Short Research Article

Laboratory Evaluation of the Insecticidal Toxins from Entomopathogenic Nematode Symbiotic Bacteria to Control Vegetable Diseases and Pests

ABSTRACT

Aims: Entomopathogenic nematode (EPN) and its symbiotic bacterium are worldwide used as microbial control agents. We isolated toxins from EPN bacteria and provided basis for using this potential resource to biological control vegetable diseases and pests.

Study Design: The toxins were extracted from 28 strains entomopathogenic nematodes bacteria. The insecticidal activities and antibiotic activities to vegetable diseases and pests were determined by bioassay.

Place and Duration of Study: College of Bioscience and Biotechnology, between May 2020 and September 2021.

Methodology: The toxins were extracted by $(NH_4)_2SO_4$ precipitation method. The insecticidal activities and antibiotic activities were evaluated by bioassay in the laboratory.

Results: The toxins of the EPN symbiotic bacteria had certain insecticidal activities on the first instar larvae of *Plutella xylostella* and *Laphygma exigua*, and strain SY5 had the most obvious insecticidal activity and antifungal activities to *Trichothecium roseurn* and *Fusarium oxysporum*.

Conclusions: The toxins of the EPN symbiotic bacteria SY5 had good insecticidal activity and antibiotic activity. Therefore, it had potential on applying to vegetable diseases and pest biocontrol.

Keywords: entomopathogenic nematodessymbiotic bacteria; toxins; extraction;

vegetable diseases and pests; bioassay

1. INRTODUCTION

Vegetables are one of the essential foods in people's daily diet, but the production of vegetables will be greatly reduced by diseases and pests. *Plutella xylostella*, *Laphygma exigua, Trichothecium roseurn* and *Fusarium oxysporum* are main pests and diseases on vegetables. The diamondback moth, *P. xylostella* (Lepidoptera: plutellidae), is one of the most serious pests of cultivated Brassicaceae (such as cabbage, cabbage, radish, and rapeseed) worldwide. *Laphygma exigua* (Lepidoptera: Noctuidae) is polyphagous, migratory, gluttonous, cosmopolitan, and intermittent pests, which damaged cabbage, tomato, pepper, eggplant, cucumber and other vegetables and plants [1-3]. Fungal diseases are considered a great problem in the vegetable production. *Trichothecium roseurn* is the disease in tomatos, and *F. oxysporum* is a worldwide distributed soil borne pathogenic fungus, which can cause fusarium wilt of melons, solanaceae, bananas, cotton, legumes and other plants [4, 5]. It is necessary to control insect pests and fungal plant disease in the vegetable production.

Entomopathogenic nematodes (EPN), natural enemy of insect, exist widely in the soil. They are non-toxic and harmless to plants, humans, animals and the environment [6]. EPN (genera Steinernema and Heterorhabditis) kill insects with the aid of a mutualistic symbiosis with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for Steinernematidae and Heterorhabditidae, respectively). *Xenorhabdus* and *Photorhabdus* secrete a wide variety of substance into the culture medium including toxins, lipases, proteases, antibiotics and lipopolysaccharides. EPN and its symbiotic bacterium are worldwide used as microbial control agents in agriculture [7-10]. In this study, the toxins were extracted from entomopathogenic nematodes bacteria. The insecticidal activities and antifungal activities to vegetable diseases and pests were determined by bioassay. The result will be helpful for the development of new microbial insecticides, insecticides toxins and will provide new ways and methods for vegetable pest and fungal plant disease biological control.

2. MATERIALS AND METHODS

2.1 Materials

The EPNs were provided by Pest Biological Control Laboratory, Shenyang Agricultural University. Twenty-eight strains of EPN symbiotic bacteria were isolated from 5 species entomopathogenic nematodes saved in the laboratory and 23 species in the soil samples collected from different regions of China. The bacterial strain names were given according to the entomopathogenic nematode hosts coded in our lab.

2.2 Production of bacterial cell and the crude extract

A single colony was inoculated into nutrient broth (18 g nutrient broth in 500 ml distilled water) in a flask and placed in a shaking incubator at 160 rpm for 40 h at 27 °C. The concentration of bacterial cells in the broth suspension was determined on a spectrophotometer at 600nmwavelength. After cultivation, the supernatant of the bacterial strains was collected by centrifugation (3400 g, 4 °C, 15min) and then $(NH_4)_2SO_4$ was added to give 85% final content. Through dialyzing and frost-desiccation, the insecticidal toxins wereobtained.

