ALLELOPATHIC EFFECTS OF AQUEOUS EXTRACTS FROM DIFFERENT PLANT PARTS OF CANADA GOLDENROD (*SOLIDAGO CANADENSIS* L.) ON SEED GERMINATION AND SEEDLING GROWTH OF KOREAN LAWNGRASS (*ZOYSIA JAPONICA* STEUD.)

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Abstract. The invasion mechanism *of Solidago canadensis* L., been extensively investigated as its one of the most destructive exotic invasive plants. The present study showed that allelopathy is beneficial to the successful invasion of invasive alien species (IAS). However, the main allelopathic part of *S. canadensis* L. is less known to the researchers. Therefore, we experimented with the allelopathic effects of extracts obtained from various parts of *S. canadensis* L. (root, litter, aboveground parts) at concentrations of 50 g L^{-1} and 150 g L^{-1} on seed germination and seedling growth of *Zoysia japonica* Steud. The results showed that the extract from the *S. canadensis* L. aboveground parts had prominent inhibition effects on Zoysiagrass seed germination rate, germination potential, vigour value, root length and plant height, and the suppression increased significantly at high concentrations. With the treatment of aqueous extract from the aboveground parts of *S. canadensis* L. (50 g L^{-1}), the content of malondialdehyde (MDA) concentration and peroxidase (POD) activity increased significantly. The root extract at the concentration of 50 g L^{-1} did not affect the parameters except POD activity, but the concentration of 150 g L^{-1} inhibited all the parameters significantly except catalase (CAT) activity. Litter aqueous extracts did not show any significant effect on any parameters of *Zoysia japonica* Steud.
Keywords: *allelochemicals, growth development, invasion, invasive species, litter extracts, enzyme activity*

Introduction

Allelopathy refers to the beneficial or harmful effects of one plant on another plant, in both crop and weed species, caused by release of biochemicals, known as allelochemicals, from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems. It can also be defined as the phytotoxicity of a component or a series of components released from plant parts by root exudation, volatilization, leaching or residue decomposition of susceptible plants (Ferguson et al., 2013; Wang et al., 2019; Anžlovar and Anžlovar, 2019). The phenomenon of allelopathy has been used to explain how some invasive species harm to native ones or even produce specific biochemicals (Callaway, 2002; Hu and Zhang, 2013; Sun et al., 2017). Furthermore, the allelopathy has been involved in the success of certain widespread plant invaders. According to novel weapons hypothesis, invasive species exude unique biochemical constituents may contribute to their successful invasion (Callaway and Aschehoug, 2000; Zhang et al., 2017).

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Allelochemicals are a subset of secondary metabolites not required for metabolism (growth and development) of the allelopathic organism. Allelochemicals with negative allelopathic effects are an important part of plant defense against herbivory (i.e., animals eat plants as their primary food) (Lorenzo et al., 2011; Ullah et al., 2021). More specifically, it was explained in the novel weapons hypothesis that through the production of biochemicals some invasive plants regenerate successfully in the invaded range. Thus these biochemicals may have the potential to cause negative effects on native plants. Moreover, the novel biochemical effects widely used in competing plants, soil biota and generalist herbivores (Wang et al., 2017a; Zhang et al., 2017; Qi et al., 2020). The basic approach used in allelopathic research for agricultural crops has been to screen for both crops and natural vegetation for their capacity to suppress weeds. To demonstrate allelopathy, plant origin, production, and identification of allelochemicals must be established as well as persistence in the environment over time in concentrations sufficient to affect plant species (Lorenzo et al., 2011; Wang et al., 2017b, 2020c).

S. canadensis L. is a perennial rhizomatic plant and belongs to Compositae (Zhang and Wan, 2017; Anžlovar and Anžlovar, 2019). In the 1930s, it introduced into China as an ornamental plants from North America and originally cultivated in Shanghai and Nanjing area, and later spread to the wild. In the 1980s it began to spread to Jiangsu and Zhejiang area. The spread of *S. canadensis* L. causes serious damage to the ecological environment of China and considered as an invasive malignant weeds (Wang et al., 2020b). At present, there are many studies found about the allelopathy of *S. canadensis* L. (Yuan et al., 2013; Wang et al., 2019) found that the extracts of *S. canadensis* L. showed greater allelopathic effects on seedlings of native plants in the new location than in the original location, and the allelopathy enhance the competitive ability of *S. canadensis* L. in the invaded range (Yuan et al., 2013). Also, they reported that the secondary metabolites of *S. canadensis* L. may increase its competitiveness through enhancing the AMF symbionts (Abhilasha et al., 2008; Wang et al., 2020a) demonstrated that root exudates of *S. canadensis* L. significantly inhibited Arabidopsis growth and it was concentration-dependent. What's more, the addition of activated carbon can relieve the inhibitory of tested plants under more realistic conditions in soil (Wang et al., 2018). Sun et al. (2006a) investigated the effects of ethanolic and aqueous extracts from leaves, stems and rhizomes of *S. canadensis* L. to mulberry, morning glory, wheat and rape seed germination and seedling growth. They found higher concentration of this extracts can both significantly inhibit seed germination and seedling growth of the four species (Wu et al., 2019).

