Repellency of the different crude extracts of *Epichloë bromicola* from *Elymus tangutorum* and enzyme activities in *Tribolium castaneum* Herbst

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Abstract: The study was to determine the repellency of crude extracts of *Epichloë bromicola* at different altitudes against *Tribolium castaneum* Herbst, and the effect of crude extracts of endophytic fungi with higher repellent activity on superoxide dismutase, catalase and cytochrome P450. The percent repellency and the activities of three enzymes were measured at the concentration of 0.25mg/mL after 2h and 4h with different crude extracts. The correlation between altitude and avoidance rate was analyzed. The stain 1, 6, and 9 at the maximum concentration of 2.5mg/mL had better repellency (66%, 59%, and 65%, respectively). The concentrations of 0.25mg/mL treatment had a higher rate of avoidance, so this concentration was selected and the enzyme activity was determined. The activities of SOD, CAT, and CYP450 were significantly increased by three crude extracts of *Epichloë bromicola* compared with the control.

1. Introduction

*Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), and it is called the red flour beetle, often infests and consumes a variety of bulk stored food products, like wheat, corn, rice¹, oats and other grains. Its odor glands can secrete benzoquinone to make grain deteriorate and mouldy, with a bad smell. First national grain storage insect research had been the discovery of traces of the red flour beetle in China.² It has been found that red flour beetle populations have strong resistance to phosphine in many countries.³ Studies have shown that red flour beetle exposed in phosphine also exacerbate the generation of hydrogen peroxide and ROS.⁴ The mobility and resource positioning capabilities of the red flour beetle which produces phosphine resistance will be reduced⁵, which compared with phosphine sensitive deficit to red flour beetle populations are less migration to the new environment.

Grass–endophyte fungus of secondary metabolites have been reported in nearly 160, Ergopeptine, Ergopeptide, Pyrrolopyrazine, Pyrrolizidine alkaloids, flavonoids, in addition to other peptides, indole derivatives, pyrimidines, sesquiterpenoids, flavonoids, as well as other classes, which besides Loline and Peramine alkaloids have strong insecticidal activity, The mixed peptides polyketones and flavonoids also showed certain insecticidal activity against aphids and mosquitoes, respectively⁶.⁷
This study used Grass–endophyte fungus insect-resistant feature, we studied crude extracts from different altitudes of *Elymus tangutorum* in 14 strains which were isolated from *Epichloe bromicola*. We measured the repellency of the red flour beetle at different concentrations, and determined the activity of superoxide dismutase, hydrogen peroxide enzyme and cytochrome P450 in the red flour beetle, based on the changes in the activity of these enzymes, the effect of crude extracts on the red flour beetle is inferred.

2. Experimental Section

2.1. Repellent effect of crude extracts

According to the method of Huang Yanzhang [8], the endophytic fungus extract of *Ernix chinensis* was dissolved and diluted with methanol, and the solution was prepared with the concentrations of 2.5mg/mL, 0.25mg/mL, 0.025mg/mL, 0.0025mg/mL and 0.00025mg/mL. Firstly, the diluent with the largest concentration was selected for preliminary screening in order to obtain the sample with better effect for the following operation. After 2 hours and 4 hours, the insect numbers on the treated and control filter papers were counted respectively, and then the percent repellency (PR) of each sample was calculated according to the formula.

\[ PR = \left( \frac{N_c - N_t}{N_c + N_t} \right) \times 100\% \]

Where NC is the number of test insects on the filter paper, head; NT for the number of test insects on the filter paper, head.

The avoidance activity of crude extracts was divided into 0 (PR = 0), I (0% < PR < 20%), II (20.1% < PR < 40%), III (40.1% < PR < 60%), SI (60.1% < PR < 80%), and GH (80.1% < PR < 100%) according to the PR level.

2.2. Effects of crude extract on SOD, CAT and cytochrome P450 in red flour beetle

The SOD activity was determined by NBT reduction inhibition method [9]. CAT activity was determined by using ammonium molybdate colorimetric method [10]. The CYP450 activity was determined according to the methods of Tiwari [11].