2.3 Bioassay

We used the larvae of *P. xylostella* and *L. exigua*, which were kindly provided by Pest Biological Control Laboratory, Shenyang Agricultural University, to detect the oral insecticidal activity of the toxins. The toxins were mixed into artificial diet at 50 μg/g of diet as the test sample. Only distilled water was given as control samples. The mixed diet (0.2g) and larvae were placed individually into 5 ml clear plastic airtight pots. Each batch had 20 larvae, and three batches were repeated. Survival and weight of larvae were recorded at 3rd, 4th, 5th day. The corrected mortality and the inhibiting rate of larval weight were calculated. The corrected mortality =100 × (treatments mortality – contrast mortality) / (1- contrast weight). The inhibiting rate of larval

weight = 100 × (contrast weight – treatments weight) / contrast weight.

The toxin (50 μg /ml)) and cooling PDA agar were mixed as 1ml: 25 ml in 9 cm Petri dishes. The control dishes were 1 ml distill water and 25 ml cooling PDA agar. The plant pathogenic fungi plug (5 mm, kindly provided by Shenyang Chemical Engineering Research College.) was added to the center of the dish after the mixed PDA solid. All dishes were incubated at 28℃. One sample had three repeats. The inhibition (the diameter of contrast and treatment) was observed and measured by the cross method at 3rd, 4th, 5th days. The inhibiting rate of plant pathogenic fungi was calculated by the formula (The inhibiting rate against plant pathogenic fungi = 100 × (the diameter of contrast –the diameter of treatments)/contrast diameter).

2.4 Statistical analysis

SPSS 23.0 software (one-way ANOVA) was used to analyze the data. The data on the corrected mortality, the inhibiting rate of larval weight and the inhibiting rate were analyzed by repeated measures ANOVA. The differences between treatments were determined using contrasts. The differences between treatments were analyzed by Duncan. All comparisons were considered as significance at $p<0.05$.

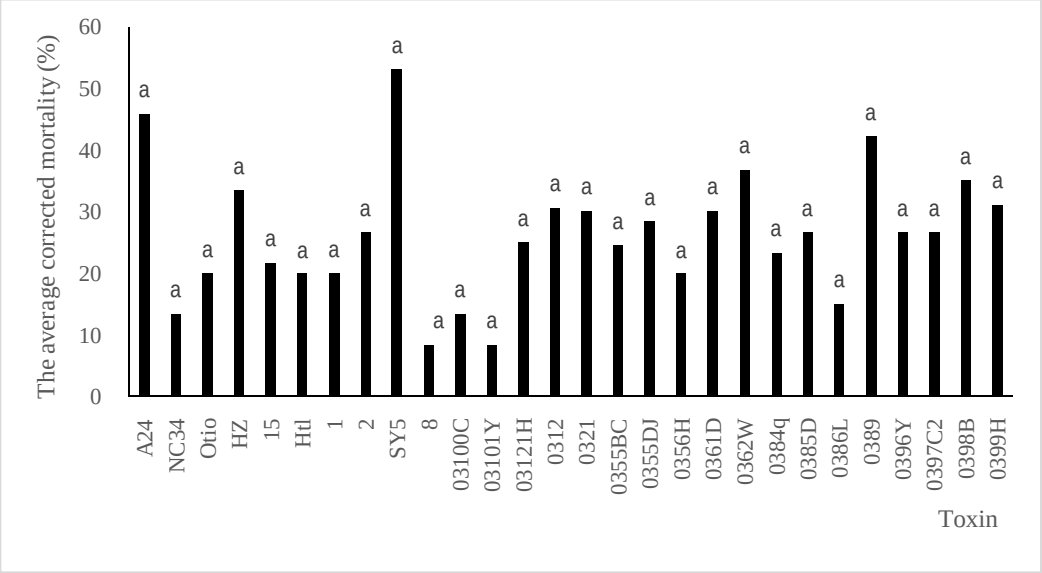
3. RESULTS AND ANALYSIS

3.1 Insecticidal activity of toxins against *P. xylostella*

The reference EPN symbiotic bacterial toxin (*X. nematophila*, *X. poinarrii*, *X. bovienii*, *P. temperate* and *P.luminescens*) exhibited highly insecticidal activities [11-16]. Bioassay results showed that the toxin of *X. nematophila* A24 had the highest oral insecticidal activities among the reference strains. The average of corrected mortality

at 3rd, 4th, 5th day and the average inhibiting rate of larval weight to *P. xylostella* were 45.83% (Fig. 1A), 77.50% (Fig. 1B), 96.67% (Fig. 1C) and 34.12% (Fig. 1D).

