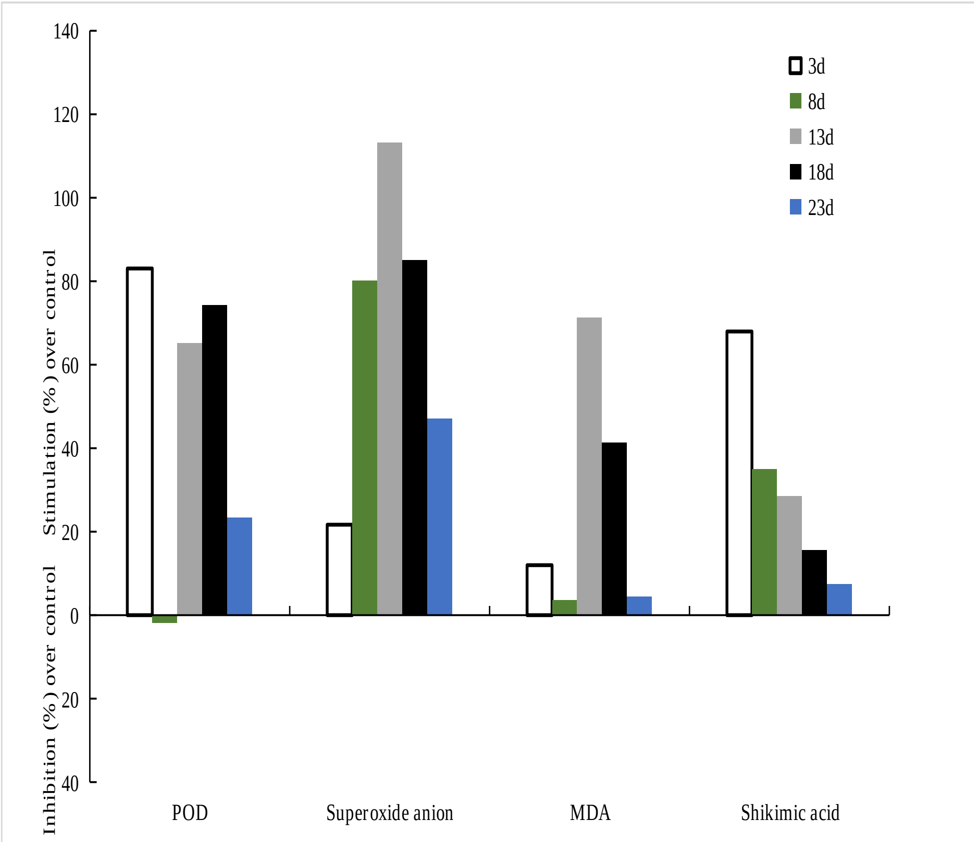

Figure 3. Stress responses of *A. philoxeroides* to the treatment of aqueous extract from *E.adenophorum* leaves.

Malondialdehyde content of alligator weed

Plants injury in stressful environments is close relation to membrane lipid peroxidation led by accumulated active oxygen, malondialdehyde (MDA) is one of the most important products on membrane lipid peroxidation. The extent of damage of membrane systems can be indirect investigated through measure the content of MDA in plant. To understand whether the treatment of *E. adenophorum* leaf extract generated injury on membrane systems of alligator weed, we measured the MDA content of alligator weed after treatment with aqueous extract from *E. adenophorum* leaves at the concentration of 0.04 $g \cdot mL^{-1}$. As shown in Fig. 3, the MDA content of alligator weed had no significant change at 8 and 23 dpi, and the content was increased gradually and higher compared with the control from 8 dpi to 18 dpi, the difference was more and weaker. The results revealed that MDA could be accumulated for some times, and then it would be recovered.

It has been proved that allelochemicals of *E. adenophorum* affects the normal working of cell membrane of plants. Ma *et al.* (7) indicated that the main reason was the injury of membrane system, caused by the accumulation of malondialdehyde (MDA). Our results showed that aqueous extract from *E. adenophorum* leaves increased the contents of superoxide anion and malondialdehyde (MDA) and peroxidase (POD) activity. Based on the investigation, we speculated that more oxygen free radical and hyperoxide were produced in the *A. philoxeroides*, which improved the peroxidase (POD) activity, to decrease the damage from the harmful compounds in the plant. These results confirmed that aqueous extract from *E. adenophorum* could lead to the membrane peroxidation, as the oxygen free radical and hyperoxide are its products.

Shikimic acid content in alligator weed

Shikimate pathway is one of important amino acid biosynthesis pathway. Shikimic acid is an important medial product in shikimic acid synthesis pathway, and its accumulation replays 5-enolpyruvyl-shikimate-3-phosphate synthase is inhibited, because the synthase can decomposite shikimic acid. The accumulation of shikimic acid will inhibit further biosynthesis in the synthesis pathway, and then influence plant growth.

The shikimic acid content in alligator weed treated by *E. adenophorum* leaf extract maintained at significantly higher level compared with the control after treatment with aqueous extract from *E. adenophorum* leaves at the concentration of 0.04 $g \cdot mL^{-1}$, but the difference was gradually reduced along with the time and became no different at 23 dpi (Fig. 3). These results showed that *E. adenophorum* leaf extract could induce the accumulation of shikimic acid of *A. philoxeroides*, which would affect the normal work of shikimate pathway and amino acid biosynthesis.

