A preliminary study on allelopathy and potential allelochemicals of root exudates from *Solanum rostratum* Dunal.

Abstract：

Aims: In order to understand the role of allelopathy in invasion success, effects of root exudates from *Solanum rostratum* Dunal on germination and primary growth of wheat and cabbage seeds were studied by vitro bioassay. These results indicated the allelopathy of root exudates and would be helpful for understanding *Solanum rostratum* Dunal invasion .

Study Design: The roots were washed clean for three times with distilled water before they were gathered in a big bucket. The container was filled with distilled water, enough to submerge the roots, and air was aerated constantly with an air pump. The water extract was collected per 24hs and for four times totally. The water extract from *Solanum rostratum* was concentrated with a rotary evaporator at 40℃ and deposited in a refrigerator at 4℃. All the root exudates were pooled around 1500mL, and the fresh weight of *Solanum rostratum* used for exudates collection was 1208g, the exudate concentration was then marked as 0.8g f w/mL.

Place and Duration of Study: *Solanum rostratum* was grown in the greenhouse on April 15th, 2019, its seeds were collected from the invaded fields in Chaoyang city (Liaoning province, China)

Methodology: Petri dish bioassay was applied to test the effects of root exudates of *Solanum rostratum* on the seeds of wheat and Chinese cabbage. For test of allelochemicals exudated through the root, The compounds in organic fraction of root exudates analysed by GC-MS.

Results: These results indicated the allelopathy of root exudates and would be helpful for understanding *Solanum rostratum* Dunal invasion .

Conclusion: Root exudates of *Solanum rostratum* contained some allelochemicals, which could inhibit the germination and radicle growth of wheat and Chinese cabbage, though the effects of root exudates on shoot growth are different, with a stimulation on Chinese cabbage while a inhibition on wheat. The difference indicated a selectivity of allelopathy effect of root exudates from *Solanum rostratum* Dunal.

Key words: *Solanum rostratum* Dunal; root exudates; Allelopathy

1. INTRODUCTION

Invasion of exotic species pose a great economic loss[1], decreases in the abundances of native species and destroy composition and function of ecosystems in invaded regions[2-4].In order to find a solution to weed problems, an increasing body of research has been focused on the mechanism understanding invasion success,

Allelopathy refers to a direct positive or negative effects of a plant on another plant or on micro-organisms by allelochemicals released into the environment[5 and has been reported to be one of main mechanisms for plant invasion[6-7]In “novel weapons hypothesis”, the allelochemicals, produced and released by invasive species, may contribute to their successful invasion[8-10The allelochemicals released by invasive species, which are noxious to native plants, can reduce the growth and even cause the death of susceptible native species, thus reducing competition and increasing resource availability for the alien species. Invasive plants may gain an advantage over their competitors[11-14] *Solanum rostratum* Dunal (Buffalobur), native to North

America and belong to the Solanaceae family, is an annual spiny weed-As an invasive weed, it has extended from central Mexico northward across the Great U.S.A. to some other countries of the world, including U.S.S.R., Australia, Canada and Korea[In China, *Solanum rostratum* was first found in Chaoyang, Liaoning Province in 1981[16 and was reported capable of invading into roadsides, riverbanks, wasted and cultivated fields in Jilin, Beijing, Hebei, Shanxi, and Xinjiang[17 It has been reported that leaves, berries, and roots of the invasive species contain some noxious substances, which could poison the livestock to death[18] (

This research preliminarily studied the effects of root exudates from *Solanum rostratum* on effect of cabbage and wheat seeds.

2 MATERIALS AND METHODS

2.1 Collection of root exudates of *Solanum rostratum* Dunal

Solanum rostratum was grown in the greenhouse on April 15th, 2010, its seeds were collected from the invaded fields in Chaoyang city (Liaoning province, China) .The plants were dug out and collected when they were at the bud stage.

The roots of 50 healthy plants with uniform growth, which fresh weight were 1208g, were washed clean for three times with distilled water before they were gathered in a big bucket. The container was filled with distilled water, enough to submerge the roots, and air was aerated constantly with an air pump. The water extract was collected per 24hs and for four times totally[19]. Finally, 1500 ml of root exudate extract was obtained, was concentrated with a rotary evaporator at 40℃ and deposited in a refrigerator at 4℃.