These toxins were extracted from 23 EPN symbiotic bacterial isolates which were gathered in different vegetation from different regions of China. Bioassay results indicated that all these bacterial strains had oral insecticidal activity to *P. xylostella*. The insecticidal activity of all toxin had no significantly different at 3rd day (Fig. 1A), and had significantly different at 4th day and 5th day. Among these strains, the toxin of SY5 had the highest oral insecticidal activity to *P. xylostella*, with 100% of the average corrected mortality at 5th day.



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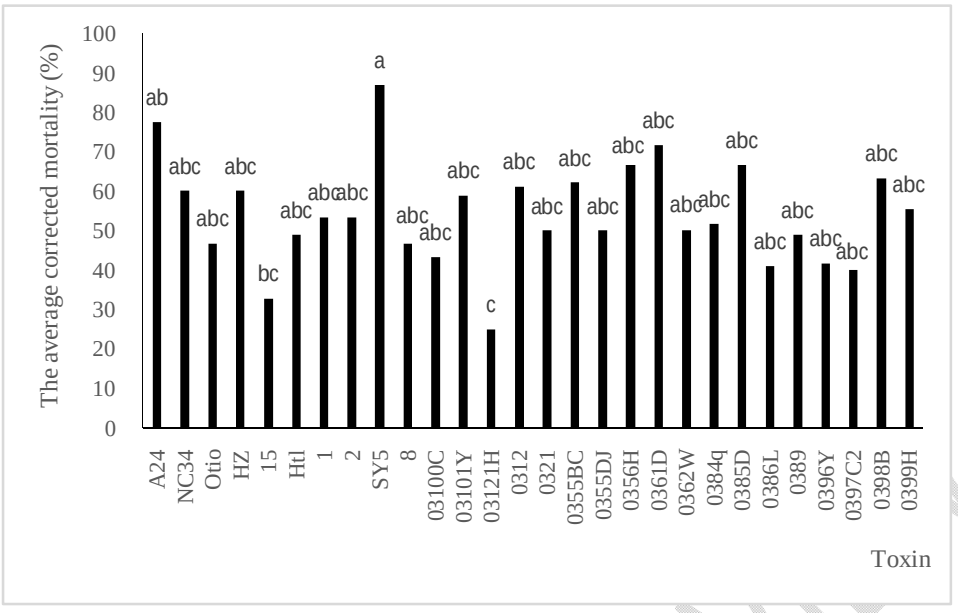
The average corrected mortality (%)
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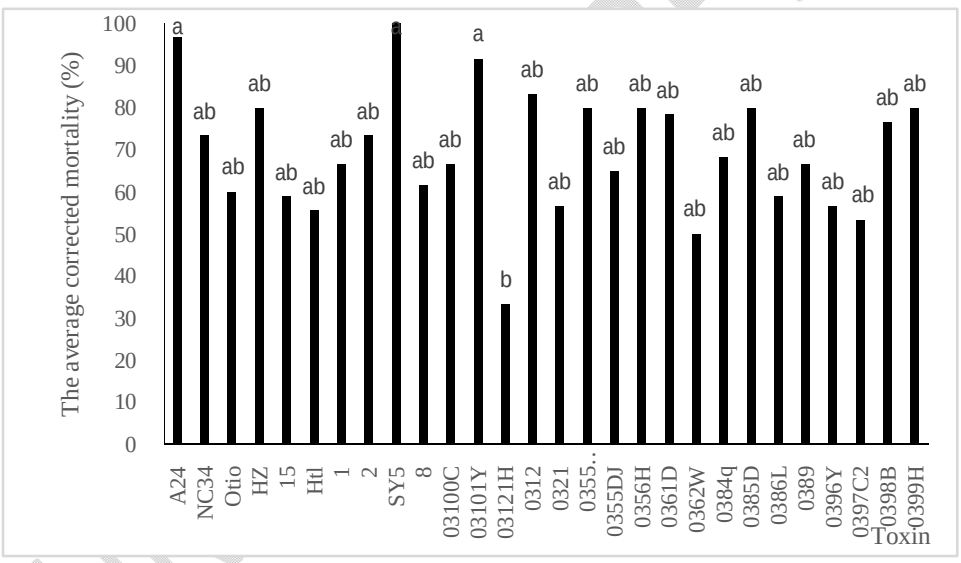
Toxin

