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A Preliminary Study on the Inhibitory Substances in Seeds of *Solanum rostratum* Dun.

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Abstract: The inhibitory substances in *Solanum rostratum* seeds were extracted with three different solvents by bioassay method and their effects were determined with cabbage seeds. The results showed that some substances extracted from the seeds, especially by water and could depress the germination and growth of the cabbage seeds. We concluded that the inhibitory substances in *S. rostratum* seeds might be one of the factors regarding to its seed dormancy.

Key words: *Solanum rostratum* · Seed extracts · Dormancy

INTRODUCTION

Buffalobur (*Solanum rostratum* Dun.) is an exotic noxious weed with potential harm. It originates from North America and has a strong capability of propagation and adaptation. As a kind of exotic poisonous weeds, it is toxic to the local crops and plants and has the potential to destroy the local ecological structure [1]. It is found that the germination rate is low and the germination time is long and the seeds have the mechanism of dormancy [2]. While Seed dormancy can reduce the competitive advantages gained by early germination and quick growth [3, 4], it can be a significant evolutionary strategy for species to hedge against environmental variability [5-11]. Seed dormancy can prevent untimely germination and ensures plant survival by adjusting vegetative development to seasonal changes in the environment [12]. This means some dormant seeds dispersed in the soil can also avoid being wept and choose to germinate in another good condition. As a result, this characteristic has brought some difficulties to prevent *S. rostratum*. So it should be helpful to break the dormancy for preventing the weed. It has been found that many treatments, such as disruption of the seed coat (scarification), a period of dry storage (after-ripening) or moist chilling (stratification), or exposure to light [13], can break the dormancy. Right choice of treatments needs a thorough understanding of its mechanism. While it has been reported that husk obstruction and morphological embryo hypoplasiawere the causes for the dormancy [2], it is still essential to do some further research. By now a lot of studies have showed that dormancy is a complication. This complication can be reflected by the likelihood that dormancy is not a single phenomenon but a condition with many contributing causes [13]. Germination can likely be inhibited by embryonic immaturity or physical or physiological constraints and whether the controlling structure or substances are embryonic or in the surrounding tissues of the seed, i.e., coat imposed [14]. The roles of phytochrome, brassinosteroids, ethylene, abscisic acid (ABA) and gibberellin(GA) in regulating dormancy and germination have been investigated intensively and there is broad agreement that these substances are important factors for establishing, maintaining or breaking dormancy [15].

The aim of this research is to determine whether the seeds contain inhibitory substances, which might be one important cause for the dormancy of *S. rostratum*.

MATERIALS AND METHORDS

Inhibitor Extraction: The *S. rostratum* seeds for extraction were collected from Chaoyang city in Liaoning provience, Northeast China, in 2008. The seeds were ground into powder first. And the potential inhibitor was extracted with four different solvents including water, ethanol, ethyl acetate and acetone. In order to determine the relationship between effect and concentration, the extractions for testing were all diluted into different concentrations from low to high.

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Preparation of Water Extraction Liquid Put: 20 grams seeds powder into a 250ml conical flask and add 100mL distilled water. 24 h later coarse extraction liquid was then centrifuged at 10000r for 10 min. Collect the supernatant extraction and adjust the volume to be 100ml by adding some distilled water. Then the extraction concentration is 0.2 g (dw)/ml and the extraction was diluted with distilled water into different concentrations (1.3×10-2, 2.5×10-2, 0.04, 0.05, 0.1 , 0.15 and 0.2 g dw•mL^{-1}).

Preparation of Ethyl Alcohol and Ethyl Acetate Extraction Liquid: 90° grams seeds powder was put into two 250ml conical flasks respectively and each conical flask was added with 100 mL ethyl alcohol or 100 mL ethyl acetate. 24 h later both coarse extractions liquid were centrifuged at 10000r for 10min, the supernatant extractions were collected respectively. And solvents were removed with rotary evaporator until each extraction was conformed into paste. The paste was diluted with distilled water into three different concentrations(0.3, 0.6and 0.9gdw•mL^{-1}) (dry powder weight/ volume).

Preparation of system extraction liquid£º90 grams seed powder were soaked with 100 mL acetone for 24 h. Coarse extraction liquid was then centrifuged at 10000 r for 10 min. The supernate was concentrated with rotary evaporator until it became paste. Then the compounds of the paste were extracted one after another by four solvents including petroleum ether, ether, ethyl acetate and ethanol. The solvents were volatilized by rotary evaporator until the extractions became pastes. Each was diluted into three levels of concentrations (0.3, 0.6 and 0.9g d w•mL^{-1}) (1/4 dry powder weight/ volume).