2.2 Effects of root exudates on seeds germination and growth

Petri dish bioassay was applied to test the effects of root exudates of *Solanum rostratum* on the ~~seeds~~ seed germination of wheat (*Triticum aestivum* L.) and Chinese cabbage (*Brassica campestris* L.). Wheat and cabbage seeds were purchased from Shenyang Agricultural University seed company. Prepare 4 extracts of root exudates with different concentrations: control group (CK)：0 g f w/mL (distilled water); I：0.1 g f w/mL (27.5 mL distilled water plus 3.5 mL root exudates); II：0.2g f w/mL (22.5 mL distilled water plus 7.5 root exudates); III：0.4 g f w/mL (27.5 mL distilled water plus 3.5 root exudates). The root exudates of four concentrations were sterilized with microporous filter membrane (0.2μm), mixed with distilled water containing agar, which were used for seed germination after solidification, and added to a culture dish with a diameter of 15cm.

The dish was placed in a 30 degree angle with the tabletop, and the seeds of 20 Chinese cabbage after sterilization were placed in a straight line 1/3 in the culture dish, as well as 15 wheat seeds.

There were three replicates for treatment of each concentration, and the replicates were kept at room temperature and shaded against the light. Germination rates were recorded daily until no further seeds germinated for three days. Then the length of the radicel and shoot were measured with a ruler. A seed was considered as having germinated when the seed coat was broken and the radicel had emerged.

RI (response index) was determined according to Williamson (1988), as followed:

$$RI=1-C/T \ (T>C) \text{ or } RI=T/C-1 \ (T<C),$$

T=Treatment response, C=Control response

RI ranges from -1 to + 1, with positive values indicating stimulation by the treatments and negative values indicating inhibition, relative to the controls. The absolute values indicate the degrees of effects.

2.3 Potential allelochemicals in root exudates tested by GC-MS

Refer to Friebe's method and make some changes[20]), for-test of allelochemicals exuded through the root, 200 mL root exudates was concentrated to about 50 mL, and was successively extracted with four organic solvents: Chloroform, ethyl acetate, acetone and ethyl ether, each 30mL for three times(totally 90 mL separately). The organic phase was collected as sample and concentrated to around 5mL with rotary evaporator.

The sample was analyzed by Aglient 5973N (GC-MS instrument), with a column HP-5 MS (30 m x 250 mm; film thickness=0. 25 μm). The column temperature was maintained at 50℃ for 1 min, and then raised to 280°C (at the rate of 10°C per min) and maintained for 15mins.; electrode stem temperature was set at 150℃ and ion source temperature at 230℃; The injector temperature was set to 250℃; E I was set at 70eV. a full scan (35-520m/z)。1 μL sample was injected by using a splitless injector, using helium as a carrier gas at alinear flow rate of 1 mL/min, and analyzed with a range of full scan (35-520m/z).

2.4 Statistical analysis

Probit analysis was conducted with Dps 2000 software. Effects of the extracts root exudates were tested through one-way ANOVA and tested by LSD at $P<0.05$. Significant difference ($P<0.05$) was marked with different letters in figures

3. RESULTS

3.1 Germination rates of wheat and Chinese cabbage

Germination rates of wheat and cabbage were overall decreased by the treatments of different concentrations of root exudates from *Solanum rostratum*, and the inhibitory effects became stronger as the concentration increased. Germination rates of wheat were: CK: 62.97%; I:51.83%; II:42.53%; III: 44.4%, and the inhibitory effect was significant ($P<0.05$) when the concentration was above 0.1 g f w/ml (fig.1). Germination rates of Chinese cabbage are: CK:50%; I:41.67%; II:43.33%; III: 26.67%, and the germination rate was decreased significant ($P<0.05$) when the concentration was above 0.2 g f w/ml (fig.2).

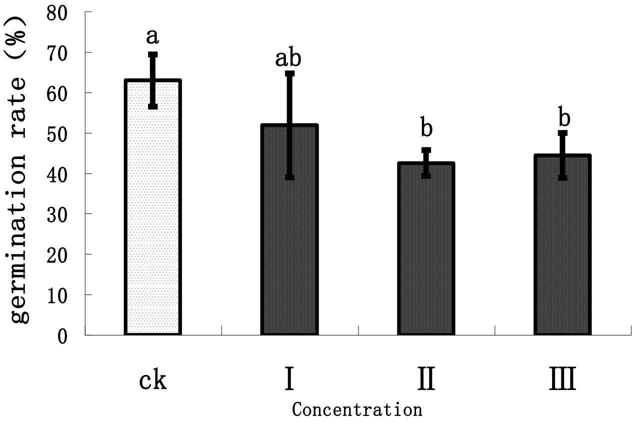

Fig.1 Germination rates of wheat (*Triticum aestivum* L.) treated by root exudates of *Solanum rostratum* Dunal.

