Insecticidal activity of the crude extract from *Epichloë bromicola* against *Rhopalosiphum padi*

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Abstract. *Rhopalosiphum padi* is a important pest in agricultural production. In order to reduce the use of chemical pesticides and improve the control effect, we reported the insecticidal activity of the crude extract from 20 strains *Epichloë bromicola* isolated from *Elymus tangutorum*. The results showed that the crude extract of *E. bromicola* had certain insecticidal activity against *R. padi*. The experimental results showed that 10 strains with good activity were 2, 3, 5, 7, 8, 11, 16, 18, 12 and 19, and their mortality rate reached more than 50% at 48h. However, the four strains of 13, 14, 15 and 17 had no insecticidal activity, but could promote the growth of *R. padi*. Among them, strain 7 had the best insecticidal activity at 48h, and the mortality rate reached 88.3%. The weakest insecticidal activity was strain 14, with a mortality rate of 21.7%. Strain 18 had the best insecticidal activity within 24h, with a mortality rate of 33.3%. At the same time, the mortality rate of 48h was significantly higher than that of 24h. In conclusion, these strains with significant insecticidal activity will be of great significance for the biological control of *R. padi* in the future.

1 Introduction

Rhopalosiphum padi is a kind of pest that has great influence on agricultural economy and is one of the main causes of crop yield and quality loss in horticulture, grain and trees[1, 2]. *R. padi* sucked the juice of leaf blades, stems and young ears of plants with piercing-sucking mouthparts, resulting in yellowing and curling of the leaf blades (up to 90%), poor plumpness of seeds, transmission of plant viruses, etc[3]. As a result, the wheat reduction reached 10% or above in China every year, especially in the outbreak year (up to 30%). Therefore, *R. padi* was considered as one of the most important aphids bringing serious damage to wheat in China[4]. It is estimated that aphids cause at least 2 per cent of all insect food loss to the world's crops each year. In addition to removing life fluids from plant screening elements, aphids are very effective vectors for viral diseases: about 60% of plant viruses are transmitted by these insects[5]. Various insecticides can be used to control aphids, but the extensive use of chemical insecticides has led to environmental pollution and the emergence of various aphid resistance populations[6].

There is an urgent need for a biological pesticide to control *R. padi.* It has been reported that the secondary metabolites produced by *E. bromicola* are not toxic to normal animal cells and provide resistance to host plants[7, 8]. Using this advantage, we studied the insect resistance of its crude extract.

In this paper, we used the same method to ferment *E. bromicola* at different altitudes to obtain crude extract, and tested the insecticidal activity of the *E. bromicola* against *R. padi* by leaf soaking method.

2 Materials and methods

These experiments were carried out at the Plant Protection Laboratory, Yuzhong Campus, Lanzhou University, Lanzhou, Gansu province.

2.1 Instruments and equipment

Rotary Evaporator (Tokyo Physiochemical Equipment Co.LTD, Tokyo, Japan); Hps-250 biochemical incubator (Harbin Donglian Electronic Technology Co.LTD, Harbin, China) Shaker incubator (Shanghai Zhichu Instrucment Co.LTD, Shanghai, China)

2.2 The experimental materials

2.2.1 Fungal Strain

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The fungal strain used in this study was isolated from the *Elymus tangutorum*. The 20 strains isolated were kept in Institute of Grassland Agriculture Protection, Lanzhou University.

2.2.2 Aphids

The aphid in the experiment was *R. padi* and provided Institute of Plant Protection, Gansu Academy of Agricultural Sciences.

2.3 General experimental procedures

2.3.1 Preparation of crude extract from E. bromicola

Twenty strains of *E. bromicola* were cultured on potato dextrose agar (PDA) at 28 ℃ for 15 days as seed, respectively. Agar plugs were used to inoculate 1000-mL Erlenmeyer flasks, each containing 400 mL of M104T (Sorbitol 100 g, Glucose 40 g, Yeast extract 3 g, Glutamic acid 10 g, Tryptophan 0.8 g, $MgSO_4.7H_2O$ 0.3 g, KH_2PO_4 1 g). The flasks were placed on an incubatory shaker at 145 rpm and 28 ℃ for 45 days. The fermentation broth of strain *E. bromicola* was separated into culture filtrate and mycelia by centrifugation. The culture and the mycelia were extracted with EtOAc (1.2 mL) and MeOH (300 mL) at room temperature (three times), respectively. Then, the EtOAc and MeOH were evaporated to dryness under vacuum on a rotar evaporator, respectively, and were combined to obtain a crude extract.

2.3.2 Insecticidal Assay

The insecticidal activity of crude extract against *R. padi* was evaluated by leaf-dip method using a previously reported procedure. The assay was repeated in an air-conditioned room at 25 ± 2 ℃. Each tested sample was dissolved in acetone at a concentration of 2 g/L and diluted with distilled water containing TW-80 (0.1 mg/L) to obtain a required concentration. Water containing TW-80 (0.1 mg/L) was used as control. Leaf disks (5 cm × 3 cm) were cut from fresh corn leaves and then dipped into the test solution for 6 s. After air-drying the treated leaf disks and 30 *R. padi* were placed individually into the disks. The mortalities were evaluated 24h and 48h after treatment. All the assays were repeated in triplicate.

2.3.3 Statistical analysis of data

Excel was used to process the original test data and SPSS22.0 was used for significance difference analysis.

3 Results and analysis

The experimental results showed that the crude extract *E. bromicola* had certain insecticidal activity. The results showed that 10 strains had good insect resistance, which were 2, 3, 5, 7, 8, 11, 16, 18, 12, and 19, respectively. The insecticidal activity of these strains was more significant than that of other strains, and the mortality rate of 48h was more than 50%.

