(19) LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Économie

(11) N° de publication : **LU508345**

# (12) BREVET D'INVENTION B1

(21) N° de dépôt: LU508345

(22) Date de dépôt: 24/09/2024

(51) Int. Cl.:
A01N 3/00, A01M 1/00, C12N 1/00

(30) Priorité:

(43) Date de mise à disposition du public: 25/03/2025

(47) Date de délivrance: 25/03/2025

(73) Titulaire(s):
SHANXI AGRICULTURAL UNIVERSITY – Taiyuan City (China), DIAO Hongliang – Taiyuan City, Shanxi (China)

(72) Inventeur(s):
MA Ruiyan – China, CAI Xiaoxia – China, XING Peixiang – China, MAO Ruixia – China, ZHANG Guisen – China, ZHOU Wenwen – China, LIU Ziyang – China, WANG Tengyu – China, DIAO Hongliang – China

(74) Mandataire(s):
Patent42 SA – 4081 Esch-sur-Alzette (Luxembourg)

## (54) A SYNERGISTIC ADDITIVE FOR FUNGAL INSECTICIDES DESIGNED FOR BARREL MIXING, AND ITS PREPARATION METHOD.

(57) The present invention discloses a synergistic additive specifically designed for use in fungal insecticides and its preparation method. The additive has a total weight of 100 parts by weight and consists of the following formulation: 10-15 parts by weight of a UV-protectant, 1-5 parts by weight of a dispersant, 0.5-2 parts by weight of a wetting agent, 5-15 parts by weight of soybean peptone, 1-5 parts by weight of oligosaccharides, 0.5-1 part by weight of chitosan oligosaccharides, 0.01-0.05 parts by weight of trace elements, 0.05-0.2 parts by weight of a polymer suspension aid, 0.5-1.5 parts by weight of a pH regulator, and the balance being deionized water. The preparation method involves four main steps: pigment grinding, preparation of the peptone solution, preparation of the polymer suspension aid solution, and blending. This additive is mainly used in combination with insecticides based on Cordyceps militaris and Cordyceps javanica.

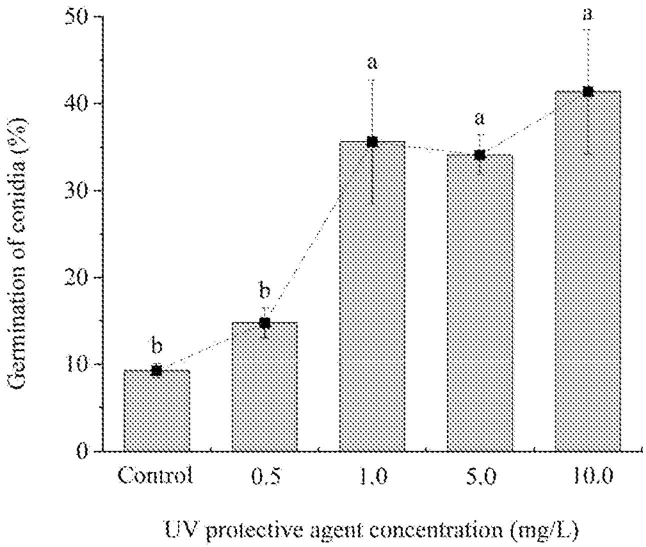


Figure 1

DESCRIPTION

LU508345

# A SYNERGISTIC ADDITIVE FOR FUNGAL INSECTICIDES DESIGNED FOR BARREL MIXING, AND ITS PREPARATION METHOD

## TECHNICAL FIELD

This invention pertains to the field of microbial pesticide additives, specifically to a synergistic additive for fungal insecticides designed for barrel mixing, and its preparation method.

## BACKGROUND

Fungal insecticides refer to biological pesticide formulations made from live fungal materials (such as spores or spore-mycelium mixtures) used to control agricultural and forestry pests. Compared to traditional broad-spectrum chemical insecticides, biological insecticides are recognized for their ecological advantages. They are highly effective in controlling specific harmful insects while having minimal impact on other plants and animals in the same environment. Fungal insecticides have the advantages of a broad host range, environmental safety, and no development of insect resistance. Over decades of development, the use of fungal insecticides has become an important part of integrated pest management and has broad prospects for future growth.

Compared to chemical pesticides, the formulation of fungal insecticides is more challenging. As live biological microorganisms, they are sensitive to external environmental factors such as temperature, humidity, and light, which affects their stability, action speed, and field efficacy. Therefore, when selecting additives for formulation, it is necessary to consider not only the physicochemical properties of the formulation but also specialized additives like light protectants, synergists, and growth promoters. Consequently, fungal insecticides use a variety of functional additives, and their formulations are generally more complex than those for chemical pesticides. Additionally, because microorganisms are live entities, their compatibility with various additives is typically poorer than with chemical pesticides. Many chemical additives can be physiologically toxic to the insecticidal fungi, and this toxicity increases with contact time. Some additives might even be completely unsuitable. Thus, selecting additives requires careful consideration of their compatibility with live microorganisms, further complicating the development of fungal insecticides.

It is evident that directly incorporating functional additives into fungal insecticide formulations presents stringent technical requirements. These additives must not only provide excellent functionality but also exhibit good biological compatibility with fungal propagules. However, most functional chemical additives struggle to meet the low toxicity requirement for long-term contact with fungi, which greatly limits the use of high-quality chemical functional additives in fungal insecticides. This characteristic not only increases the difficulty and cost of developing effective fungal insecticides but also significantly impacts their long-term storage stability and field efficacy.