Note: Different lowercase letters indicate significant differences among species at *P* < 0.05 according to ANOVAs (Tukey HSD test). The same as below.

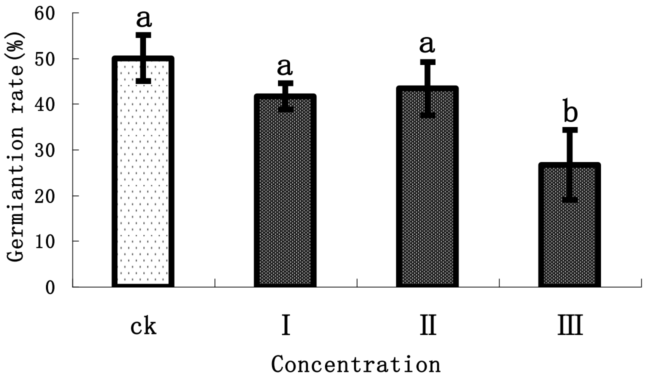

Fig. 2 Germination rates of Chinese cabbage (*Brassica campestris* L.) treated by root exudates of *Solanum rostratum*

3.2 Radicle length of wheat and Chinese cabbage

Totally, radicle growth of wheat and cabbage were inhibited by various concentrations of root exudates from *Solanum rostratum*, and the inhibitory effects became greater as the concentration increased. Radicle length (cm) of wheat were as follows: 5.24(CK), 4.27(I), 4.29(II) and 1.62 (III), and the inhibitory effect was significant (P＜0.05) when the concentration reached as high as 0.1 g f w/ml (fig.3). Radicle length (cm) of Chinese cabbage were as follows: 5.71(CK), 4.76(I), 3.41(II) and 2.64(III), and the radicle growth was significantly inhibited (P＜0.05) when the concentration was above 0.2 g f w/ml (fig.4).

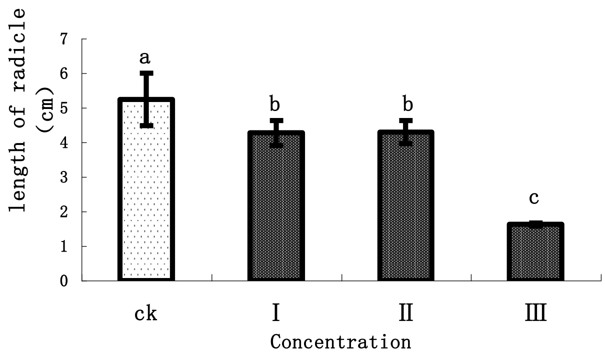

Fig.3 Radicle length of wheat treated by root exudates of *Solanum rostratum* Dunal.

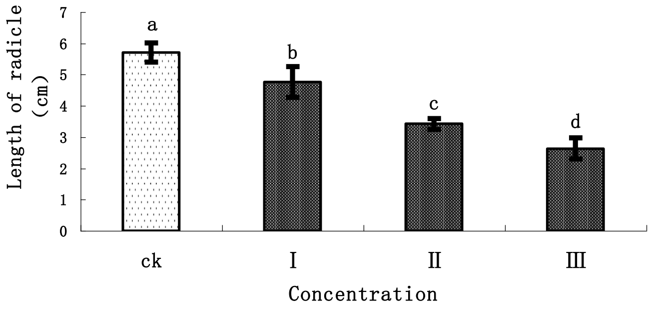

Fig.4 Radicle length of Chinese cabbage treated by root exudates of *Solanum rostratum*

3.3 Shoot length of wheat and Chinese cabbage

Indicated by the results, effects of root exudates on shoot growth of wheat and cabbage came out to be different. The shoot growth of wheat was inhibited while the shoot growth of Chinese cabbage was stimulated. Shoot length（cm）of wheat were as follows: 3.11 (CK), 2.49 (I), 2.54 (II) and 1.75 (III)，and the inhibitory effect was significant ($P<0.05$) when the concentration reached as high as 0.1 g f w/ml and the effects strengthened as concentration increased (fig.5). Shoot length（cm）of Chinese cabbage were as follows: 2.67 (CK), 2.99 (I), 2.91 (II) and 3.33 (III)，and the radicle growth was stimulated by root exudates, but the effects was negatively significant ($P>0.05$) (fig.6).