B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Toxin

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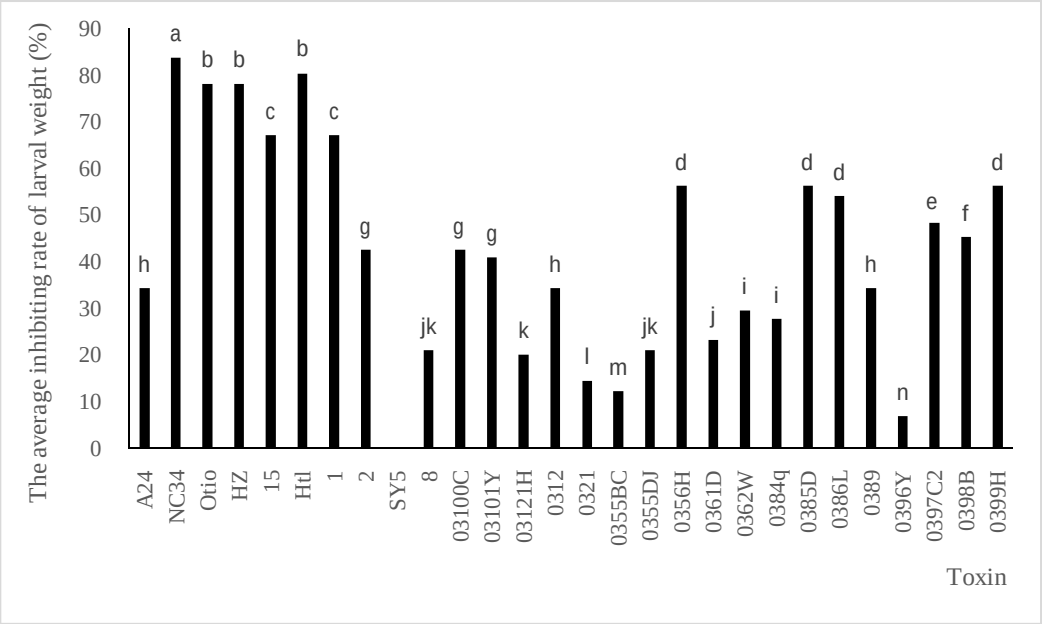


D

Fig.1 Insecticidal activity of toxins against *P. xylostella*

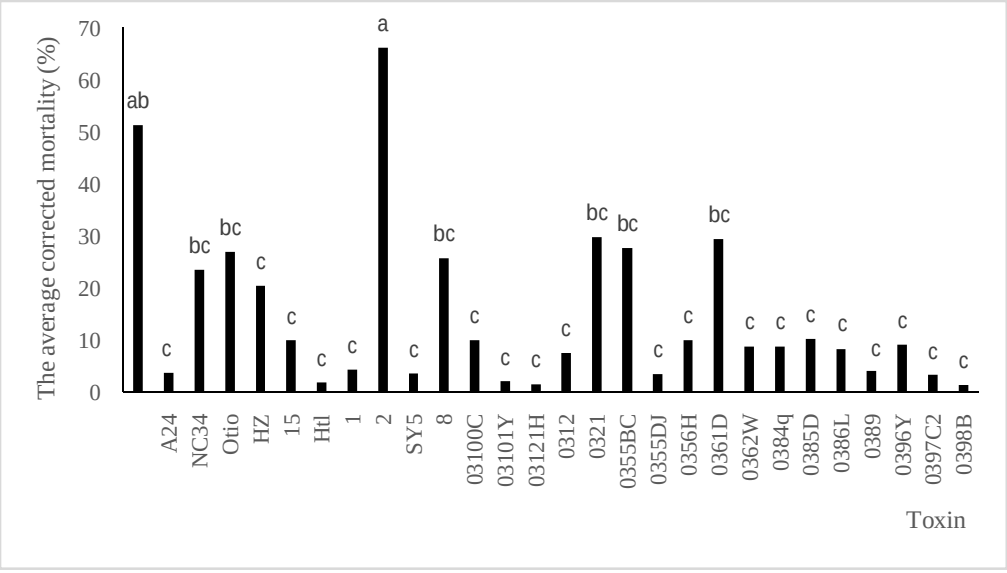
A. The average corrected mortality of 3d