One approach to overcoming these technical challenges is to physically separate fungal propagules from certain functional additives and mix them at the time of use, known as the barrel-mixing additive technology. Developing and using barrel-mixing synergistic additives can, to some extent, reduce the biological compatibility requirements for additives, thereby expanding the range of usable functional additives for fungal insecticides. This approach effectively avoids the physiological toxicity of most chemical additives when in long-term contact with fungal propagules and represents an ideal solution for fungal insecticide additives.

In summary, developing a barrel-mixing synergistic additive that is based on extensive short-term low-toxicity evaluations, and that provides light protection and synergistic effects after application, with self-dispersibility, high storage stability, and ease of use, is a key research direction for improving the performance and field efficacy of fungal insecticide formulations.

## SUMMARY

The objective of the present invention is to provide a specialized barrel-mixing synergistic additive for fungal insecticides and its preparation method, addressing the issues mentioned in the background technology.

To achieve this objective, the invention offers the following technical solution:

A specialized barrel-mixing synergistic additive for fungal insecticides, where the total weight of the additive is 100 weight parts, and the formulation includes: 10-15 weight parts of a light protectant; 1-5 weight parts of a dispersant; 0.5-2 weight parts of a wetting agent; 5-15 weight parts of soybean peptone; 1-5 weight parts of oligosaccharides; 0.5-1 weight part of chitosan oligosaccharides; 0.01-0.05 weight parts of trace elements; 0.05-0.2 weight parts of a polymer suspension aid; 0.5-1.5 weight parts of a pH regulator; the remaining parts being deionized water.

Preferably, the light protectant is a self-dispersing aqueous yellow pigment paste, specifically one of Yellow M-GR or Yellow M-HR produced by Zhejiang Namei New Materials Co., Ltd.

Preferably, the dispersant is a naphthalene sulfonate formaldehyde condensate-based polymer surfactant, specifically one of D-425, D-450, or D-400 produced by AkzoNobel.

Preferably, the wetting agent is a water-soluble organic silicon wetting agent, specifically OFX-0193 produced by Dow Corning.

Preferably, the oligosaccharides are selected from xylo-oligosaccharides, sucrose oligosaccharides, algal oligosaccharides, and fructo-oligosaccharides, with a molecular weight of less than 2000 Da, or a combination of these.

Preferably, the trace elements are selected from manganese sulfate, copper sulfate, and ferrous sulfate, either singly or in combination.

Preferably, the polymer suspension aid is one of Carbomer 940, Carbomer 941, or Carbomer 970.

Preferably, the pH regulator is one of triethanolamine, diethanolamine, or ethanolamine.

A preparation method for a specialized barrel-mixing synergistic additive for fungal insecticides, including the following steps:

S1. Pigment paste grinding: According to the formulation, weigh the pigment paste, dispersant, and wetting agent. First, dissolve the dispersant and wetting agent in deionized water in a weight ratio equal to the pigment paste. Add this solution to the pigment paste and stir thoroughly. Then, place the material in a sand mill for grinding. The sand mill conditions are as follows: zirconium beads with a diameter of 0.2-0.3 mm, a filling rate of 70-75%, and grind until the pigment particle size D90 ≤ 110 nm or D100 ≤ 230 nm.

S2. Soybean peptone solution preparation: According to the formulation, weigh soybean peptone, oligosaccharides, chitosan oligosaccharides, and trace elements. Add soybean peptone to hot deionized water at 60-65°C in a weight ratio of 1:3 and stir until completely dissolved. After cooling to room temperature, add oligosaccharides, chitosan oligosaccharides, and trace elements, and stir until fully dissolved.

S3. Polymer suspension aid solution preparation: According to the formulation, weigh the polymer suspension aid and add it to hot deionized water at 40-45°C in a weight ratio of 1:50, mixing thoroughly. While stirring, add the pH regulator until the pH reaches 7-8.

S4. Mixing and formulation: Place the prepared polymer suspension aid solution under stirring conditions. Slowly add the ground pigment paste while stirring until evenly mixed. Then, slowly add the prepared soybean peptone solution while stirring until well blended. Next, add the pH regulator while stirring until the pH of the mixture is 7-8. Finally, add the remaining deionized water to reach a total weight of 100 parts and stir until homogeneous to obtain the additive product.

Compared to existing technology, the benefits of the present invention are:

The specialized barrel-mixing synergistic additive for fungal insecticides, primarily used in conjunction with Beauveria bassiana and Metarhizium anisopliae insecticides, provides light protection and synergistic effects for the fungal live bodies after application. It features excellent self-dispersibility, high stability, and ease of use. It avoids the toxicity of chemical additives to live fungal bodies from long-term contact and provides environmental protection and synergistic effects for fungal insecticide live propagules when used together. This enhances the efficacy of the fungal insecticide and extends its efficacy duration. The production process of the additive is simple and cost-effective, with the product demonstrating excellent self-dispersibility, high storage stability, and convenience of use.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a spectrum showing the UV protection effect of the pigment paste yellow M-GR of the present invention on the conidia of Cordyceps sinensis IF-1106;

Figure 2 is a spectrum showing the UV protection effect of the pigment paste yellow M-GR of the present invention on the conidia of Cordyceps sinensis IF-1106.