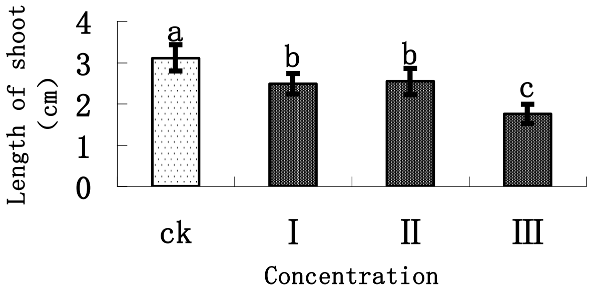

Fig.5 Shoot length of wheat treated by root exudates of *Solanum rostratum* Dunal.

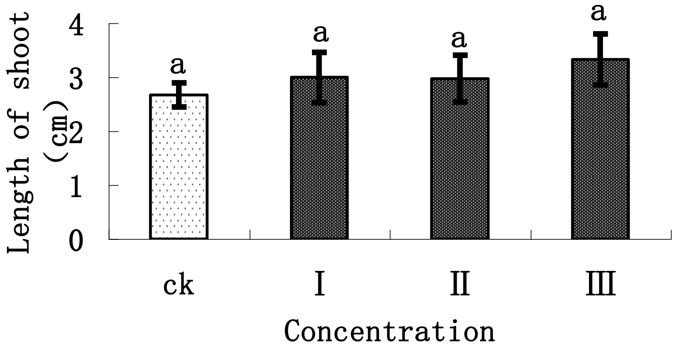

Fig.6 Shoot length of Chinese cabbage treated by root exudates of *Solanum rostratum* Dunal.

3.4 Response index of wheat and Chinese cabbage

RI values of wheat were negative, which indicates that root exudates inhibited the germination and growth of radicle and shoot. When the concentration was as high as 0.4g fw/mL, obvious difference of absolute RI values occurred in sequence: RI (radicle length) ＞ RI(shoot length) ＞ RI (germination rates) (fig.7). Unlikely to wheat, RI value (shoot length) of Chinese cabbage was positive, while the RI values of germination rates and radicle length are negative. The RI values manifested that the growth of shoot of cabbage was stimulated but the germination and radicle growth were inhibited by root exudates. And the inhibitory effect on radicle growth was stronger, suggested by absolute RI value (fig.8).

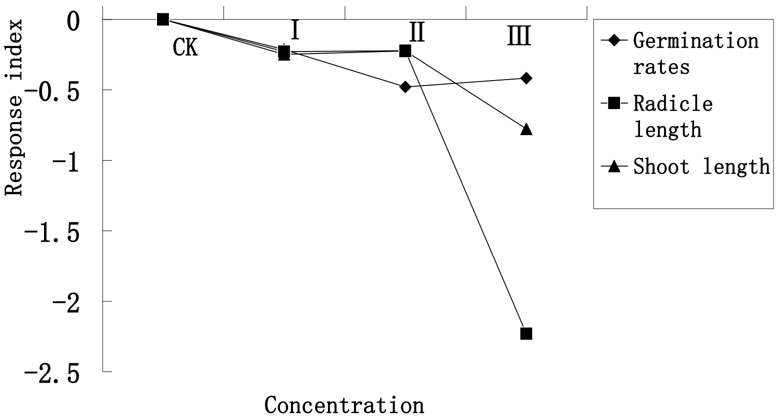

Fig.7 Response index of root exudates from *Solanum rostratum* Dunal on wheat

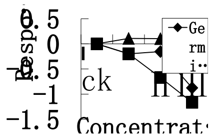

Fig.8 Response index of root exudates from *Solanum rostratum* Dunal on Chinese cabbage

3.5 Analysis of allelochemicals by GC-MS

In organic fraction of root exudates, 23 different compounds were detected, including 14 sorts of saturated and in saturated alkane, 7 esters, 1 amine and 1 phenols. From all the compounds were selected 7 esters, 1 amine and 1 phenol, which were potential allelochemicals, and their retention time were marked in total ion flow graph of GC-MS (Fig.9). The chemical names of the potential allelochemicals were listed in table 1.

