

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

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Abstract

Rhopalosiphum padi is an important pest in agricultural production. In order to reduce the use of chemical pesticides and improve the control effect, the study reported the insecticidal activity of the crude extract from 18 strains *Epichloë bromicola* isolated from *Elymus tangutorum*. The results showed that the crude extract of *E. bromicola* displayed insecticidal activity against *R. padi*. The results showed that 6 strains with good activity were strains 3, 4, 5, 7, 8, and 18, and their mortality rate (65%, 71.7%, 75%, 88.3%, 60%, and 70.0%) reached more than 60% at 48h. However, the five strains of 4, 13-15, 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%. At the same time, the mortality rate of 48h was significantly higher than that of 24h. In conclusion, the crude extract of strain 7 with significant insecticidal activity will be of great significance for the biological control of *R. padi* in the future.

Keywords: *Epichloë bromicola*, *Rhopalosiphum padi*, crude extract, insecticidal activity

Abbreviations

R. padi: *Rhopalosiphum padi*, *E. bromicola*: *Epichloë bromicola*, Co., Ltd: company limited, EtOAc: ethyl acetate, MeOH: methanol, TW-80: tween-80.

Introduction

R. 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 insect resistance to host plants [7, 8]. Using this advantage, Using the same fermentation method, 18 strains of *E. bromicola* were fermented and extracted to obtain crude extract this paper used the same method to ferment *E. bromicola* at different altitudes to obtain crude extract, and tested their insecticidal activity against *R. padi* by leaf soaking method.

Materials and Methods

General experimental procedures

Instruments and equipment: Rotary Evaporator (Tokyo Physicochemical 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). Fungal strain: *E. bromicola* used in this study was isolated from the *Elymus tangutorum*, and the 18 strains isolated were kept in Institute of Grassland Agriculture Protection, Lanzhou University. Aphid: The aphid in the experiment was *R. padi* and provided Institute of Plant Protection, Gansu Academy of Agricultural Sciences.

Preparation of crude extract from *E. bromicola*

Eighteen strains of *E. bromicola* were cultured on potato dextrose agar (PDA) at 28°C 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 3g, Glutamic acid 10 g, Tryptophan 0.8 g, MgSO₄·7H₂O 0.3 g, KH₂PO₄ 1g). The flasks were placed on an incubatory shaker at 145 rpm and 28°C 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.

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°C. 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.

Statistical analysis of data

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

Results and discussion

The experimental results showed that the crude extract *E.bromicola* showed insecticidal activity against *R. padi*. The results showed that 10 strains had weak 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%.

Mortality rate of *R. padi* with crude extract for 24 h

The mortality rate of crude extract of 18 strains displayed weak insecticidal activity against *R. Padi* after 24 h. Among these crude extract of tested strains, the insecticidal activity of crude extract from strains 11 and 18 against *R. Padi* were better than other strains, and their mortality rate were 31.7% and 33.3%, respectively. The insecticidal activity of crude extract from strains 2, 3, 7-9, 12, 16 ex-

hibited moderate insecticidal activity by comparing with strains 11 and 18, and their mortality rate were 20.0%, 25.0%, 21.7%, 26.7%, 21.7%, 21.7%, and 25.0%, respectively. The remaining strains (1, 4, 5, 6, 10, 13-15, 17) showed weak activity, and their mortality rate ranged from 6.67% to 18.3%. The inhibition rate of crude extract of strain 17 was the same as that of blank was the same as that of CK, which indicated that this crude extract had no inhibition on *R. padi* at 24h.

According to Duncan's multiple comparison method, the difference of killing rate of 18 strains was not significant. The mortality rate of 18 strains and CK 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 in CK and strains (18 and 11). There is no difference between CK and 17. The difference of other treatments was not significant.

Mortality rate of *R. padi* with crude extract for 48 h

After 48 h, the mortality rate of aphids was significantly higher than that of 24 h. Among these crude extracts, the mortality rate of strains 3, 4, 5, 7, 8, 18 were 65%, 71.7%, 75%, 88.3%, 60%, and 70.0%, respectively. The results indicated that these strains displayed obvious biological activities. The insecticidal activity of crude extract from strains 2, 9-12, and 16 exhibited moderate insecticidal activity, and their mortality rate were 40.0%, 43.3%, 46.7%, 55.0%, 53.3%, and 51.7%, respectively. The remaining strains (1, 4, 6, 13-15, and 17) showed weak activity, and their mortality rate ranged from 21.7% to 36.7%.

The mortality rate of 18 strains and CK 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.

Mortality rate between 24h and 48h

As shown in Table 1, the mortality rate of 48h was significantly higher than that of 24h. As shown in Fig. 1, the mortality rate of crude extract from 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 13-15 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.

Strains	24h (%)	48h (%)	Difference (%)
CK	6.67	25.00	18.33
1	15.00	40.00	25.00
2	20.00	65.00	45.00
3	25.00	71.70	46.70
4	15.00	31.70	16.70
5	11.70	75.00	63.30
6	10.00	36.70	26.70
7	21.70	88.30	66.60
8	26.70	60.00	33.30
9	21.70	43.30	21.60
10	18.30	46.70	28.40
11	31.70	55.00	23.30
12	21.70	53.30	31.60
13	16.70	26.70	10.00

14	13.30	21.70	8.40
15	11.70	23.30	11.60
16	25.00	51.70	26.70
17	6.67	23.70	17.03
18	33.30	70.00	36.70

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

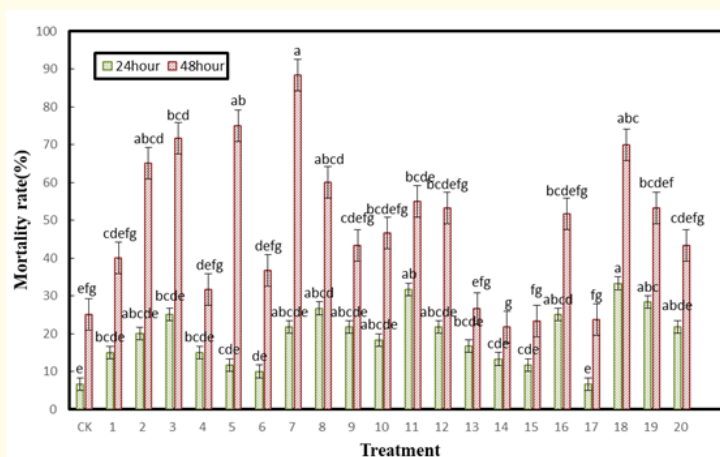


Figure 1: Effects of *E. bromicola* on the insecticidal rate of *R. padi* (different lowercase letters indicate the difference between different treatments ($P < 0.05$)).

Conclusion

In this paper, the insecticidal activity of the crude extracts of 18 *E. bromicola* was tested against *R. padi*. Among these tested crude extracts, strain 7 exhibited significant insecticidal activity by comparing with other strains, and its mortality rate was 88.3%. The results indicated that this crude extract of strain 7 has the potential of biological control of *R. padi*. And the crude extract can be further isolated and purified, and more effective pure compounds can be obtained to further improve the insecticidal activity.

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Conflict of interest

The authors declare that they do not have any conflict of interest associated with this work.

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