Bioassay Experiment: For application in the Petri dish bioassays, 5 ml extractions of each concentration were respectively added into the Petri dishes (9cm diameter) with two layers of filter paper in. Determination was conducted with 20 equidistant seeds set in each glass Petri dish. Controls comprised 20 seeds grown in Petri dishes fitted with filter paper and distilled water (5 ml). The were three replicates for every treatment. Replicates were kept at room temperature. Germination rates were recorded daily until no further seeds germinated for three consecutive days. Then the length of the radicel was measured with a ruler. A seed was considered as having germinated when the seed coat was broken and the radicel had emerged. The radicel length was recorded as relative length ((radicel length/mean radicel length of control) ×100%).

Statistical Analysis: Probit analysis (SPSS 13.0) was used to determine whether the increasing concentrations of each extraction differ in the inhibitory effects on the germination and radicel growth. Effects of the extracts from four different solvents were tested with one-way ANOVA followed by LSD test at $P \leq 0.05$. In the figure shown in the results, different letters above the symbols indicate significant differences ($P<0.05$) between germination rates or relative radcel length.

RESULTS

Our bioassay experiments revealed that the seed extracts from different solvents all showed some effects on the germination and radicel growth of cabbage seeds.

Water Extracts: The germination rates of seeds treated with the water extracts decreased from 68.3% to 54% as the treatment concentration increased. when the concentration was above 0.025 g•mL^{-1}, the rates became to be significantly lower than that of the control ($P<0.05$) (Fig. 1). In Fig. 2, we can see that seed radicel growth was depressed significantly by the water extracts. As the concentrations went up the relative radicel length of cabbage seeds decreased obviously as follow: 6.2%, 5.7%, 5%, 3.8%, 3.8%, 3.8%, 1.3%. The length of the radicel are significantly shorter than control ($P<0.05$).

Ethanol and Ethyl Acetate Extracts: The treatment of ethanol and ethyl acetate extracts both decreased the seeds germination rates as the treatments concentrations rose. However, the inhibitory effect of ethanol extracts was obviously stronger. The rates decreased by ethyl acetate extracts were 87.4 %, 86.6 % and 68 %. It became to be significantly lower than that of the control ($P<0.05$) when the concentration was above 0.6g•mL^{-1}. The rates decreased by ethyl alcohol extracts were56%, 46% and 36% and were all significantly lower than that of the control ($P<0.05$) (Fig. 3). As the situation of the germination, seeds radicel growth was depressed by both extracts. The inhibitory effects both increased as extracts concentrations increased. The length decreased by ethanol extracts was 96.4%, 90.94% and 81.84%. It became to be significantly lower than that of the control ($P<0.05$), when the concentration was above 0.6 g•mL^{-1}. The rates decreased by ethanol extracts were 27.3 %, 14.5% and12.7 % and all significantly lower than that of the control ($P<0.05$) (Fig. 4).