3.1 Treatment of *R. padi* with crude extract for 24 hours

The mortality rate of *R. padi* at 24h was 33.3% and 6.67%, respectively in strain 18 and strain 17. Strain 17 had the same 6.67% as CK, so strain 17 had no inhibition on *R. padi* at 24h. The mortality rate of *R. padi* was 33.3%, 31.7%, and 28.3%, respectively in strain 18, 11 and 19.

According to Duncan's multiple comparison method, the difference of killing rate of 30 strains was not significant.

The *R. padi* mortality of 20 strains and CK 24h ranged from large to small (18>11>19>8>3=16>7=9=12=20>2>10>13>4>4>15=5>6>CK=17). Multiple comparison by Duncan's method found that there were significant differences among 17, CK, 18 and 11. There is no difference between CK and 17; The difference of other treatments was not significant.

3.2 Treatment of *R. padi* with crude extract for 48 hours

The mortality rate of *R. padi* at 48 h was 88.3% and 21%，7%, respectively in strain 7 and strain 14. Strains 14, 15, and 17 had lower mortality rate than CK, so the three strains had the opposite effect and promoted aphid growth.

The mortality rate of *R. padi* was 88.3%, 75%, and 71.7%, respectively in strain 7, 5 and 3.

The *R. padi* mortality of 20 strains and CK 48h ranged from large to small (7>5>3>18>2>8>12=19>16>10>9=20>1>6>4>13>CK17>15>14). According to Duncan's method, there were significant differences among 2, 3, 5, 7, 8, 11, 12, 18, 19, and 14. There was no significant difference between 14 and other treatments.

3.3 Comparison of *R. padi* mortality at 24h and 48h

As shown in **Table 1.**, the mortality rate of 48h was significantly higher than that of 24h. It can be seen from the **Fig. 1.** that the mortality of strains 3, 5, and 7 at 24h and 48h was significantly different, which was 46.7%, 63.3% and 66.6%, respectively. This indicates that these three strains will play a greater role after 24h, with more obvious insect resistance. The differences of strains 13, 14, and 15 were small, 10%, 8.4% and 11.6%, respectively. Compared with CK by 18.33%, these three strains promoted the growth of *R. padi* between 24h and 48h.

4 Conclusion

In this paper, the insecticidal activity of 20 *E. bromicola* of *Elymus tangutorum* on *R. padi* was studied, and the time variables were set for 24h and 48h. We found that *E. bromicola* were generally resistant to this *R. padi* and found the crude extract of strain 7 had the best insecticidal activity, up to 88.3%.

Therefore, strain 7 can be used to inhibit *R. padi*. In the future, this crude extract can be further isolated and purified, and more effective pure compounds can be obtained to further improve the insecticidal activity.

Table 1. Mortality rate of *R. padi* at 24h and 48h

Strains	24h (%)	48h (%)	Difference (%)
CK	6.67	25	18.33
1	15	40	25
2	20	65	45
3	25	71.7	46.7
4	15	31.7	16.7
5	11.7	75	63.3
6	10	36.7	26.7
7	21.7	88.3	66.6
8	26.7	60	33.3
9	21.7	43.3	21.6
10	18.3	46.7	28.4
11	31.7	55	23.3
12	21.7	53.3	31.6
13	16.7	26.7	10
14	13.3	21.7	8.4
15	11.7	23.3	11.6
16	25	51.7	26.7
17	6.67	23.7	17.03
18	33.3	70	36.7

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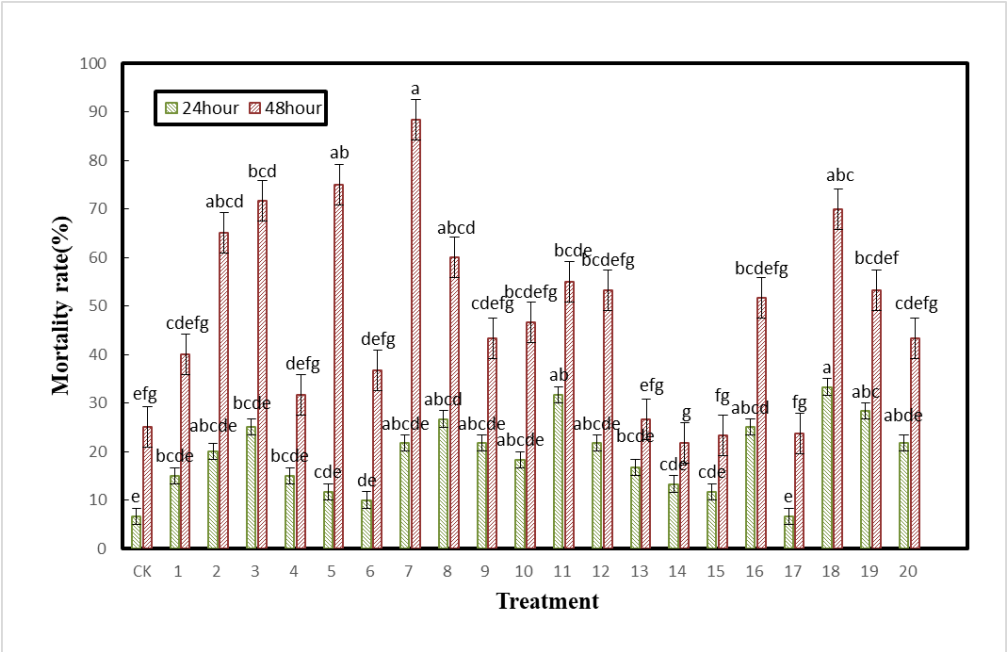

Fig. 1 Effects of *E. bromicola* on the insecticidal rate of *R. padi*

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