However, these studies mainly focus on the competitiveness between different plant species or the organic solvent of the whole plant or an organization tissue to evaluate the allelopathic effect, but whether these so-called allelochemical can release to the soil environment through the leaching and decomposition of litter and have a real ecological effect also deserves further research. Some researches already demonstrated the aqueous extracts allelopathy in different parts of invasive plants (Gao et al., 2009; Li and Jin, 2010; Wei et al., 2020), but rarely the allelopathy of *S. canadensis* L. has been noticed Therefore, our study use aqueous extracts of different parts of *S. canadensis* L. applied to *Zoysia japonica* Steud. (Zoysiagrass) seeds germination and seedling inhibition experiments to clarify its allelopathic inhibition mechanism and provide a basis to the assessment of actual ecological effect of allelochemical from *S. canadensis* L. to the soil environment.

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Materials and methods

Plant materials

There were two species used in this experiment i.e. *S. canadensis* L. and *Zoysia japonica* Steud. (Zoysiagrass). The *Zoysiagrass* seeds was used in the experiment bought from seeds market in Zhenjiang of Jiangsu Province, China. In December 2013, root, litter, aboveground parts of *S. canadensis* L. were collected from wild in Zhenjiang of Jiangsu Province, China (32.171058° N, 119.540535° E).

Preparation of S. canadensis L. extracts

The plant samples of *S. canadensis* L. were separated into root, litter and aboveground parts; each plant part was cut into 2-3cm pieces, which were soaked for 48 h with 1L distilled water and then filtered. The final concentration of the root, litter, and aboveground parts aqueous extracts of *S. canadensis* L. was 400 g L^{-1}, 150 g L^{-1}, 180 g L^{-1}, respectively. All the aqueous extracts stored at 4°C.

Effects of aqueous extracts on seed germination and seedling growth of Zoysiagrass

Thirty seeds of *Zoysiagrass* were sown in Petri dishes (9 cm in diameter) containing 10 mL aqueous extracts (the root, litter, aboveground parts aqueous extracts of *S. canadensis* L.) or 10 mL distilled water (control). Two concentrations were set for each aqueous extract i.e. 50 g L^{-1} and 150 g L^{-1} with five replicates per treatment. The Petri dishes were cultivated at room temperature (20°C) and the germination rate was measured daily for 5 days.

Lipid peroxidation and enzyme analyses

Growth parameters, content of malondialdehyde (MDA) as well as catalase (CAT) and peroxidase (POD) activity were assayed to evaluate the effects of allelochemicals and aqueous extracts from different parts of *S. canadensis* L. Lipid peroxidation was determined in 0.5 g fresh whole plants by measuring the content of MDA, a production of lipid peroxidation, by the thiobarbituric acid reaction (Gossett et al., 1994). 0.5 g whole plants were collected and ground with mortar with pestle on the ice immediately, suspended in 2 ml 50 mM phosphate buffer (PBS) at pH 7.8. Extracts were centrifuged at 12,000 × g for 20 min (4°C) and the supernatant was used for the determination of enzyme activity. CAT activity was assayed in a reaction solution (3 ml) containing 0.3% H_2O_2 and distilled water. The reaction was started by adding 100 µl crude extracts to the reaction solution and the activity was followed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption. POD activity was assayed in a reaction solution (3 ml) including 50 mM PBS at pH 7.0, 0.2% guaiacol, 0.3% H_2O_2 and 100 µl crude extracts. The increasing absorbance on account of oxidation of guaiacol was measured at 420 nm (Xu et al., 2015).

Determination of indexes and methods

In accordance with the rules of the international seed germination (We considered radicle length is half of the seed as germination). Determination of germination index formula is (Gao et al., 2009):

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$$Germination\ rate\ (\%) = (no.of\ germinated\ seeds \div total\ no.of\ seeds) \times 100 \quad \text{(Eq.1)}$$

$$Germination\ potential\ (\%) = (4th\ day\ no.of\ germinated\ seed \div total\ no.seeds) \times 100 \quad \text{(Eq.2)}$$

Daily germination number in the 4th day was the maximal (Jing, 2006).

$$Vigour\ value\ (V) = \left(\frac{a}{1} + \frac{b}{2} + \frac{c}{3} + \frac{d}{4} + \cdots \frac{x}{n}\right) \times [\frac{100}{S}] \quad \text{(Eq.3)}$$

where a, b, c… respectively, represent the number of seeds germinated after 1, 2, 3 days of inhibition, x is the number after (n) days and (S) is total number of germinated seeds (El-Soud et al., 2013).