## DETAILED DESCRIPTION OF THE INVENTION

In order to make the purpose, technical solution and advantages of the present invention more clearly understood, the present invention is further described in detail below in conjunction with specific embodiments. It should be understood that the specific embodiments described herein are only used to explain the present invention and are not used to limit the present invention.

Example 1

A specialized barrel-mixing synergistic additive for fungal insecticides, with a total weight of 100 parts by weight, includes the following formulation: 10–15 parts by weight of a light protectant; 1–5 parts by weight of a dispersant; 0.5–2 parts by weight of a wetting agent; 5–15 parts by weight of soybean peptone; 1–5 parts by weight of oligosaccharides; 0.5–1 part by weight of chitosan oligosaccharides; 0.01–0.05 parts by weight of trace elements; 0.05–0.2 parts by weight of a polymer suspension aid; 0.5–1.5 parts by weight of a pH regulator. The remainder being deionized water

The light protectant is a self-dispersing aqueous yellow pigment paste, specifically one of the products named Yellow M-GR or Yellow M-HR produced by Zhejiang Namei New Materials Co., Ltd. The dispersant is a naphthalene sulfonate-formaldehyde condensate-type polymer surfactant, specifically one of the products named D-425, D-450, or D-400 produced by AkzoNobel. The wetting agent is a water-soluble silicone wetting agent, specifically the product named OFX-0193 produced by Dow Corning. The oligosaccharides are selected from wood oligosaccharides, sucrose oligosaccharides, algal oligosaccharides, and fructose oligosaccharides with a molecular weight <2000 Da, either individually or in combination. The trace elements are selected from manganese sulfate, copper sulfate, and ferrous sulfate, either individually or in combination. The polymer suspension aid is one of Carbomer 940, Carbomer 941, or Carbomer 970. The pH regulator is one of triethanolamine, diethanolamine, or ethanolamine.

Preparation method for a special bucket additive for fungal insecticides:

S1. Pigment paste grinding:

According to the formulation, weigh out the pigment paste, dispersant, and wetting agent. Dissolve the dispersant and wetting agent in deionized water in a weight ratio equal to the pigment paste. Add this solution to the pigment paste and stir thoroughly. Then, grind the mixture in a sand mill under the following conditions: zirconia beads with a diameter of 0.2-0.3 mm, a filling rate of 70-75%, and grind until the pigment particle size is D90 ≤ 110 nm or D100 ≤ 230 nm.

S2. Preparation of peptone solution:

Weigh out the soybean peptone, oligosaccharides, chitosan oligosaccharides, and trace elements according to the formulation. Add the soybean peptone to hot deionized water at 60-65°C in a weight ratio of 1:3. Stir until fully dissolved, cool to room temperature, then add the oligosaccharides, chitosan oligosaccharides, and trace elements, and stir until completely dissolved.

S3. Preparation of polymer suspension aid solution:

Weigh out the polymer suspension aid according to the formulation. Add it to hot deionized water at 40-45°C in a weight ratio of 1:50 and mix thoroughly. While stirring, add a pH adjuster until the pH value reaches 7-8.

S4. Mixing and modulation:

Place the prepared polymer suspension aid solution under stirring. Slowly add the ground pigment paste while stirring until evenly mixed. Then, slowly add the prepared peptone solution and stir until uniformly mixed. Next, add a pH adjuster while stirring until the pH value of the mixture is 7-8. Finally, add the remaining deionized water to reach a total weight of 100 parts and stir thoroughly to obtain the additive product.

In this embodiment, the additive formulation includes:13 parts by weight of Yellow M-2G; 5 parts by weight of D-425; 1 part by weight of OFX-0193; 10 parts by weight of soybean peptone; 1 part by weight of algal oligosaccharides; 1 part by weight of sucrose oligosaccharides; 1 part by weight of chitosan oligosaccharides; 0.01 parts by weight of manganese sulfate; 0.01 parts by weight of copper sulfate; 0.01 parts by weight of ferrous sulfate; 0.1 parts by weight of Carbopol 940; 1.2 parts by weight of triethanolamine; 66.67 parts by weight of deionized water.

According to the above formulation, first dissolve 13 parts by weight of deionized water with D-425 and OFX-0193. Add this to Yellow M-2G and stir thoroughly until well mixed. Then, grind the material in a sand mill under the following conditions: zirconia beads with a diameter of 0.2-0.3 mm, a filling rate of 75%, and grind until the pigment particle size is D90 ≤ 110 nm or D100 ≤ 230 nm to obtain the pigment paste. Add soybean peptone to 30 parts by weight of hot deionized water at 60-65°C, and stir thoroughly until completely dissolved. After cooling to room temperature, add algal oligosaccharides, sucrose oligosaccharides, chitosan oligosaccharides, manganese sulfate, copper sulfate, and ferrous sulfate. Stir until all components are fully dissolved to obtain the peptone solution. Add Carbopol 940 to 5 parts by weight of hot deionized water at 40-45°C and mix thoroughly. While stirring, add triethanolamine until the pH value reaches 7-8 to obtain the Carbopol 940 solution. Under stirring, slowly add the ground pigment paste to the Carbopol 940 solution and mix well. Then, slowly add the peptone solution and mix thoroughly. Finally, adjust the pH to 7-8 by adding triethanolamine while stirring. Add the remaining deionized water to reach 100 parts by weight and stir evenly to obtain the additive product.