B. The average corrected mortality of 4d

C. The average corrected mortality of 5d

D. The average inhibiting rate of larval weight

2.2 Insecticidal activity of toxins against *L. exigua*



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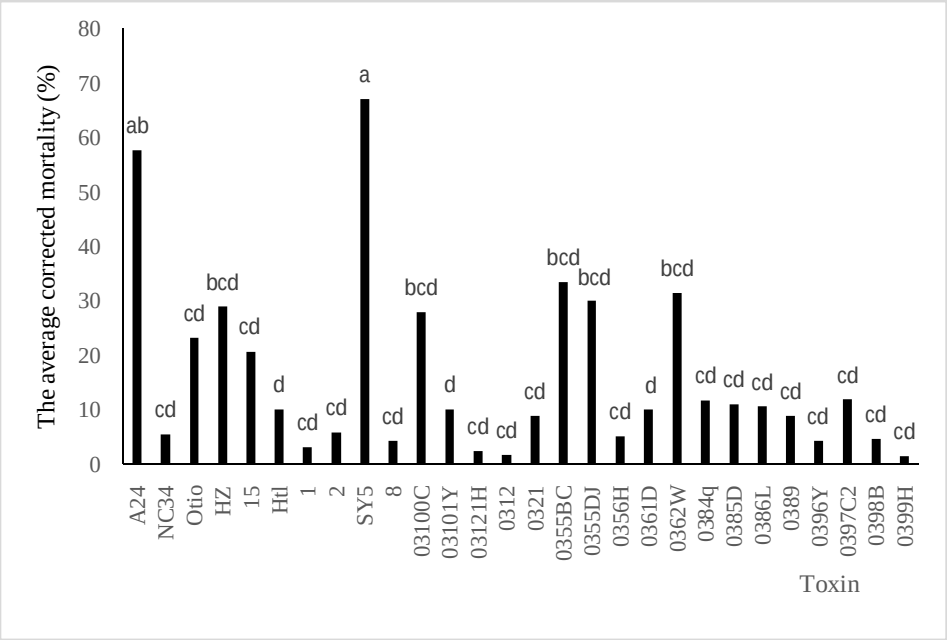
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Otio
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03101Y
03121H
0312
0321
0355BC
0355DJ
0356H
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Toxin