In addition, selected 10 seedlings randomly from each Petri dishes in the 6th day and measured the root length and plant height. Aboveground parts extracts at the concentration of 150 g L^{-1} absolutely inhibited the growth of *Zoysiagrass* seedlings. Thus we cannot get the data of MDA, CAT and POD in *Zoysiagrass* seedlings.

Statistical analysis

Measurements were performed by using five replicates randomly. All measurements were examined statistically through SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) and Origin Pro 9.0. Variance analysis with two crossed fixed factors was applied to discriminate the effects of aqueous extracts of *S. canadensis* L. on the seed germination of *Zoysiagrass* varied with different types and concentrations of the extracts. Data were presented in means ± SE. All the parameters in the control and treatments were compared for each aqueous extract using an ANOVA and (Newman-Keuls or Student–Newman–Keuls) tests to determine the significant differences ($P < 0.05$). The graphs were produced in origin pro9.

Results

Effect of the extracts on the seed germination of Zoysiagrass

The effect of the aqueous extracts of *S. canadensis* L. on the seed germination of *Zoysiagrass* varied with different types and concentrations of the extracts. As compared with the control, the aqueous extract from the *S. canadensis* L. litter (50 g L^{-1} and 150 g L^{-1}) showed no significant influence to the germination rate, germination potential and vigour value of *Zoysiagrass* seeds ($P > 0.05$; *Figs. 1,2,3*). The 50 g L^{-1} extract from the root had no obvious effect to Zoysiagrass seeds germination rate, germination potential and vigour value ($P > 0.05$), but the effects of 150 g L^{-1} extract were significantly inhibitory ($P < 0.05$; *Figs. 1,2,3*). The extract from the aboveground parts of *S. canadensis* L. showed significant inhibitory effect ($P < 0.05$), and the suppression strengthened with the increasing of the extract concentration (*Figs. 1,2,3*). The allelopathic effects of aboveground parts extract on *Zoysiagrass* seeds were the strongest, followed by the root extract, and smallest for the litter extract. At the concentration of 50 g L^{-1}, only

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aboveground parts extract significantly decreased the germination rate, germination potential and vigour index of *Zoysiagrass* (P < 0.05). Root extract and aboveground parts extract inhibited germination rate, germination potential and vigour index obviously at 150 g L^{-1} (P < 0.05), and aboveground parts extract showed stronger inhibitory effect (*Table 1*).

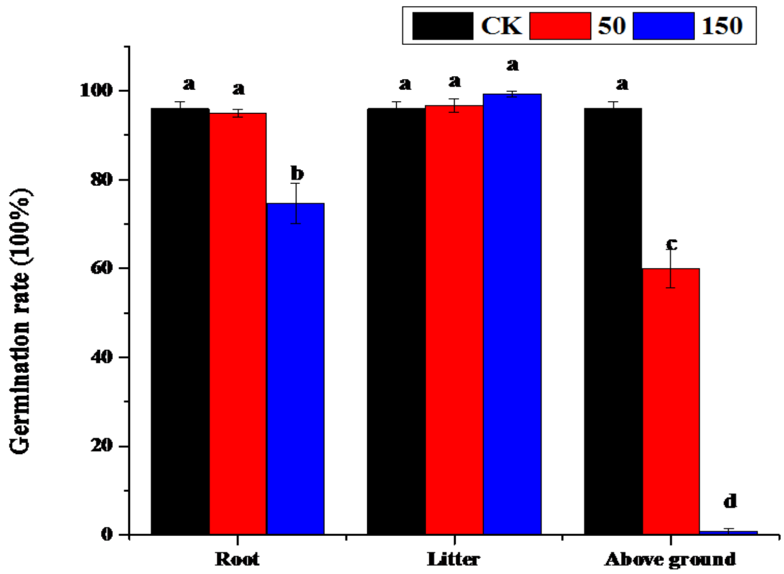

Figure 1. *Effects of the aqueous extracts of S. canadensis L. on the germination rate of Zoysiagrass seeds. Data with different superscript letters indicate a significant difference (P < 0.05). The concentrations of aqueous extract are given in g L^{-1}*

