Influence of synergism with *Bacillus thuringiensis* and *Beauveria bassiana* on diamondback moth larvae

Xue Chun-Mei [1,a], Sun Yan-Bin[2,b], Zhang Yue-Hua[2,c*]

[1]College of Life Science, Jiamusi University Jiamusi, 154007, PR China;

[2]College of Science, Jiamusi University Jiamusi, 154007, PR China

[a]jmsxcm@126.com, [b]63145964@qq.com,[c*]Corresponding Author:751520177@.com

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Abstract: [Objective] Researching the effects of different concentrations of *B.* thuringiensis and *B. bassiana* mixed liquor on the mortality of Plutella xylostella larvae in Jiamusi. **[Methods]** Employing PBS buffer diluted the *B. thuringiensis* and *B. bassiana* into different concentrations of suspension, the mixture of *B. thuringiensis* and *B. bassiana* was mixed into seven different concentration gradients by orthogonal analysist, and determination the mortality of P. xylostella larvae at different concentrations, and then screened more suitable larval LD_{50} proportion and concentration. **[Results]** According to the observation and analysis, when the ratio of *B. thuringiensis* and *B. bassiana* was 3: 1 in 96h, the P. xylostella larvae mortality rate reached 72%, among them, the median lethal rate of larvae in 10^{-1}~10^{-6}g/mL was higher. **[Conclusion]** The lethal effect of each mixture on the diamondback moth larvae was different. When the mixture ratio of *B. thuringiensis* and *B. bassiana* was 3: 1 and the concentration was 10^{-1}~10^{-6}g/mL, the lethal effect on insect larvae was obvious.

Introduction

Lepidoptera insects Plutella xylostella larvae mainly endanger cabbage, broccoli and other vegetables [1], resulting in decreased vegetable production, farmers reduce income. In the process of pesticide removal in the field, chemical control and biological control methods are commonly used. However, unreasonable application of chemical pesticides has resulted in serious environmental pollution[2], which affects the quality and output of vegetables to varying degrees[3]. *B. thuringiensis* is an effective microbial insecticide in biological control. It can produce insecticidal crystal protein, α-exotoxin, β-exotoxin and other substances in the process of growth and metabolism[4]. Insecticidal rate, no pollution to humans and animals and the environment, etc[5]. But there are easy to produce resistance, persistent and poor stability and other drawbacks. However, Beauveria bassiana has the characteristics of wide distribution, strong infectivity and epidemic spread in nature. However, due to the unstable quality and application effect of Beauveria bassiana powder and its insecticidal speed Slower, affecting the enthusiasm of forest farmers to use. *B. thuringiensis* and *B. bassiana* alone have some limitations. In this paper, the *B. thuringiensis* and *B. bassiana* mixed with seven different concentrations of gradient mixture on the diamondback moth larvae mortality in order to use biological control measures to control field pests, which agricultural pollution prevention and control of Northeast China and the promotion of biological pesticides are of great significance.

Materials and methods

Test materials

Xian lou *Bacillus thuringiensis* powder (Bt) and Tian li *Beauveria bassiana* powder (Bb). Plutella xylostella larvae collected from Jiamusi City, Heilongjiang Province, Jiamusi University Jianan Experimental Farm vegetable cultivation experiment base.

Medium production

Beef extract peptone medium: beef extract 5.0g/L, peptone 10.0g/L, NaCl 5.0g/L, agar 20.0g/L, distilled water volume to 1.0L, pH 7.2-7.4. PDA medium: potato 200.0g/L, glucose 20.0g/L, agar 20.0g/L, distilled water volume to 1.0L, pH natural.

PBS buffer preparation

Weigh 8.0gNaCl, 0.2gKCl, 3.63gNa2HPO4 • 12H2O, 0.24gKH2PO4 dissolved in 900mL distilled water, with hydrochloric acid to adjust the pH to 7.4, water 1.0L.

Sterile buffer preparation

The PBS buffer and distilled water, 1.0mL pipette tip and the prepared medium together into an autoclave at a pressure of 121 ˚C, 0.1MPa sterilization 20min. Under sterile conditions *B. thuringiensis* and *B. bassiana* were weighed 1.000g with analytical balance, into 10.0mL PBS buffer, the concentration of 10^{-1}g/mL, followed by dilution to 10^{-10}g/mL and Set up a control experiment, concentration gradient dilution with sterile distilled water, each experiment was repeated 2 times.

Strains coated

The suspension of *B. thuringiensis* concentration of each gradient was applied to 15.0mL beef extract peptone medium. *B. bassiana* suspension of each concentration gradient was applied to 15.0mL PDA medium, and control experiments were designed with distilled water, experimental group and control group repeated 5 times. The coated uniform culture dish into the incubator, 29˚C for 2 days.

Mixed liquid preparation

B. thuringiensis and *B. bassiana* were made into a single dose and mixed agent, the mixture Bt: Bb codenamed A_1, A_2, A_3, A_4, A_5, the ratio was 1: 1, 1: 2, 2: 1, 1: 3 and 3: 1.

