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Short communication

Repellent, insecticidal and phytotoxic activities of isoalantolactone from *Inula racemosa*

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Abstract

Isoalantolactone, a natural product isolated from traditional Chinese medicinal herb roots of *Inula racemosa* Hook. f. (Fam. Compositae), has been shown to possess strong antifungal activities. The present investigation showed that isoalantolactone also exhibited repellent and toxic activities against rice weevil [*Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)] based on a food preference apparatus and a poisoned food technique. The toxicity of isoalantolactone to rice weevil was dose dependent, whereas the repellency was not. Isoalantolactone showed strong phytotoxic effects on seed germination and seedling growth of wheat at a concentration of 500 $\mu g\,ml^{-1}$ for 60 h; however, this side effect could be reduced markedly by shortening the treating time at this concentration. Results indicate that isoalantolactone might be considered for wheat seeds preservation in control of storage weevils.
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Keywords: Isoalantolactone; Repellent; Insecticidal; Phytotoxic

1. Introduction

Around the world, it is still a big challenge to prevent stored grains from insect ingestion. The damages of insect ingestion not only cause severe losses of grains, but also bring up bad quality resulting in seed germination rate decrease, poisoning of human beings and livestock, etc. Therefore, search for new chemicals highly effective, easily biodegradable and less toxic to human beings will be expected to be more advantageous in control stored grains from insect damage during the storage period.

Aluminum phosphide, one of the common fumigants, is a poison extensively used all over the world, particularly in the developing countries as a grain preservative. However, recently some reports have appeared in the literature in which serious toxic effects of this agent to lungs, heart and blood vessels causing pulmonary edema, shock and arrhythmias have been reported (Abder-Rahman, 1999; Khosla et al., 1992; Singh et al., 1991). Thus, it is urgent to look for a new less toxic chemical as well as natural products, instead of this poisonous fumigant.

Isoalantolactone, a natural product isolated from traditional Chinese medicinal herb roots of *Inula racemosa* Hook. f., was disclosed to possess strong antifungal activities (Liu et al., 2001; Picman and Schmeider, 1993; Tan et al., 1998). As a traditional Chinese medicine, the herb roots of *I. racemosa* usually were used to invigorate the spleen, regulate the function of the stomach, relieve the depression of the liver qi, alleviate pain especially between the neck and the shoulders and to prevent abortion (Jiangsu College of New Medicine, 1977; Tsarong, 1994). However, there are no reports on the insecticidal action of isoalantolactone against storage insects. The present work was undertaken to evaluate the possibility and potentiality of isoalantolactone in the control of storage grain pests.

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2. Methods and materials

2.1. Extraction and isolation of isoalantolactone

Isoalantolactone was isolated from roots of *I. racemosa* collected in July 1992 from Luqu County, Gansu Province, China, and identified by Prof. G. L. Zhang, with a specimen logged under the number GC 9216 in the Herbarium of Lanzhou University, Lanzhou 730000, China, following procedures described earlier (Tan et al., 1998). Basically, the air-dried roots (1.2 kg) of *I. racemosa* were extracted twice for 48 h at room temperature with MeOH–Et2O–petrol (2:2:1). Distillation of MeOH in vacuo from the filtrate afforded a black gum. This gum was separated by repeated column chromatography (CC) on silica gel and gel filtration over Sephadex LH-20 to afford finally lactone. The identification of the lactone (Fig. 1) was accomplished by a combination of spectroscopic methods, such as infrared (IR) spectra, electron impact ionization mass (EIMS), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and distortionless enhancement by polarization transfer (DEPT).

2.2. Determination of rice weevil repellency

Repellent properties of isoalantolactone on rice weevil [*Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)] were determined using a food preference apparatus (Xie et al., 1995). The acetone dissolved isoalantolactone was mixed uniformly with the wheat seeds to procure the doses as follows: 50, 75, 100 and 125 $g\,g^{-1}$ (w/w), followed by standing at 25 ± 1 °C for 12 h so as to allow the solvent to evaporate completely. An appropriate amount of acetone was used as the negative control.