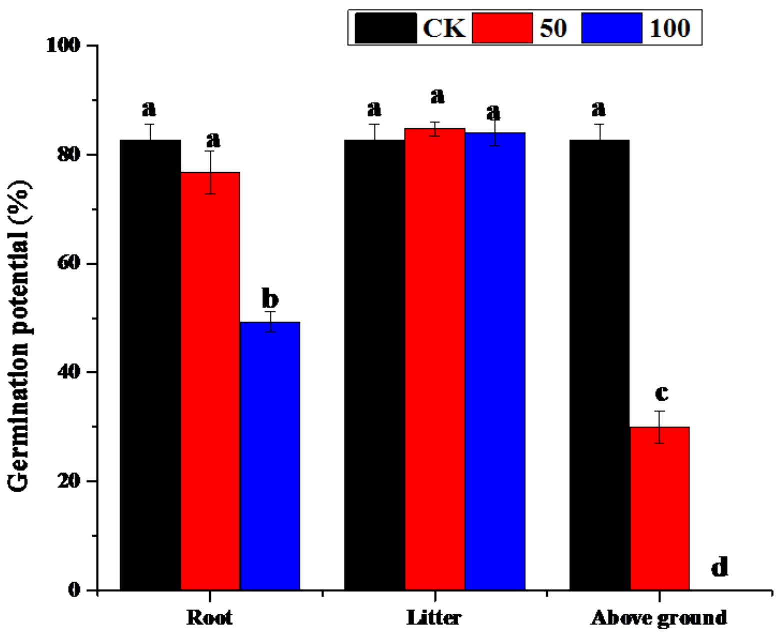

Figure 2. *Effects of the aqueous extracts of S. canadensis L. on the germination potential of Zoysiagrass seeds. Data with different superscript letters indicate a significant difference (P < 0.05). The concentrations of aqueous extract are given in g L^{-1}*

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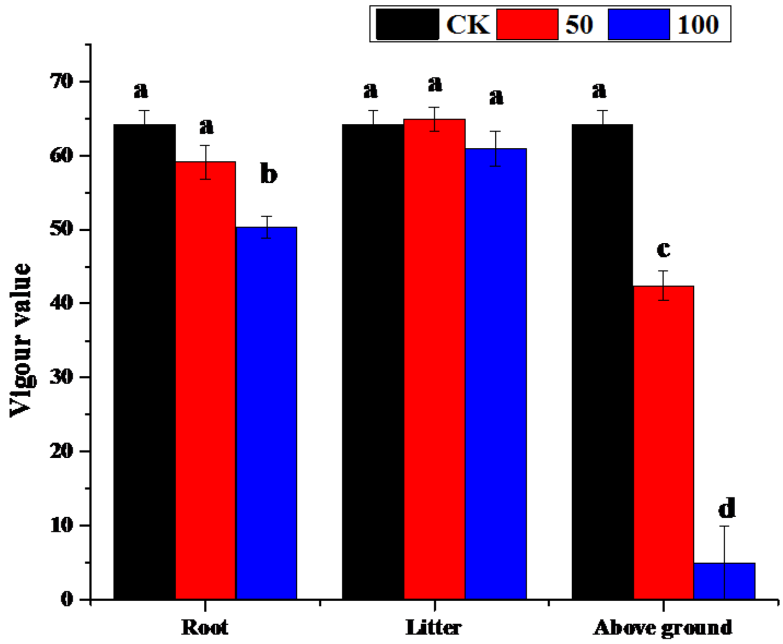

Figure 3. *Effects of the aqueous extracts of S. canadensis L. on the vigour value of Zoysiagrass seeds. Data with different superscript letters indicate a significant difference (P < 0.05). The concentrations of aqueous extract are given in g L^{-1}*

Table 1. *Summary of S-N-K tests of effect of treatment with different parts of S. canadensis L. indifferent concentrations*

	ck	Root		Litter		Aboveground part	
		50 g L^{-1}	150 g L^{-1}	50 g L^{-1}	150 g L^{-1}	50 g L^{-1}	150 g L^{-1}
GR	96.0±1.633a	95.3±0.816a	74.7±4.546b	96.7±1.491a	99.3±0.667a	60.0±4.346c	0.7±0.667d
GP	82.7±2.867a	76.7±3.944a	49.3±1.944b	84.7±1.333a	86.0±2.449a	30.0±2.981c	0d
VI	64.2±1.916a	59.1±2.270a	50.3±1.461b	64.9±1.652a	60.9±2.364a	42.4±2.019c	5.0±5.000d
PH	2.4±0.097a	2.4±0.116a	1.4±0.130b	2.3±0.113a	2.6±0.117a	0.8±0.100c	0.0±0.020d
RL	2.2±0.085a	2.2±0.075a	1.7±0.099b	2.1±0.076a	2.2±0.088a	0.4±0.064c	0.0±0.013d
MDA	2.0±0.113ab	1.4±0.085ab	1.7±0.177ab	2.1±0.131ab	1.9±0.294ab	2.7±0.304a	
CAT	9.2±0.467bc	8.0±0.686c	8.2±0.410c	10.3±0.787ab	11.0±0.338a	8.7±0.266bc	
POD	34.7±1.000b	24.6±1.060c	25.0±1.046c	32.2±2.439b	29.6±1.034bc	55.1±3.483a	