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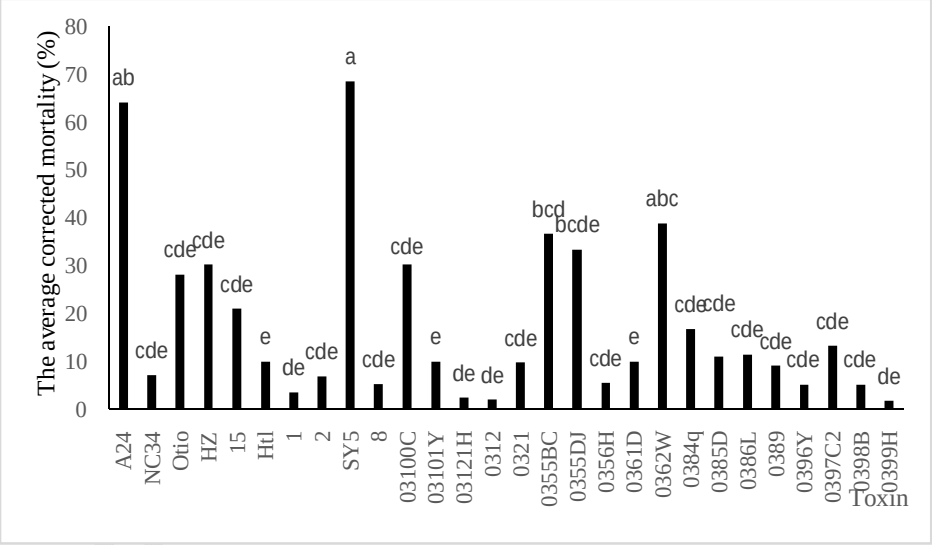
The average corrected mortality (%)
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Otio
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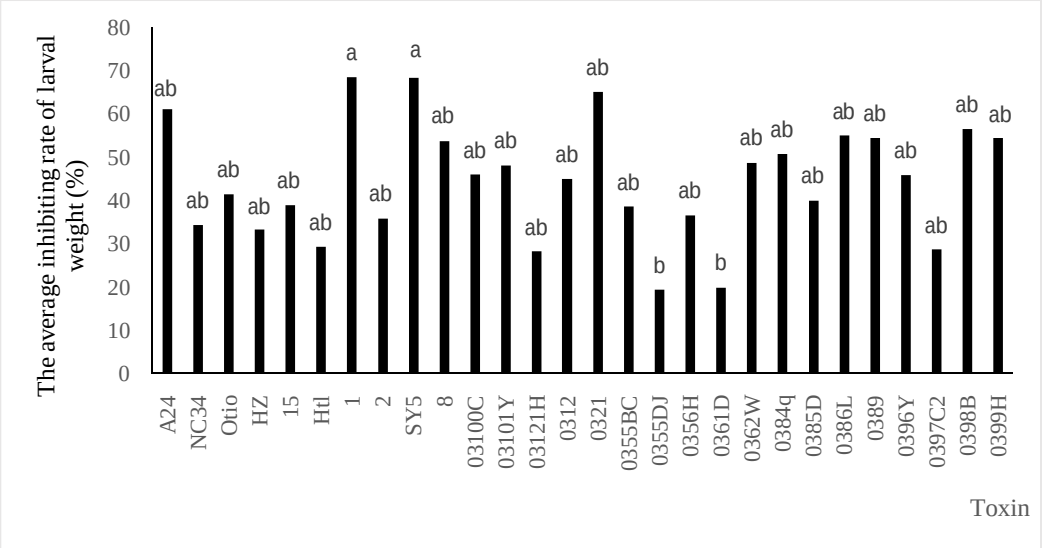


D

Fig.2 Insecticidal activity of toxins against *L. exigua*

A. The average corrected mortality of 3d

B. The average corrected mortality of 4d

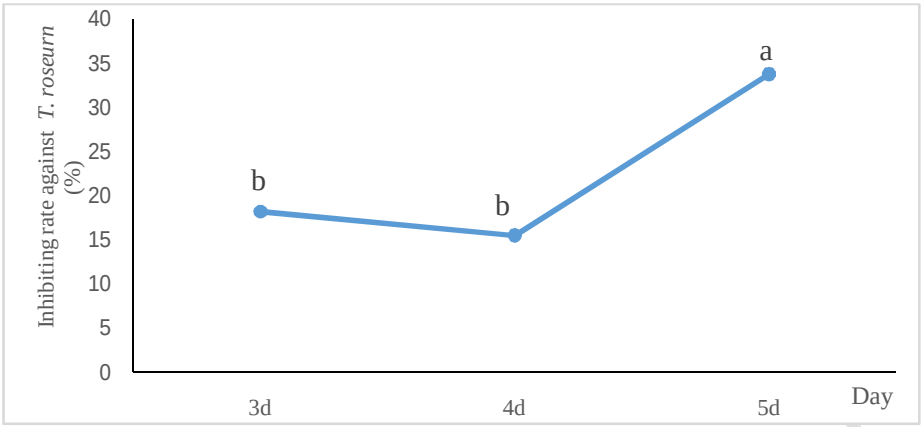
C. The average corrected mortality of 5d

D. The average inhibiting rate of larval weight

At 3rd day, 4th day and 5th day, SY5 have the most obvious insecticidal activity among the 28 strains of symbiotic bacteria toxin (Fig. 2). The average corrected mortality rates were 66.14 % (Fig. 2A), 67.03% (Fig. 2B) and 68.52 % (Fig. 2C), respectively. The average corrected mortality to *L. exigua* of SY5 toxin was not significantly different from the broth of strains A24 and 0362W, but its average inhibiting rate of larval weight was more than these two strains (Fig. 2D).