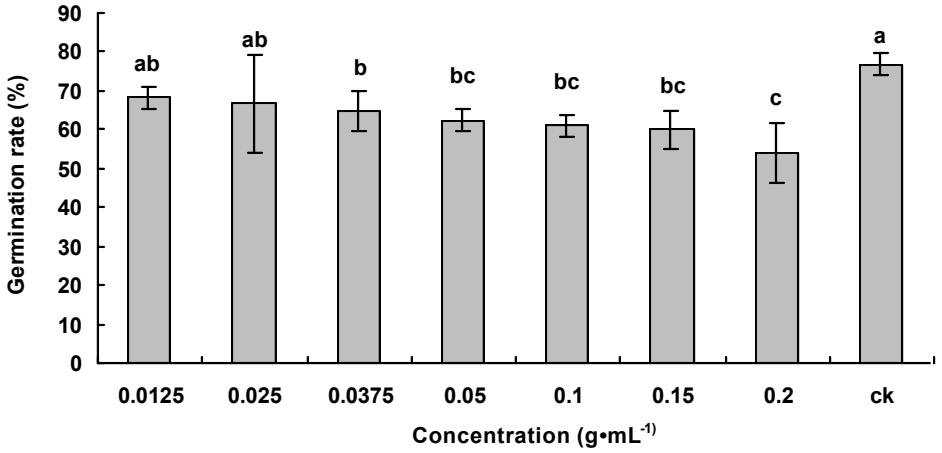

Fig. 1: Relative radicel length of Chinese cabbage treated by water extracts

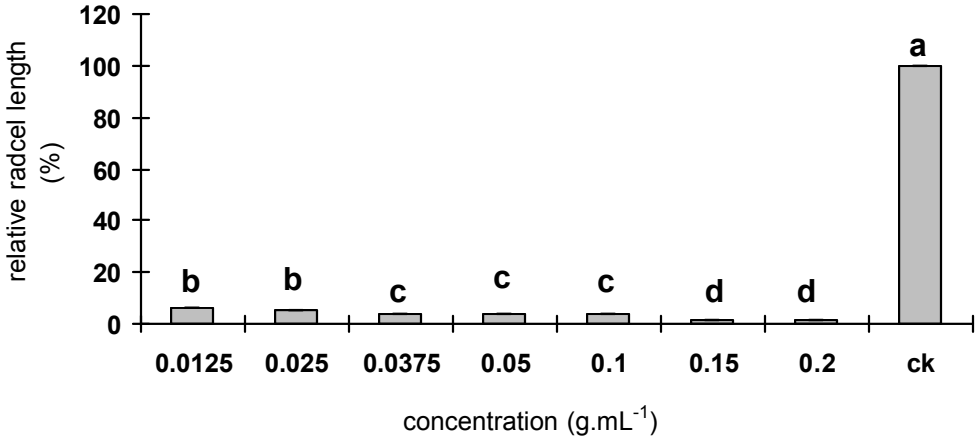

Fig. 2: Germination rates of Chinese cabbage treated by water extracts

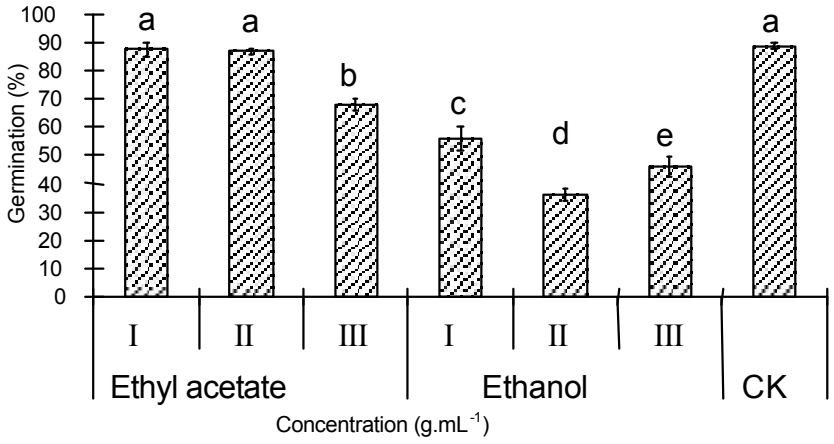

Fig.3: Germination rates of Chinese cabbage treated by organic extracts

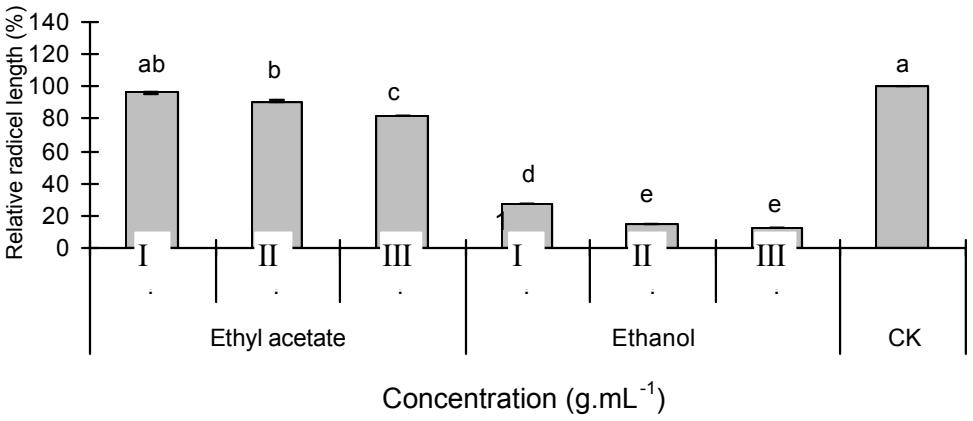

Fig. 4: Relative radicel length of Chinese cabbage treated by organic extracts

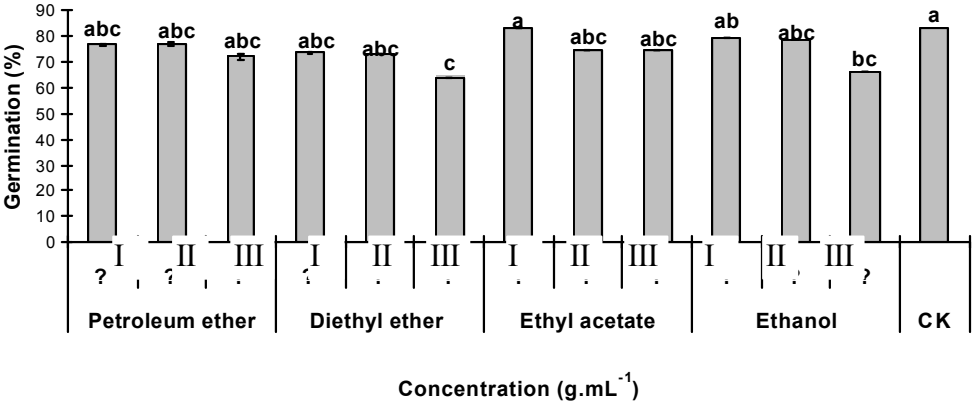

Fig. 5: Germination rates of Chinese cabbage treated by system organic extracts

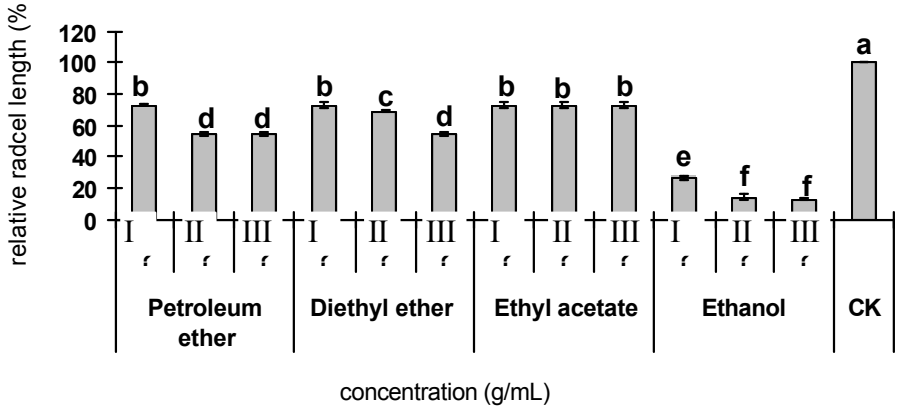

Fig. 6: Relative radicel length of Chinese cabbage treated by system organic extracts

System Extracts: The seed germination rates were decreased by treatments of different extracts but not significantly on the whole. except by the treatments of diethyl ether and ethanol extracts at the concentration of 1.2g•mL^{-1} ($P<0.05$) (Fig. 5). Dissimilarly the radicel growth were depressed by the extracts significantly ($P<0.05$). when concentrations reached as high as 0.6 g•mL^{-1}, the effects decreased as the concentrations went up, except by the extracts of ethyl acetate (no significant changes in radicel length as concentration went up). The radicel length were reduced most significantly by the treatments of ethanol extracts ($P<0.05$), (Fig. 6).

DISCUSSION