Food preference chambers, which were divided into five equal sections by brass partitions, were used to conduct the multiple-choice bioassay to test the repellency of isoalantolactone to *S. oryzae* (Xie et al., 1995). A total amount of 100 g of treated wheat seeds with a specific concentration of isoalantolactone, including the acetone-treated negative control, was poured randomly into each section. One hundred unsexed adult weevils (1–2 week old) were introduced into each food preference chamber and maintained at 27 ± 1 °C and 60 ± 5% RH for 24 h. Another food preference chamber with the same sections was used to determine the weevil repellency of a common fumigant aluminum phosphide (as the positive control) purchased from market of Penlai, Shandong (55–60% tablet produced by Penglai Chemical Inc.) at a concentration of 0.05 $\mu g\,g^{-1}$. Among the five sections, one section contains 100 g of fumigant-treated wheat seeds and other four sections contain the same amount of wheat seeds treated with acetone as negative control. After 24 h, the contents of each sector were vacuumed and the numbers of the weevils at each treated or control diet were counted. The data were presented in terms of the mean percentage of settling response (insect numbers in each section $\times$ 100/total insects) $\pm$ SE. The entire experiment was carried out at three separate times and each time with five replicates (total was 15 replicates). Results showed that the wheat seeds pre-treated with isoalantolactone were strongly repellent to *S. oryzae*; however, the repellency was not dose dependent. The mean percent of settling responses of *S. oryzae* to the isoalantolactone were 12.87 ± 1.41, 12.41 ± 1.36, 12.13 ± 1.76 and 13.22 ± 1.93% at doses of 50, 75, 100 and 125 $\mu g\,g^{-1}$ (isoalantolactone/wheat seeds, the moisture content of wheat seeds was 12%, measured using the WILE 65 digital grain moisture tester, made in Flexible Drive Agencies), respectively. The settling response was 49.45 ± 2.65% to the negative control and 10.83 ± 1.76% to the positive control of aluminum phosphide in three separate experiments. The settling response of *S. oryzae* to given doses of isoalantolactone was markedly less than that of the acetone negative control ($p < 0.01$) and similar to that of aluminum phosphide ($p > 0.05$). This finding increased the potential practical value of isoalantolactone for wheat seed protection from the attack of weevil.

2.3. Insecticidal activity of isoalantolactone against rice weevil

The insecticidal activity of isoalantolactone against *S. oryzae* was determined with a poisoned food technique (Xie et al., 1995). Wheat seeds were treated with different concentrations of isoalantolactone from 200 to 3000 $\mu g\,g^{-1}$ (isoalantolactone/wheat seeds). The same method and positive and negative controls mentioned in the repellency test were adopted in grain treatments. Twenty grams of the pre-treated wheat seeds were placed in a glass vial, into which 15 unsexed adult weevils (1–2 week old) were introduced. Each treatment was cultured at 27 ± 1 °C and 60 ± 5% RH with three replicates. The mortality of adult weevils was assessed after 5, 10, 15 and 20 days, and the Abbotts formula was used to correct mortality relative to that of the negative control (Xie et al., 1995). Results showed that *S. oryzae*

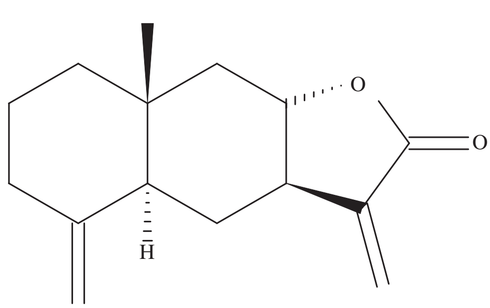

Fig. 1. Chemical structure of isoalantolactone.

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