Example 2

This exampleis based on example1, with the following differences:

The additive formulation used in this exampleconsists of 10 parts by weight of Yellow M-2G, 3 parts by weight of D-425, 0.5 parts by weight of OFX-0193, 15 parts by weight of soybean peptone, 2 parts by weight of algal oligosaccharides, 1 part by weight of chitosan oligosaccharides, 0.01 parts by weight of manganese sulfate, 0.01 parts by weight of copper sulfate, 0.01 parts by weight of ferrous sulfate, 0.1 parts by weight of Carbopol 970, 1.5 parts by weight of ethanolamine, and 66.87 parts by weight of deionized water.

According to the above formulation, first dissolve 10 parts by weight of deionized water with D-450 and OFX-0193. Add this solution to Yellow M-2G and stir thoroughly until well mixed. Then, grind the mixture in a sand mill under the following conditions: zirconia beads with a diameter of 0.2-0.3 mm, a filling rate of 75%, and grind until the pigment particle size is D90 ≤ 110 nm or D100 ≤ 230 nm to obtain the pigment paste. Add soybean peptone to 45 parts by weight of hot deionized water at 60-65°C and stir thoroughly until completely dissolved. After cooling to room temperature, add algal oligosaccharides, chitosan oligosaccharides, manganese sulfate, copper sulfate, and ferrous sulfate. Stir until all components are fully dissolved to obtain the peptone solution. Add Carbopol 970 to 5 parts by weight of hot deionized water at 40-45°C and mix thoroughly. While stirring, add ethanolamine until the pH value reaches 7-8 to obtain the Carbopol 970 solution. Under stirring, slowly add the ground pigment paste to the Carbopol 970 solution and mix well. Then, slowly add the peptone solution and mix thoroughly. Finally, adjust the pH to 7-8 by adding ethanolamine while stirring. Add the remaining deionized water to reach 100 parts by weight and stir evenly to obtain the additive product.

Example 3

This example is based on example 1, with the following differences:

The additive formulation used in this embodiment consists of 15 parts by weight of Yellow M-HR, 3 parts by weight of D-425, 0.5 parts by weight of OFX-0193, 12 parts by weight of soybean peptone, 2 parts by weight of algal oligosaccharides, 1 part by weight of fructose oligosaccharides, 0.5 parts by weight of chitosan oligosaccharides, 0.01 parts by weight of manganese sulfate, 0.01 parts by weight of copper sulfate, 0.02 parts by weight of ferrous sulfate, 0.15 parts by weight of Carbopol 941, 1.5 parts by weight of triethanolamine, and 64.31 parts by weight of deionized water. According to the above formulation, first dissolve 15 parts by weight of deionized water with D-425 and OFX-0193. Add this solution to Yellow M-HR and stir thoroughly until well mixed. Then, grind the material in a sand mill under the following conditions: zirconia beads with a diameter of 0.2-0.3 mm, a filling rate of 75%, and grind until the pigment particle size is D90 ≤ 110 nm or D100 ≤ 230 nm to obtain the pigment paste. Add soybean peptone to 36 parts by weight of hot deionized water at 60-65°C and stir thoroughly until completely dissolved. After cooling to room temperature, add algal oligosaccharides, fructose oligosaccharides, chitosan oligosaccharides, manganese sulfate, copper sulfate, and ferrous sulfate. Stir until all components are fully dissolved to obtain the peptone solution. Add Carbopol 941 to 7.5 parts by weight of hot deionized water at 40-45°C and mix thoroughly. While stirring, add triethanolamine until the pH value reaches 7-8 to obtain the Carbopol 941 solution. Under stirring, slowly add the ground pigment paste to the Carbopol 941 solution and mix well. Then, slowly add the peptone solution and mix thoroughly. Finally, adjust the pH to 7-8 by adding triethanolamine while stirring. Add the remaining deionized water to reach 100 parts by weight and stir evenly to obtain the additive product.

Example 4

This example is based on example 1, with the following differences:

The additive formulation used in this embodiment consists of 15 parts by weight of Yellow M-HR, 5 parts by weight of D-400, 1 part by weight of OFX-0193, 10 parts by weight of soybean peptone, 1 part by weight of algal oligosaccharides, 0.5 parts by weight of xylan oligosaccharides, 0.5 parts by weight of fructose oligosaccharides, 0.8 parts by weight of chitosan oligosaccharides, 0.01 parts by weight of copper sulfate, 0.01 parts by weight of ferrous sulfate, 0.1 parts by weight of Carbopol 940, 1.2 parts by weight of diethanolamine, and 66.67 parts by weight of deionized water. According to the above formulation, first dissolve 15 parts by weight of deionized water with D-400 and OFX-0193. Add this solution to Yellow M-HR and stir thoroughly until well mixed. Then, grind the material in a sand mill under the following conditions: zirconia beads with a diameter of 0.2-0.3 mm, a filling rate of 75%, and grind until the pigment particle size is D90 ≤ 110 nm or D100 ≤ 230 nm to obtain the pigment paste. Add soybean peptone to 20 parts by weight of hot deionized water at 60-65°C and stir thoroughly until completely dissolved. After cooling to room temperature, add algal oligosaccharides, xylan oligosaccharides, fructose oligosaccharides, chitosan oligosaccharides, copper sulfate, and ferrous sulfate. Stir until all components are fully dissolved to obtain the peptone solution. Add Carbopol 940 to 5 parts by weight of hot deionized water at 40-45°C and mix thoroughly. While stirring, add diethanolamine until the pH value reaches 7-8 to obtain the Carbopol 940 solution. Under stirring, slowly add the ground pigment paste to the Carbopol 940 solution and mix well. Then, slowly add the peptone solution and mix thoroughly. Finally, adjust the pH to 7-8 by adding triethanolamine while stirring. Add the remaining deionized water to reach 100 parts by weight and stir evenly to obtain the additive product.