All the data present in mean ± SE, and data with different superscript letters behind indicate a significant difference (P < 0.05). Independent samples S-N-K tests were performed using SPSS version 17.0 (SPSS Inc., Chicaogo, IL). GR is germination rate, GP is germination potential, VI is vigour index, PH is plant height, RL is root length. The control treatment was conducted for each plant extracts

Effect of the extracts on the growth of the Zoysiagrass seedlings

Aqueous extract of *S. canadensis* L. litter (50 g L^{-1} and 150 g L^{-1}) and the 50 g L^{-1} extract from the root had no obvious impacts on the root length and plant height of *Zoysiagrass* seedlings (P > 0.05), but the aqueous extracts from root (150 g L^{-1}) and aboveground parts (50 g L^{-1} and 150 g L^{-1}) had significant inhibition on *Zoysiagrass* seedlings (P < 0.05), and the degree of suppression increased at a high concentration (150 g L^{-1}) (*Figs. 4,5*). Aboveground parts extract with the concentration of 50 g L^{-1} decreased the plant height and root length of *Zoysiagrass* while root extract and litter

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extract have no effects on them (P < 0.05). At the concentration of 150 g L^{-1}, root extract and aboveground parts extract significantly inhibited plant height and root length (P < 0.05), and aboveground parts extract had more negative effects (*Table 1*).

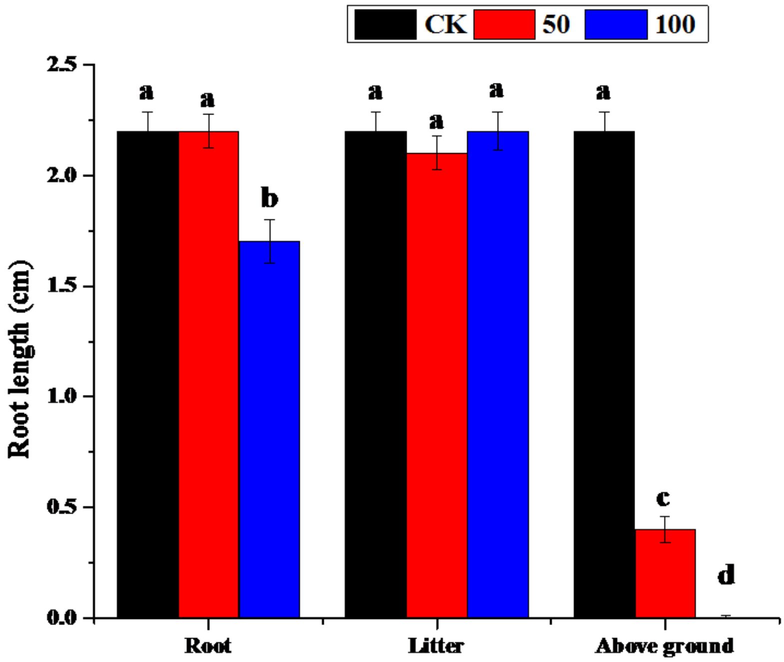

Figure 4. *Effects of the aqueous extracts of S. canadensis L. on the root length of Zoysiagrass seedlings. Data with different superscript letters indicate a significant difference (P < 0.05). The concentrations of aqueous extract are given in g* L^{-1}