Our results showed that the extracts from different solvents could depressed the germination and the radicel growth of the cabbage seeds, which indicated that the might be some inhibitory substances in the seeds of *S. rostratum*. From the results we can see that effects of most extracts are dose-dependent and the depressing effects increased as the concentration of treatments increased except the extracts from ethyl acetate. The effects did not change significantly with the increase of the concentration. As for the depressing capacity, we provided a primary analysis of the inhibitory capacity of extracts from different solvents. The effects showed some difference among the extracts from the different solvents. The water extracts and ethanol extracts showed stronger effects than other extracts. Maybe that was because that the inhibitory substances are water-soluble and ethanol-soluble, or that the substances solved in the water and ethanol are more effective. In order to know the causes for that phenomenon, it needs some more research. Our experiments provided a reference for the extraction methods which should be helpful for the further research. It has been reported that the substances in the seeds of some species are involved in the process of seed dormancy. Work with the A. thaliana ecotype Cvi indicates that whether a seed will germinate or remain dormant may depend on the intrinsic balance of GA and ABA biosynthesis and catabolism [16]. And some other plant hormones, ethylene, brassinosteroids (BR), auxin and cytokinins are involved in regulating gene expression during the induction, maintenance and release of dormancy [17]. But the components of main inhibitory substances in the seeds need further determination and the mechanism of dormancy needs more analysis on the basis of further research.

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