The fungal insecticide additive provided by the invention features a simple production process and low production cost. The product has good self-dispersibility, high storage stability, and ease of use. When used in conjunction with fungal insecticides, this additive can provide environmental light protection, promote germination under low humidity, and supply necessary nutrients for growth. It enhances the efficacy of the fungal insecticide and extends the duration of its effectiveness, and the main performance indicators are shown in Table 1;

Table 1 Main technical indicators of the present invention

| Performance index | Index value |
|---|---|
| D90 | ≤110nm |
| PH value | 7-8 |
| UV protection value | 650-800 |

To further illustrate the invention, but not limited to it, the following four experiments were conducted for verification:

1. UV protection of organic yellow pigment dispersion system on bacterial spores

The spore germination rate after UV-B (wavelength 312 nm) irradiation at 2 J/cm² was used to evaluate and determine the ultraviolet protection effect of the organic yellow pigment. The specific test method is as follows: Prepare spore suspensions with a

concentration of $5\times10^5$ spores/mL and place them under UV-B (312 nm) ultraviolet light. LU508345
After irradiation, transfer 1 μL of the suspension to a PDA plate, add 1 mL of sterile water, and spread evenly with a spreader. Incubate at 25°C for 72 hours and record the number of individual colonies formed after germination. Each treatment was repeated three times, and the spore germination rate was calculated.

Refer to Figure 1 for the ultraviolet protection effect of the pigment paste Yellow M-GR on the conidiospores of Beauveria bassiana IF-1106 and Figure 2 for the effect of Yellow M-GR on the same spores. Figure 1 shows the effect before grinding (D90 ≤ 300 nm), while Figure 2 shows the effect after grinding (D90 ≤ 110 nm). The results indicate that Yellow M-GR has a significant ultraviolet protection effect on the conidiospores of Beauveria bassiana IF-1106. The protection effect after grinding is notably higher than before grinding, with the best ultraviolet protection effect achieved at a pigment concentration of 5 mg/L in the ground system (particle size D90 ≤ 110 nm), where the spore germination rate reached 87.45%.

2. Evaluation of UV protection effects of additives

3. Prepare spore suspensions of Beauveria bassiana IF-1106 with 2% glucose and 1% peptone, and dilute the additive product from Example 1 10,000 times. Add 2% glucose and 1% peptone to the additive test solution to reach a spore concentration where there are approximately 50 spores per field of view under a microscope (10×40 magnification). Expose the spore suspension to UV-B (wavelength 312 nm) with a dose of 2 J/cm². After irradiation, incubate the spore suspension at 25°C for 12 hours. Examine the spore survival rate under a microscope. Each treatment was repeated four times, and three fields of view were observed each time. Record the number of germinated spores and the total number of spores in each field of view to calculate the spore survival rate. The ultraviolet protection effect value of the additive was calculated using the formula $p = (F_a - F_0) / F_0 \times 100$, where $F_a$ is the germination rate of the spore suspension with the additive after ultraviolet irradiation, and $F_0$ is the germination rate of the spore suspension without the additive after ultraviolet irradiation. Refer to Table 2 for the ultraviolet protection effect of the additive.

Table 2 UV protection effect of additives

| Auxiliary agent | Spore germination rate after UV irradiation (%) | UV protection value P |
|---|---|---|
| CK | 9.8±1.2 | — |
| Example 1 | 85.3±7.3 | 770.4 |
| Example 2 | 78.6±5.2 | 702.1 |
| Example 3 | 75.7±10.6 | 672.4 |
| Example 4 | 81.9±9.7 | 735.7 |

The results showed that under indoor conditions, after using the additive, the germination rate of Cordyceps sinensis IF-1106 spores was significantly increased after UV-B (wavelength 312nm) 2J/cm2 dose energy irradiation compared with the control without additives, indicating that the additive product has a good UV protection effect on the strain spores.

3. Evaluation of indoor efficacy enhancement

Sterilized solutions were prepared using 0.1% Tween-80 in sterile water and a 10,000-fold dilution of the additive product from Example 1. Spore suspensions of Beauveria bassiana IF-1106 and Metarhizium anisopliae IJ-tg19 were prepared at concentrations of $10^6$, $10^7$, and $10^8$ spores/mL. These suspensions were sprayed evenly onto plates containing sterilized fresh cabbage leaves (each leaf was inoculated with 20 newly molted wingless aphids of the same age). The spraying was performed 20 times, and the plates were sealed with sealing film. Each treatment included 20 aphids, with the experiment repeated five times. A 0.1% Tween-80 solution was used as a blank control treatment. After spraying and inoculation, the plates were placed in a light incubator (25°C, 12L:12D) for rearing. The number of dead aphids was recorded daily, and the cumulative corrected mortality rate and lethal dose effect values were calculated.