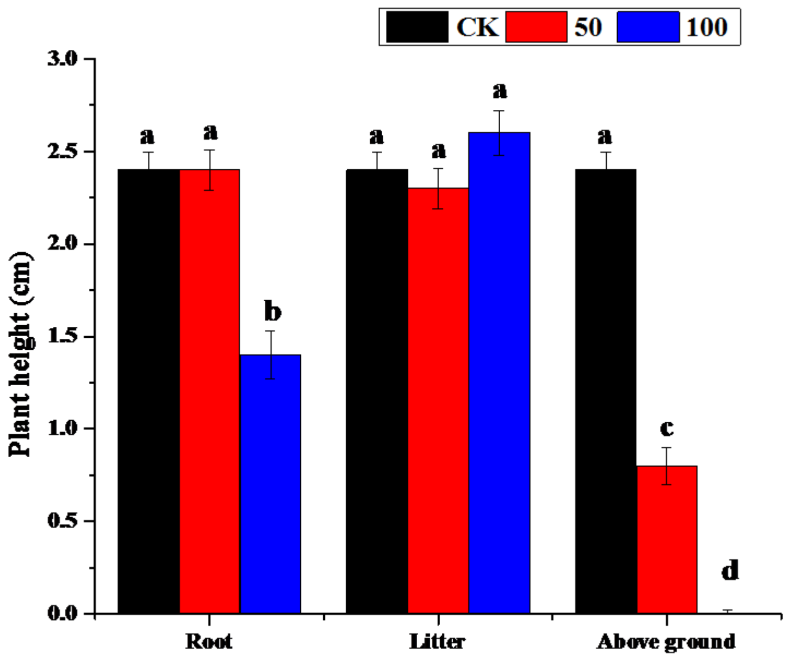

Figure 5. *Effects of the aqueous extracts of S. canadensis L. on the plant height of Zoysiagrass seedlings. Data with different superscript letters indicate a significant difference (P < 0.05). The concentrations of aqueous extract are given in g* L^{-1}

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Effect of the extracts on the MDA concentration of the Zoysiagrass seedlings

MDA content was a measurement of lipid peroxidation. The effect of the aqueous extracts of *S. canadensis* L. on the MDA concentration of the *Zoysiagrass* seedlings varied with different types of the extracts (*Fig. 6*). At 50 g L^{-1}, the root aqueous extract significantly decreased the MDA concentration of the *Zoysiagrass* seedlings ($P < 0.05$), but at 150 g L^{-1} the suppression was not significant compared with the control ($P > 0.05$). The aqueous extract of *S. canadensis* L. litters (50 g L^{-1} and 150 g L^{-1}) showed no obvious effects ($P > 0.05$). The 50 g L^{-1} aboveground extracts increased the MDA concentration obviously ($P < 0.05$).

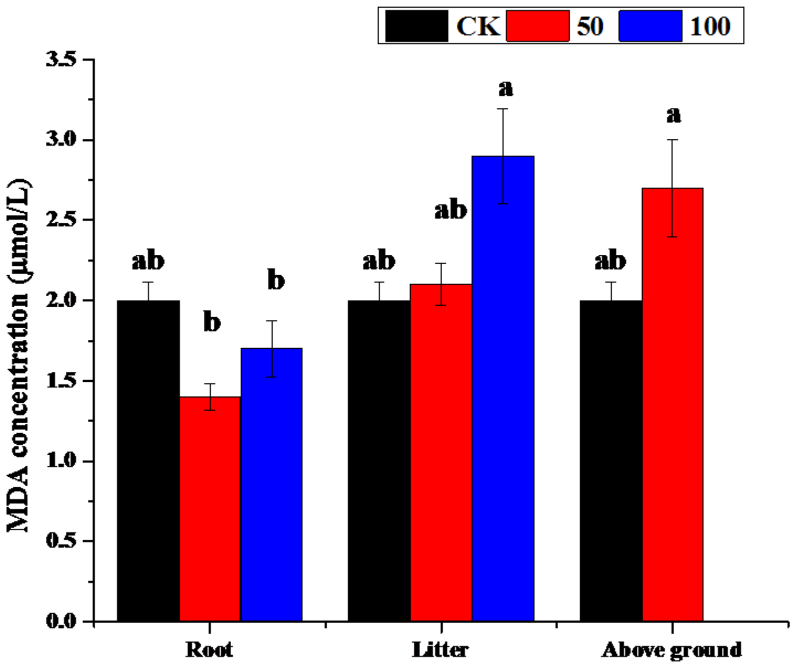

Figure 6. *Effects of the aqueous extracts of S. canadensis L. on the MDA concentration of Zoysiagrass seedlings. Data with different superscript letters indicate a significant difference (P < 0.05). The concentrations of aqueous extract are given in g L^{-1}*

Effect of the extracts on CAT and POD activity of the Zoysiagrass seedlings

The aqueous extracts of root, litter and aboveground parts from *S. canadensis* L. had no significant difference to the CAT activity of the *Zoysiagrass* seedlings ($P > 0.05$; *Fig. 7*). The extract of *S. canadensis* L. root (50 g L^{-1} and 150 g L^{-1}) significantly inhibited the activity of POD, while aboveground parts extract (50 g L^{-1}) obviously increased it (*Fig. 8*; $P < 0.05$). The litter extract of *S. canadensis* L. did not affect the POD activity ($P > 0.05$).

Relationship between different parameters of Zoysiagrass

Table 2 showed the pearson correlation analysis of all the parameters present in the experiment. MDA did not have any relationship with all the parameters, and POD had no obvious relationship with germination potential, plant height and as well as with root length. But beyond of this, the other parameters have significantly correlation with others.