Taken together, the toxin of strain SY5 had the highest virulence to *P. xylostella* and *L. exigua*. Therefore, SY5 was selected as the most highly virulent symbiotic bacteria for further study.

2.3 Antifungal activities of the toxin to *T. roseurn* and *F. oxysporum*



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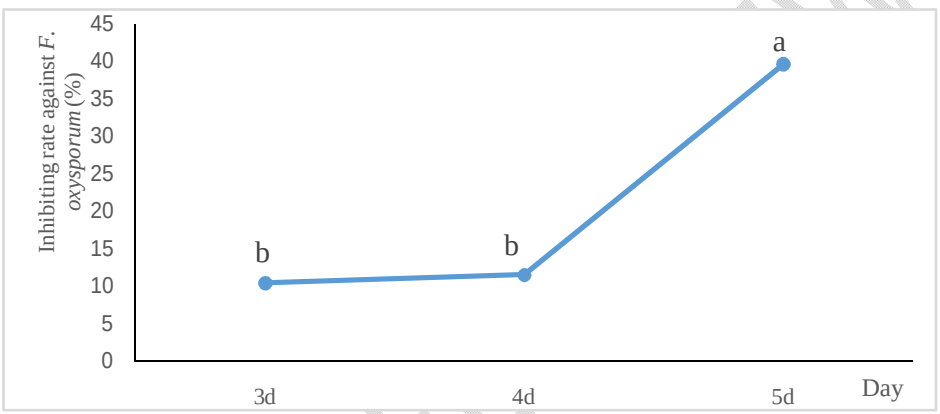

B

Fig3. Antifungal activities of the toxin to *T. roseurn* and *F. oxysporum*

A. The inhibiting rate against *T. roseurn*

B. The inhibiting rate against *F. oxysporum*

Our results showed that the toxin of the symbiotic bacteria SY5 had antifungal activity against *T. roseurn* and *F. oxysporum.* As shown in Fig 3, the antifungal activities increased over time. The inhibiting rate against *T. roseurn* and *F. oxysporum* at 5th day were 33.82% and 39.66%, which significantly raised compare to the inhibiting rate of 3d and 4d.

4. DISCUSSION

Green food and biological pesticides are the priority development agenda in the agricultural production, and the research and development of biological control,

ecological control and other alternative control technologies are the main way to achieve this agenda. *Bacillus thuringiensis* (Bt) is the most wildly used biocontrol bacteria. With the extensive using of Bt products, the resistance of agricultural pests (such as *P. xylostella*) is becoming more and more obvious[17]. So, it is necessary to find some new biocontrol resource for the pest and plant disease control.

As an important biological control resource, EPN have been used to control a variety of agricultural, forestry, grassland, flower, and sanitary pests such as grubs, leeks, and cutworms [17-19]. EPNs and their bacteria have a wide range of parasitic pests, and can produce different types of insects toxins. Study on such bacteria and their insecticidal substances is helpful for developing new microbial insecticides, insecticides toxins and genes. In different strain types and species, the antibiotic production of *Xenorhabdus* and *Photorhabdus* are different qualitatively and quantitatively [20-24]. In this study, the toxin was the extracellular protein of native isolated *Xenorhabdus* and *Photorhabdus*. Our result showed that the insecticidal activities of toxin were changed differently among different strains. The toxin of SY5 showed higher larva mortality than the other stains. The insecticidal activities of symbiotic bacteria SY5 toxin against the two pest were different, which may be due to the different ability of different insects to respond to the toxins. The strain also showed well antifungal activity against two vegetable disease, *T. roseurn* and *F. oxysporum*.

At present, there have been many reports on the toxins and genes of the symbiotic bacteria of entomopathogenic nematodes [25-28]. The strains used in this experiment were all collected and isolated in China. Insecticidal activity substances are separated, purified and identified in order to discover new insecticidal substances and insecticidal genes. The results will provide new materials for the development of new microbial insecticides, insecticidal genes and new materials for the biological control of vegetable production.

5. CONCLUSION

In this study, the toxins from 28 strains symbiotic bacteria were extracted. The highly

virulent strains SY5 was screened by bioassay. This strain had the highest insecticidal activities against *P. xylostella* and *L. exigua*, and good antifungal activities against *T. roseurn* and *F. oxysporum*. The study provided biocontrol resource for vegetable pest and disease control.

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