Refer to Table 3 for the indoor biological test synergistic effect of adjuvants for result analysis;

Table 3 Indoor biological test synergistic effect of adjuvants

| Index | | Measured value | |
|---|---|---|---|
| | | No additives | With additives |
| IF-1106 | 3d median lethal concentration (LC50) | $1\times10^{10}$ spores/mL | $5.8\times10^{8}$ spores/mL |
| | 106 spores/mL median lethal time (LT50) | 5.5d | 4.18d |
| IJ-tg19 | 3d median lethal concentration (LC50) | $0.9\times10^{7}$ spores/mL | $6.2\times10^{6}$ spores/mL |
| | 106 spores/mL median lethal time (LT50) | 3.5d | 3.2d |

The results showed that under indoor conditions, the insecticidal efficacy and lethality rate of the two insecticidal fungal spore suspensions were significantly improved after the use of adjuvants, indicating that the adjuvant product has a certain effect in promoting the insecticidal efficacy.

4. Field application efficacy evaluation

A spore suspension with a concentration of $5\times10^{7}$ spores/mL was prepared using a custom-made wettable powder of Beauveria bassiana, and the additive product (diluted 10,000-fold) was added to the suspension to prepare Test Spray Liquids #1 to #5. All spray liquids were prepared in 5-liter batches, and the solution was thoroughly mixed before spraying. An electric backpack sprayer was used for even application, with treatments administered twice: the first application and again 3 days after the first application. The trial involved three investigations of results, conducted 3 days, 7 days, and 14 days after the first application to assess the number of surviving pests. Prior to treatment, the pest population on sample plants was recorded. Survey method: Each treatment was divided into two sampling areas (upper and lower). In each area, five sampling points were used, and at each point, two plant samples were selected. The total pest count for each plant was recorded at each sampling point.

Formulas for Calculation: The reduction rate of pests and the control efficacy were calculated using the following formulas:

Pest reduction rate = (number of live insects before application - number of live insects after application) / number of live insects before application × 100

Control effect = (pest reduction rate in the treatment area - insect reduction rate in the blank control area) / (100 - insect reduction rate in the blank control area) × 100;

For details, please refer to Table 4 for the field efficacy test of the invention adjuvant preparation combined with rose smoke-colored Cordyceps wettable powder to control greenhouse aphids;

Table 4 Field efficacy test of the invention adjuvant preparation combined with rose smoke-colored Cordyceps wettable powder to control greenhouse aphids

| Number | Treatment | Control efficiency | | |
|---|---|---|---|---|
| | | 3 days after application | 7 days after application | 14 days after application |
| 1# | Agent | (25.5±2.5)b | (46.9±3.1)b | (66.7+4.7)b |
| 2# | Agent + Example 1 | (33.8±2.6)ab | (64.4+ 3.3)a | (79.8+2.6)a |
| 3# | Agent + Example 2 | (34.0±4.8)ab | (61.4+4.6)a | (78.7±1.5)a |
| 4# | Agent + Example 3 | (36.7+2.9)a | (60.9±6.2)a | (78.0±4.8)a |
| 5# | Agent + Example 4 | (31.4+2.7)ab | (62.5+5.1)a | (78.3±2.7)a |

The results show that the control effect of rose smoke-colored Cordyceps wettable powder is significantly improved after the adjuvant is combined with adjuvants, especially in the early and middle stages of application, which can effectively improve the control effect of fungal insecticides on aphids.

The results indicate that the combination of Beauveria bassiana wettable powder with the additive significantly improves control efficacy, especially in the mid to late stages of application, effectively enhancing the effectiveness of the fungal insecticide against aphids.

In summary, the invention relates to obtaining a specialized bucket-mix enhancer for fungal insecticides used with Beauveria bassiana and Metarhizium anisopliae. This enhancer provides environmental light protection and efficacy enhancement for the live fungal propagules after application, thereby promoting the effectiveness of the fungal insecticide and extending its efficacy. The invention also includes the preparation method for this specialized bucket-mix enhancer, designed for use with Beauveria bassiana and Metarhizium anisopliae fungal insecticides. It ensures that the enhancer provides environmental light protection and efficacy enhancement for the live fungal propagules, promoting the effectiveness of the fungal insecticide and prolonging its efficacy. LU508345

Furthermore, the invention encompasses the product of this specialized bucket-mix enhancer, which, when used with Beauveria bassiana and Metarhizium anisopliae fungal insecticides, provides environmental light protection and efficacy enhancement for the live fungal propagules, promoting the effectiveness of the fungal insecticide and extending its efficacy.

The production process of the specialized bucket-mix enhancer is simple and cost-effective. The product features excellent self-dispersion, high storage stability, and ease of use. When used in combination with fungal insecticides, it offers environmental light protection, promotes germination and growth under low humidity, and enhances the efficacy and longevity of the fungal insecticide.

Finally, it should be noted that the above is only a preferred embodiment of the present invention and is not intended to limit the present invention. Although the present invention has been described in detail with reference to the aforementioned embodiments, it is still possible for those skilled in the art to modify the technical solutions described in the aforementioned embodiments or to make equivalent substitutions for some of the technical features therein. Any modifications, equivalent substitutions, improvements, etc. made within the spirit and principles of the present invention should be included in the protection scope of the present invention.

## CLAIMS



1. A synergistic additive specifically for fungal insecticides, characterized by having a total weight of 100 parts by weight, with the following formulation: 10-15 parts by weight of a UV-protectant, 1-5 parts by weight of a dispersant, 0.5-2 parts by weight of a wetting agent, 5-15 parts by weight of soybean peptone, 1-5 parts by weight of oligosaccharides, 0.5-1 part by weight of chitosan oligosaccharides, 0.01-0.05 parts by weight of trace elements, 0.05-0.2 parts by weight of a polymer suspension aid, 0.5-1.5 parts by weight of a pH regulator, and the balance of deionized water.