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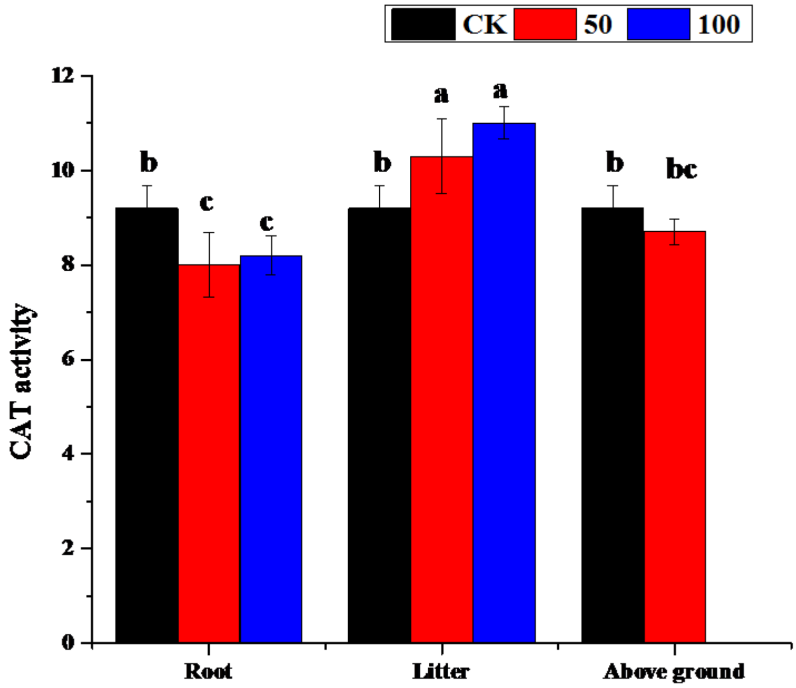

Figure 7. *Effects of the aqueous extracts of S. canadensis L. on the CAT activity of Zoysiagrass seedlings. Data with different superscript letters indicate a significant difference (P < 0.05). The concentrations of aqueous extract are given in g L^{-1}*

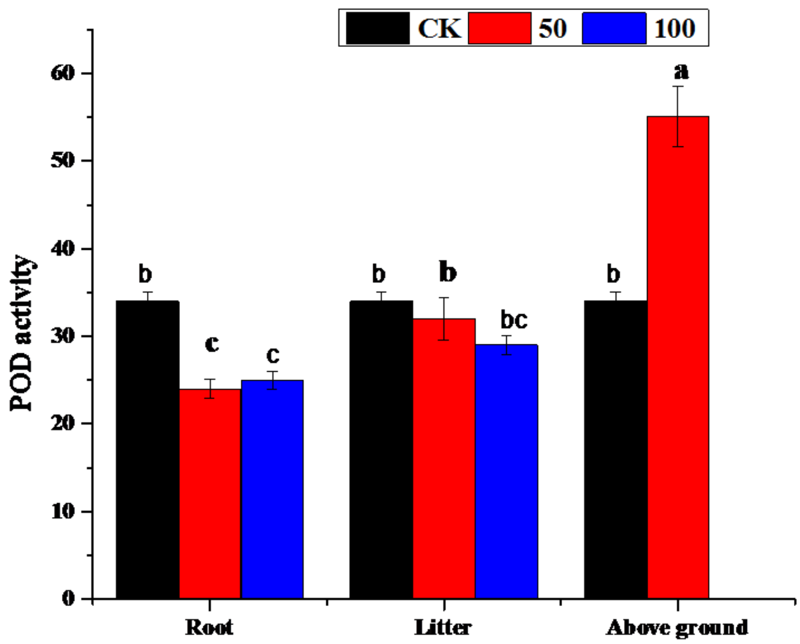

Figure 8. *Effects of the aqueous extracts of S. canadensis L. on the POD activity of Zoysiagrass seedlings. Data with different superscript letters indicate a significant difference (P < 0.05). The concentrations of aqueous extract is given in g L^{-1}*

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Table 2. *Pearson correlation analysis of all the parameters present in the experiment*

	GR	GP	VI	PH	RL	MDA	CAT	POD
GR	1	.946**	.945**	.931**	.897**	.267	.890**	.522**
GP		1	.925**	.973**	.931**	.298	.795**	.324
VI			1	.899**	.852**	.311	.858**	.518**
PH				1	.956**	.321	.745**	.279
RL					1	.036	.699**	.159
MDA						1	.265	.458**
CAT							1	.709**
POD								1

Behind each data, asterisks (*) show significant differences (P < 0.05) between each parameter. The analysis was performed using SPSS version 17.0 (SPSS Inc., Chicaogo, IL). GR is germination rate, GP is germination potential, VI is vigour index, PH is plant height, RL is root length