CONCLUSIONS

We found that application of aqueous extracts from the leaves of *E. adenophorum* influenced the cell structure, cell membrane, activity of key enzymes and amino acid biosynthesis in alligator weed (*A. philoxeroides*). These results indicate that the aqueous extracts from the *A. philoxeroides* might be potential source of natural herbicide compounds against the alligator weed. Hence, further research is required to find the active allelochemicals and their allelopathic suppression mechanism.

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REFERENCES

1. Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. and Vivanco, J.M. (2003). Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science* **301:** 1377-1380.
2. Chipman, D.M., Duggleby, R.G. and Tittmann, K. (2005). Mechanisms of acetohydroxyacid synthases. *Current Opinion in Chemical Biology* **9:** 475-481.
3. Christoffoleti, P.J., Nissen, S., Gaines, T. and Peres, L.E.P. (2015). Auxinic herbicides, mechanisms of action, and weed resistance: a look into recent plant science advances. *Agricultural Sciences* **72:** 356-362.
4. Fan, H., Guo, S., Jiao Y., Zhang, R. and Li, J. (2007). Effects of exogenous nitric oxide on growth, active oxygen species metabolism, and photosynthetic characteristics in cucumber seedlings under NaCl stress. *Frontiers of Agriculture in China* **1:** 308-314. (Chinese).
5. Gao, J., Xiao, Q., Yin, L. and He, G. (2008). Isolation of cDNA clones for genes up-regulated in drought-treated *Alternanthera philoxeroides* root. *Molecular Biology Reports* **35:** 485-488.
6. Hegedus, A., Erdei, S. and Horvath, G. (2001). Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Science* **160:** 1085-1093.
7. Jin, H.M., Guo, F.X. and Wen, X.Y. (2012). Inhibitory effects of leachate from *Eupatorium adenophorum* on germination and growth of *Amaranthus retroflexus* and *Chenopodium glaucum*. *Acta Ecologica Sinica* **32:** 50-56. (Chinese).
8. Liu, X., Ouyang, C., Wang, Q. and Li, Y. (2016). Evaluation of antibacterial and antifungal properties of 9-oxo-10,11-dehydroageraphorone extracted from *Eupatorium adenophorum*. *Journal of Plant Diseases & Protection* **123:** 93-99.
9. Longstreth, D.J., Bolanos, J.A. and Smith, J.E. (1984). Salinity effects on photosynthesis and growth in *Alternanthera philoxeroides* (Mart.) Griseb. *Plant Physiology* **75:** 1044-1047.
10. Zhang, M., Fu, D.M., Chen, H.B. and Deng, T.T. (2010). Preliminary study on allelopathic effects and mechanism of *Eupatorium adenophorum* to wheat and rape seedlings. *Journal of Zhejiang University (Agriculture and Life Sciences)* **36:** 547-553.
11. Proksch P. (1990). Ontogenetic variation of biologically active natural prosucts in *Ageratina adenophora*. *Phytochemisty* **29:** 453. (Chinese)
12. Yang, Q.S.,Yang, J. and Liu, H.M. (2006). The research progress on the chemical components and bioactivity of *Eupatorium adenophorum* as alien invasive species. *Journal of Beijing Normal University* **42:** 70-73. (Chinese)
13. Ruf, M. and Brunner, I. (2003). Vitality of tree fine roots: Re-evaluation of the tetrazolium test. *Tree Physiology* **23:** 257-263.
14. Sanders, N.J., Weltzin, J.F. and Crutsinger, G.M. (2007). Insects mediate the effects of propagule supply and resource availability on a plant invasion. *Ecology* **88:** 2383-2391.
15. Sang, W., Zhu, L. and Axmacher, J. (2010). Invasion pattern of *Eupatorium adenophorum* Spreng in southern China. *Biological Invasions* **12:** 1721-1730.
16. Wang, Y.B., Guang, L.Z. and Luo, J.Z. (2014). Allelopathic effects of *Eupatorium adenophorum* (Spreng.) on Eucalyptus. *Eucalypt Science & Technology* **1:** 27-31.
17. Whaley, C.M., Wilson, H.P. and Westwood, J.H. (2007). A new mutation in plant ALS confers resistance to five classes of ALS-inhibiting herbicides. *Weed Science* **55:** 83-90.
18. Yang, G., Wan, F., Liu, W. and Zhang, X. (2006). Physiological effects of allelochemicals from leachates of *Ageratina adenophora* (Spreng.) on rice seedlings. *Allelopathy Journal* **18:** 237-246.
19. Yu, X., Yu, D. and Ma, K. (2004). Relationships between allelopathy and invasiveness by *Eupatorium adenophorum* at different sites. *Chinsese Journal of Plant Ecology* **28:** 773-780. (Chinese).
20. Zhao, J., Williams, C.C. and Last, R.L. (1998). Induction of Arabidopsis tryptophan pathway enzymes and camalexin by amino acid starvation, oxidative stress, and an abiotic elicitor. *Plant Cell* **10:** 359-370.
21. Zhou, J., Dong, B.C. and Alpert, P. (2012). Effects of soil nutrients heterogeneity on intraspecific competition in the invasive, clonal plant *Alternanthera philoxeroides*. *Annals of Botany* **109:** 813-818.
22. Zhu, X., Zhang, J. and Ma, K. (2011). Soil biota reduce allelopathic effects of the invasive *Eupatorium adenophorum*. *PLos One* **6:** 1-6.