2. The synergistic additive for fungal insecticides according to claim 1, wherein the UV-protectant is a self-dispersing water-based yellow pigment slurry.

3. The synergistic additive for fungal insecticides according to claim 1, wherein the dispersant is a polymer surfactant based on naphthalene sulfonate-formaldehyde condensates.

4. The synergistic additive for fungal insecticides according to claim 1, wherein the wetting agent is a water-soluble organic silicon wetting agent.

5. The synergistic additive for fungal insecticides according to claim 1, wherein the oligosaccharide is one or more of xylan oligosaccharide, sucrose oligosaccharide, alginate oligosaccharide, or fructose oligosaccharide with a molecular weight of less than 2000 Da.

6. The synergistic additive for fungal insecticides according to claim 1, wherein the trace elements are one or more of manganese sulfate, copper sulfate, or ferrous sulfate.

7. The synergistic additive for fungal insecticides according to claim 1, wherein the polymer suspension aid is one of Carbomer 940, Carbomer 941, or Carbomer 970.

8. The synergistic additive for fungal insecticides according to claim 1, wherein the pH regulator is one of triethanolamine, diethanolamine, or ethanolamine.

9. A preparation method for the synergistic additive for fungal insecticides according to any one of claims 1-8, characterized by comprising the following steps:

S1. pigment slurry grinding: according to the formulation by mass, weigh the UV-protectant, dispersant, and wetting agent; first, dissolve the dispersant and wetting agent in deionized water in an amount equal to the weight of the UV-protectant, and add this solution to the UV-protectant; stir thoroughly to mix, then place the material in a sand mill for grinding; set the sand mill to the following conditions: zirconium bead diameter of 0.2-0.3 mm, a filling rate of 70-75%; grind until the pigment particle size reaches D90 ≤ 110 nm or D100 ≤ 230 nm;

S2. peptone solution preparation: according to the formulation by mass, weigh the soybean peptone, oligosaccharides, chitosan oligosaccharides, and trace elements; add the soybean peptone into 60-65°C hot deionized water at a mass ratio of 1:3, stirring thoroughly until fully dissolved; after cooling to room temperature, add the oligosaccharides, chitosan oligosaccharides, and trace elements, stirring until completely dissolved;

S3. polymer suspension aid solution preparation: according to the formulation by mass, weigh the polymer suspension aid; add it into 40-45°C hot deionized water at a mass ratio of 1:50, mixing thoroughly; while stirring, add the pH regulator until the pH value reaches 7-8;

S4. mixing and adjustment: under stirring, slowly add the ground pigment slurry to the prepared polymer suspension aid solution, mixing thoroughly; then, slowly add the prepared peptone solution, stirring evenly; while stirring, adjust the pH by adding more pH regulator until the mixture reaches a pH of 7-8; finally, add the remaining

LU508345

deionized water to make up 100 parts by weight and stir evenly to obtain the final additive product.

## REVENDICATIONS



1. Additif synergique spécifiquement destiné aux insecticides fongiques, caractérisé en ce qu'il contient au total 100 parties en poids, la composition suivante : 10 à 15 parties en poids d'un agent de protection contre les UV, 1 à 5 parties en poids d'un dispersant, 0,5 à 2 parties en poids d'un agent mouillant, 5 à 15 parties en poids de peptone de soja, 1 à 5 parties en poids d'oligosaccharides, 0,5 à 1 partie en poids d'oligosaccharides de chitosane, 0,01 à 0,05 partie en poids d'oligoéléments, 0,05 à 0,2 partie en poids d'un adjuvant de suspension polymère, 0,5 à 1,5 partie en poids d'un régulateur de pH, le reste étant de l'eau déionisée.

2. Additif synergique destiné aux insecticides fongiques selon la revendication 1, dans lequel l'agent de protection contre les UV est une suspension pigmentaire jaune à base d'eau autodispersante.

3. Additif synergique pour insecticides fongiques selon la revendication 1, dans lequel le dispersant est un tensioactif polymère à base de condensats de naphtalène sulfonate-formaldéhyde.

4. Additif synergique pour insecticides fongiques selon la revendication 1, dans lequel l'agent mouillant est un agent mouillant organique de silicium soluble dans l'eau.

5. Additif synergique pour insecticides fongiques selon la revendication 1, dans lequel l'oligosaccharide est un ou plusieurs oligosaccharides de xylane, oligosaccharides de saccharose, oligosaccharides d'alginate ou oligosaccharides de fructose ayant un poids moléculaire inférieur à 2000 Da.

6. Additif synergique pour insecticides fongiques selon la revendication 1, dans lequel les oligo-éléments sont un ou plusieurs parmi le sulfate de manganèse, le sulfate de cuivre ou le sulfate ferreux.

7. Additif synergique pour insecticides fongiques selon la revendication 1, dans lequel l'adjuvant de suspension polymère est l'un parmi le Carbomer 940, le Carbomer 941 ou le Carbomer 970.

8. Additif synergique pour insecticides fongiques selon la revendication 1, dans lequel le régulateur de pH est l'un parmi la triéthanolamine, la diéthanolamine ou l'éthanolamine.