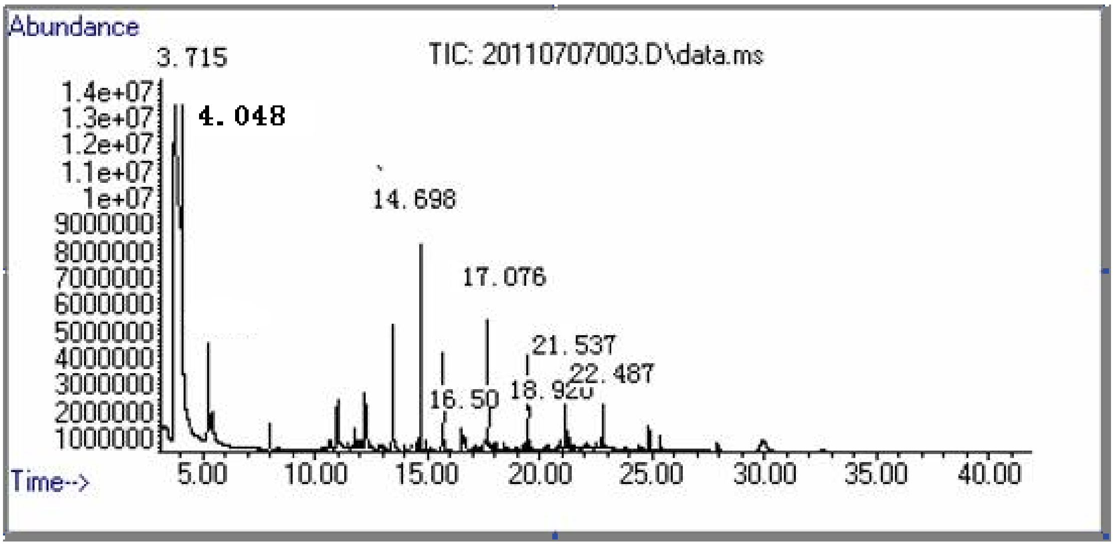

Fig.9 Total ion flow graph of organic fraction of root exudates by GC-MS

Tab.1 Potential allelochemicals in root exudates of *Solanum rostratum* Dunal

Chemical names of potential allelochemicals	*Retention Time (min)*	*Peak Area (%)*	**Molecular Formular**
1-Butanol,3-methyl-, acetate	3.715	53.18	83
1-Butanol,2-methyl-, acetate	4.048	7.82	90
Thenyldiamine	14.698	3.58	86
1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	16.509	1.66	83
Pentadecanoic acid, 14-methyl-, methyl ester	17.076	0.26	83
Eptadecanoic acid,16-methyl-, methyl ester	18.920	0.13	95
Phenol,2,2'-methylene bis[6-(1,1-dimethylethyl)-4-methyl-	21.537	0.11	98
Bis(2-ethylhexyl)phthalate	22.487	0.15	82

4. DISCUSSION

Our research revealed that root exudates of *Solanum rostratum* contained some allelochemicals, which could inhibit the germination and radicle growth of wheat and Chinese cabbage, though the effects of root exudates on shoot growth are different, with a stimulation on Chinese cabbage while a inhibition on wheat. The difference indicated a selectivity of allelopathy effect of root exudates from *Solanum rostratum* Dunal. In the natural world, the allelochemicals released into the soil could inhibit the seed germination and growth of the other species, and reduce the ability of gaining resources of other plants[21-23]

The compounds in organic fraction of root exudates analyzed by GC-MS included 4 straight chain esters, 2 phthalate esters, 1 amine and 1 phenol. Of those compounds, the phthalate esters, amine (also a sulfide) and phenol belonged to potential allelochemical class listed by Rice[24]– And in some other Solanaceae plants, similar compounds have been determined as allelochemicals[25-27]As for the straight chain esters, their structures were similar to some allelochemcals determined in eggplant, such as Hexadecane acid methyl ester and heptadecane acid methyl ester[25] Though the functional groups of the substances analyzed as allelochemicals were similar to those allelochemicals, the branched-chain groups were still different.

The results of our research showed that *Solanum rostratum* potentially inhibit the growth of the surrounding plants, which would be important in evaluating its invasion success. However, the major effective compounds functioning of allelopathy of *Solanum rostratum* Dunal were still not confirmed, and there were still new compounds found[10 29 constituents of the essential oil extracted from *Solanum rostratum* exhibited significant suppressive action on seed germination and seedling development of test species[28]. Further works, such as the allelopathy of other organs and allelopathy in the field, interaction mechanism between allelochemicals and soil microorganisms, between allelochemicals and parasitic plants, were still needed to be determined.

5. CONCLUSION

Root exudates of *Solanum rostratum* contained some allelochemicals, which could inhibit the germination and radicle growth of wheat and Chinese cabbage, though the effects of root exudates on shoot growth are different, with a stimulation on Chinese cabbage while a inhibition on wheat. The difference indicated a selectivity of allelopathy effect of root exudates from *Solanum rostratum* Dunal.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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