Indoor biological determination

The Plutella xylostella larvae were immersed in different concentrations of the mixture for 10s, air-dried, placed in a diameter of 9cm Petri dishes, each with a mixture of 10 treated with distilled water as a control. The collected fresh young cabbage leaves were washed dry weighed 2.0g were dipped into different mixes 10s, removed into the Petri dishes. Placed at 25˚C feeding, daily replacement of fresh young cabbage leaves, respectively, at 24h, 48h, 72h and 96h statistics larval mortality. All data are processed using SPSS16.60 software.

Result analysis

Buffer effect comparison

B. thuringiensis and B. bassiana were diluted with PBS buffer solution and distilled water for 24 h, respectively. The numbers of Bt and Bb colonies diluted with PBS buffer were 298 cfu/mL and 276cfu/mL. The number of colonies diluted with distilled water was 224cfu/mL and 210cfu/mL, respectively, and the number of colonies diluted with PBS was significantly higher than that of distilled water, indicating that PBS buffer was more diluted than distilled water Liquid better protect the integrity of microorganisms (see Table 1).

Table 1. B. thuringiensis and B. bassiana were diluted in water and PBS, respectively

The fungus powder	Buffer	concentration[g/mL]									
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Bt	Distilled water	224	178	156	104	65	48	31	18	9	3
	PBS	298	250	202	152	129	86	52	27	12	6
Bb	Distilled water	210	165	104	85	65	34	22	9	3	1
	PBS	276	233	165	103	67	44	32	11	5	2

Determination of different mixture properties

The result of applying 4d mixture showed that the mixture of *B. thuringiensis* and *B. bassiana* mixed with 7 kinds of mixture with different proportions was higher than that of single dose of Bt and single dose of Bb. The larvae did not die within 24 hours. The mortality rate of single dose of Bt was higher than that of single dose of Bb within 48 hours. The mortality rate was 16% under the Bt: Bb = 1: 3 ratio and the mortality rate under Bt: Bb = 2: 1 ratio The mortality rate of larvae reached 15% at 22% Bt: Bb = 3: 1, and the larval mortality increased within 72 hours. At this time, the dead larvae lost their luster compared with the control larvae, There are dark brown spots of varying sizes. No mortality was observed in the 96h control group. The mortality rate was as high as 72% for the Bt: Bb = 3: 1 ratio, exceeding 15% for the Bt: Bb = 1: 3 ratio (Figure 1).

2.3 Bt: Bb=3: 1the ratio of the concentration of each comparison

Experimental data show that with the extension of time, the mortality of diamondback moth larvae gradually increased. When the ratio of *B. thuringiensis* and *B. bassiana* was 3: 1, the larvae did not die within 24 hours. The larvae gradually died within 10^{-1}g/m ~10^{-8}g/mL within 48h, and larvae died within 72h Gradually increased within 96h 10^{-1}g/mL~10^{-6}g/mL concentration has reached 60% to 80%, to achieve the experimental determination of the standard (Figure 2).

Conclusion analysis

According to the indoor toxicity test of *B. thuringiensis* and *B. bassiana* on the common pests in North conservatory, the results showed that the mortality of Plutella xylostella larvae was higher within 96h under the A_5 ratio, The mortality of larvae was between 60% and 70% at the concentration of 10^{-5}g/mL~10^{-6}g/mL. Comparing the results of experiment and production cost, the two concentrations accord with the standard of this experiment and could be applied in the process of removing pesticide in field.

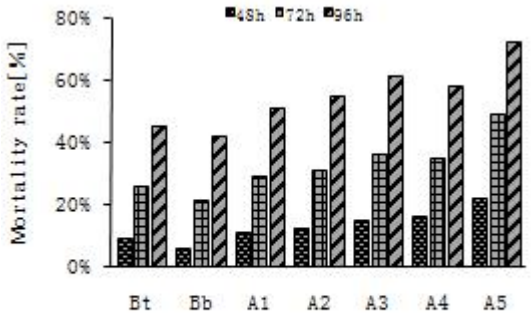

Figure 1. Comparison of mortality of Plutella xylostella with different agents

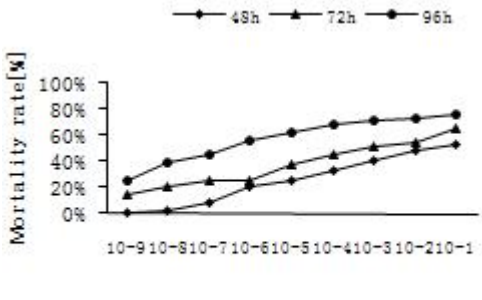

Figure 2. 3:1 Plutella xylostella larval mortality

In this experiment, the virulence of the northern pests were determined, the application of *B. thuringiensis* and *B. bassiana* in the field planting and fruit tree planting can effectively prevent and reduce pest insects such as diamondback moth caused by the phenomenon of production, but the growth of field pests in the environment There is a certain gap between the indoor environment and the natural environment for the growth of insects, and the effect of *B. thuringiensis* and *B. bassiana* is easily affected by the environmental conditions such as ultraviolet radiation, light and air temperature, and the related research is still needed in practical application .

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