Discussion

The success of some of the worst plant invaders depend on the mechanism of allelopathy (Callaway and Aschehoug, 2000), including *Mikania micrantha* (Chen et al., 2009), *Ageratina adenophora* (Zhou et al., 2013) and *Fallopia japonica* (Dommanget et al., 2014). The significant inhibition that the aqueous extracts from the *S. canadensis* L. root and aboveground parts imposed on the germination rate, germination potential, vigour value, root length and plant height of *Zoysiagrass* seedlings indicated the existence of allelopathic water-soluble substances in the extracts. These results in accordance with the previous findings that aqueous extracts of *S. canadensis* L. suppress seed germination and seedling growth of some plants (Sun, 2006; Ledger et al., 2015). Moreover, some studies represented that the effects of different parts extracts of *S. canadensis* L were varied, and low concentrations of the extracts always showed positive effects on seed germination and seedling growth of native plants, and high concentration demonstrated negative effects on native seed germination and seedling growth (Sun et al., 2006b; Huang et al., 2009; Megenhardt, 2015). To some extent, our result in accordance with it, root extract and litter extract had no effect on Zoysiagrass seed germination, plant height and root length at low concentration (50 g L^{-1}) and inhibited these parameters at high concentration (150 g L^{-1}). Aboveground parts extract inhibited these parameters of *Zoysiagrass* at both 50 g L^{-1} and 150 g L^{-1}, and the inhibition strengthened with the increasing concentration (*Table 2*). In one of proceedings studies it was noted that growth rate reduced of target species in the presence of both aqueous and ethanolic extract of *S. canadensis* L. at different concentrations (Możdżeń et al., 2020). Similarly, in another study, leaf extracts with high concentration significantly decreased root length, leaf shape index, germination percentage, germination potential, germination index, germination vigor index, and germination rate index of lettuce (Wang et al., 2019).

Besides the reduction in growth of *Zoysiagrass* seedlings, the membranes were affected by *S. canadensis* L. aqueous extracts. Allelochemicals can impair the cell membranes through direct interaction with membrane constituents or due to impairment of some metabolic functions necessary to maintain the membrane functions (Bertin et al., 2003). Higher concentrations of aboveground part aqueous extracts of *S. canadensis* L. caused more serious lipid peroxidation damage, and the result was consistent with some other research (Han et al., 2012). But the root extract of *S. canadensis* L. caused obvious

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reduction of MDA, and it showed that the root extract can relieve the lipid peroxidation of the Zoysiagrass seedlings. Through the results, we speculated that there were some allelochemicals in aqueous extracts of root, litter and aboveground parts of *S. canadensis* L. These alllelochemicals could harm to cell membrances of the *Zoysiagrass* seedlings through direct interaction with a constituent of the menbrance or as a result of an impairment of some metabolic function necessary to the maintenance of membrane function.

As previous researches showed, CAT can be induced in plant species and CAT can produce and metabolise H_2O_2. What's more, CAT activity is directly regulated by the concentration of H_2O_2 and the accumulation of H_2O_2 can stimulate the CAT activity (Fornazier et al., 2002). Both the aqueous extracts of *S. canadensis* L. had no significant effects on CAT activity of *Zoysiagrass* seedlings, suggesting that the aqueous extracts could not induce and promote the metabolise of H_2O_2 in *Zoysiagrass* seedlings.

Aqueous extracts of *S. canadensis* L. strongly affects the activity of peroxidases in both root and aboveground parts. But it showed pronounced increase of POD activity in aboveground parts and decrease in root. Previous studies in Eucalyptus extracts and other allelochemicals on plant seedling illustrated that allelochemicals absorbed by plant cells should be antidotal and the antidotal action and other responses of plant cells caused the increase of POD activity. The decrease of POD activity of *S. canadensis* L. root extract, indicating the allelochemicals in the root injury the POD enzyme by toxicity. But some other researches gained the opposite results, higher concentration levels of *Pogostemon cablin* aqueous extracts might have exceeded the rate of detoxification which then result in inhibitory effect on plant growth and dramatically decreasing on POD activity (Xu et al., 2015; Meiners et al., 2017).

Conclusion

In conclusion, the aqueous extracts of *S. canadensis* L. could influence the *Zoysiagrass* seeds germination and seedlings growth through water-soluble allelopathic substances. And the regulation of the anti-oxidase activity of CAT and POD and the oxidization of the cell membranes may make a contribution to the effects. Our demonstration of suppression of Zoysiagrass by aqueous extracts of *S. canadensis* L. suggest that allelopathy plays a more important role than other mechanisms do in the out-competition of *S. canadensis* over other plants, and make it invasive in new habitats and offered an approach to explain the invasive of *S. canadensis* L. but the invasive mechanism still needs further study.

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