2.3. Statistical Data Analysis

Excel 2016 and SPSS Statistics 25.0 software were used for data analysis. One-way ANOVA and Duncan's new complex range method were used to test the significance of difference.

3. Results and discussion

3.1. Avoidance of crude extracts against *Rhizophora chinensis*

The repellent effect of crude extracts treated with 2.5mg/mL against the red flour beetle were different, ranging from 26% to 66%, they all had a certain avoidance effect. Strains 1, 9 and 6 had better avoidance rates, which were 66%, 65% and 59%, respectively. The first two strains had IV level of avoidance activity, and the last one was a fat level. Therefore, these three kinds of samples were mainly used in subsequent experiments.

The avoidance rates of strains 1, 6 and 9 against the red flour beetle at 0.25mg/mL, 0.025mg/mL, 0.0025mg/mL and 0.00025mg/mL, respectively, are shown in Table 1. The three strains were all 0.25mg/mL, and PR was higher when treated, so this concentration was selected as the appropriate concentration for this experiment. Strains 1, 6 and 9 showed significant differences at the lowest concentration.
### Table 1 Effects of different concentrations from the three crude extracts on the percentage repellency against *Tribolium castaneum* Herbst

| Treatment     | 2h          | 4h          |
|--------------|-------------|-------------|
|              | 0.25mg/mL   | 0.025mg/mL  | 0.0025mg/mL | 0.00025mg/mL | 0.25mg/mL | 0.025mg/mL | 0.0025mg/mL | 0.00025mg/mL |
| Strain 1     |             |             |             |             |           |             |             |             |
| 0.25mg/mL    | 64±14aA     | 28±12aAB    | 24±9bB      | 24±9bB      |           |             |             |             |
| 0.025mg/mL   | 47±11aAAB   | 44±9aA      | 38±5abA     | 35±5abA     |           |             |             |             |
| 0.0025mg/mL  | 40±17aA     | 33±9aA      | 44±9aA      | 42±9aA      |           |             |             |             |
| 0.00025mg/mL | 59±15aA     | 46±15aA     | 40±5aA      | 45±10aA     | 63±15aA   | 45±10aA     | 43±2aA      | 33±15aA     |

3.2. *Activity of crude extract on SOD, CAT and cytochrome P450 in Rhizophora chinensis*

Epichloë bromicola of crude extracts from *Elymus tangutorum* against red flour beetle's SOD, CAT, CYP450 activity are shown in figure 1 (A), (B), (C), strain 1, 6 strains, and strains 9 test after three sample processing worm SOD activity were higher than control, respectively is 210.12 U/μg, 133.06 U/μg and 62.56 g U/μg, including 1 strains and strain 6 processing compared with significant difference, 3.97 times and 2.51 times for comparison. CAT activity of the test insects decreased after sample treatment of strain 1, and increased after sample treatment of strain 9 and strain 6, with no significant difference compared with the control. The CYP450 activity of strains 1, 6 and 9 after treatment was 1.43, 1.27 and 1.49 times higher than that of the control, and the differences were significant compared with the control (P<0.05).

![Fig. 1 Effects of crude extracts of strain 1, 6, and 9 on activitives of SOD (A), CAT (B), and CYP450 (C) in *Tribolium castaneum* Herbst](image)

4. Conclusion

The research results show that the strain 1, 6, and 9 of crude extracts have a certain avoidance effect on of the red flour beetle. Song [12] found that the secondary metabolites from the crude extracts of endophytic fungi from *Elymus tangutorum* had no toxicity to normal bovine kidney cells, indicating that the strain of *Epichloë bromicola* isolated from *Epichloë bromicola* may have low toxicity. The results of this study indicated that strains 1, 6, and 9 showed better repellent effect and had the potential to be developed as botanical insect repellent.
On the one hand, crude extracts of *Epichloë bromicola* can protect the crops against the red flour beetles, decrease economic losses, on the other hand, the main purpose of insect repellent is to drive away insects, at the same time food security can be guaranteed, we don't have to worry about that they may be harmful to people through the food chain.

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