9. Procédé de préparation de l'additif synergique pour insecticides fongiques selon l'une quelconque des revendications 1 à 8, caractérisé en ce qu'il comprend les étapes suivantes :

S1. broyage de la suspension pigmentaire : selon la formulation en masse, peser le protecteur UV, le dispersant et l'agent mouillant ; tout d'abord, dissoudre le dispersant et l'agent mouillant dans de l'eau déionisée en une quantité égale au poids du protecteur UV, et ajouter cette solution au protecteur UV ; remuer soigneusement pour mélanger, puis placer le matériau dans un broyeur à sable pour le broyage ; régler le broyeur à sable dans les conditions suivantes : diamètre des billes de zirconium de 0,2 à 0,3 mm, taux de remplissage de 70 à 75 % ; broyer jusqu'à ce que la taille des particules de pigment atteigne D90 ≤ 110 nm ou D100 ≤ 230 nm;

S2. préparation de la solution de peptone : selon la formulation en masse, peser la peptone de soja, les oligosaccharides, les oligosaccharides de chitosane et les oligo-éléments ; ajouter la peptone de soja dans de l'eau déionisée chaude à 60-65°C dans un rapport massique de 1:3, en remuant soigneusement jusqu'à dissolution complète ; après refroidissement à température ambiante, ajouter les oligosaccharides, les oligosaccharides de chitosane et les oligo-éléments, en remuant jusqu'à dissolution complète;

S3. préparation de la solution d'aide à la suspension de polymère : selon la formulation en masse, peser l'aide à la suspension de polymère ; l'ajouter dans de l'eau déionisée chaude à 40-45°C dans un rapport massique de 1:50, en mélangeant soigneusement ; tout en remuant, ajouter le régulateur de pH jusqu'à ce que la valeur du pH atteigne 7-8;

S4. mélange et ajustement : sous agitation, ajouter lentement la suspension de pigments broyés à la solution d'aide à la suspension de polymère préparée, en mélangeant soigneusement ; ensuite, ajouter lentement la solution de peptone préparée, en remuant uniformément ; tout en remuant, ajuster le pH en ajoutant plus de régulateur de pH jusqu'à ce que le mélange atteigne un pH de 7-8 ; enfin, ajouter l'eau déionisée restante pour constituer 100 parties en poids et remuer uniformément pour obtenir le produit additif final.

**FIGURES**



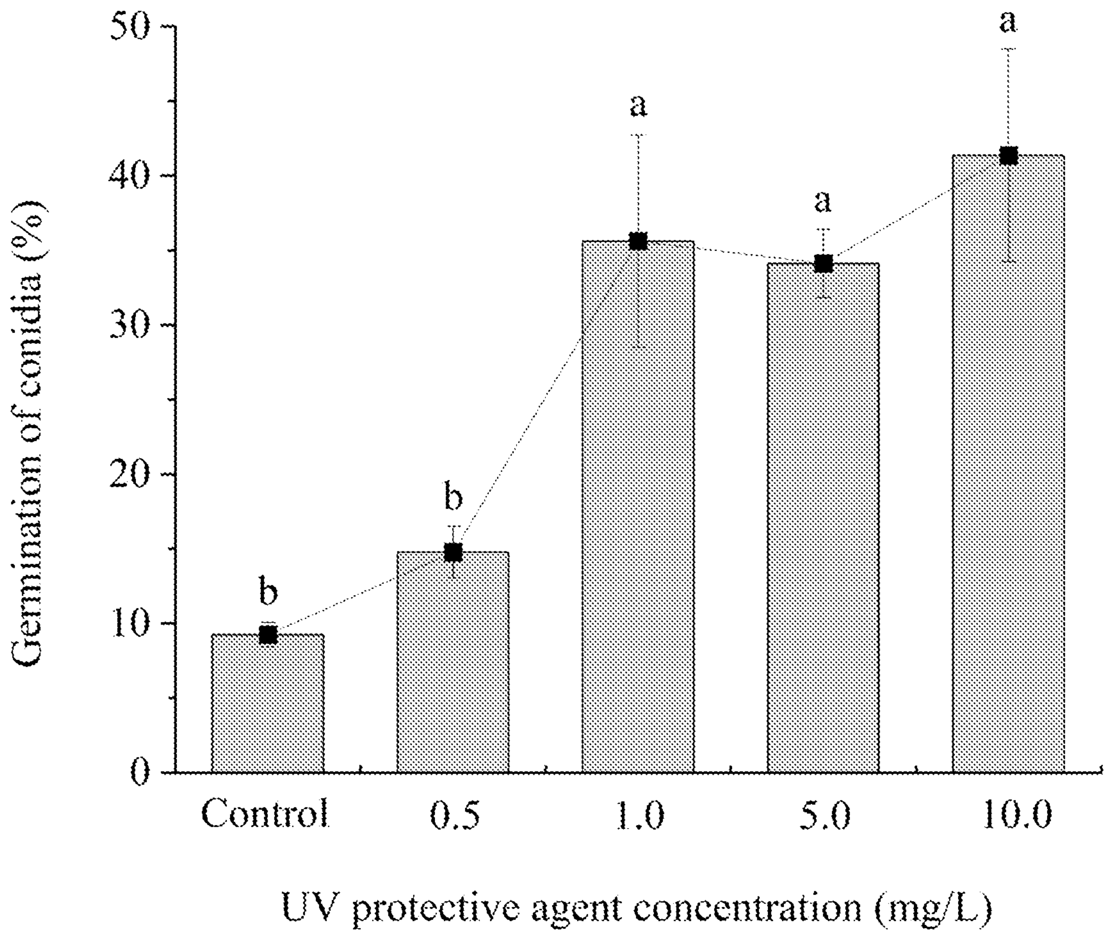


**Figure 1**

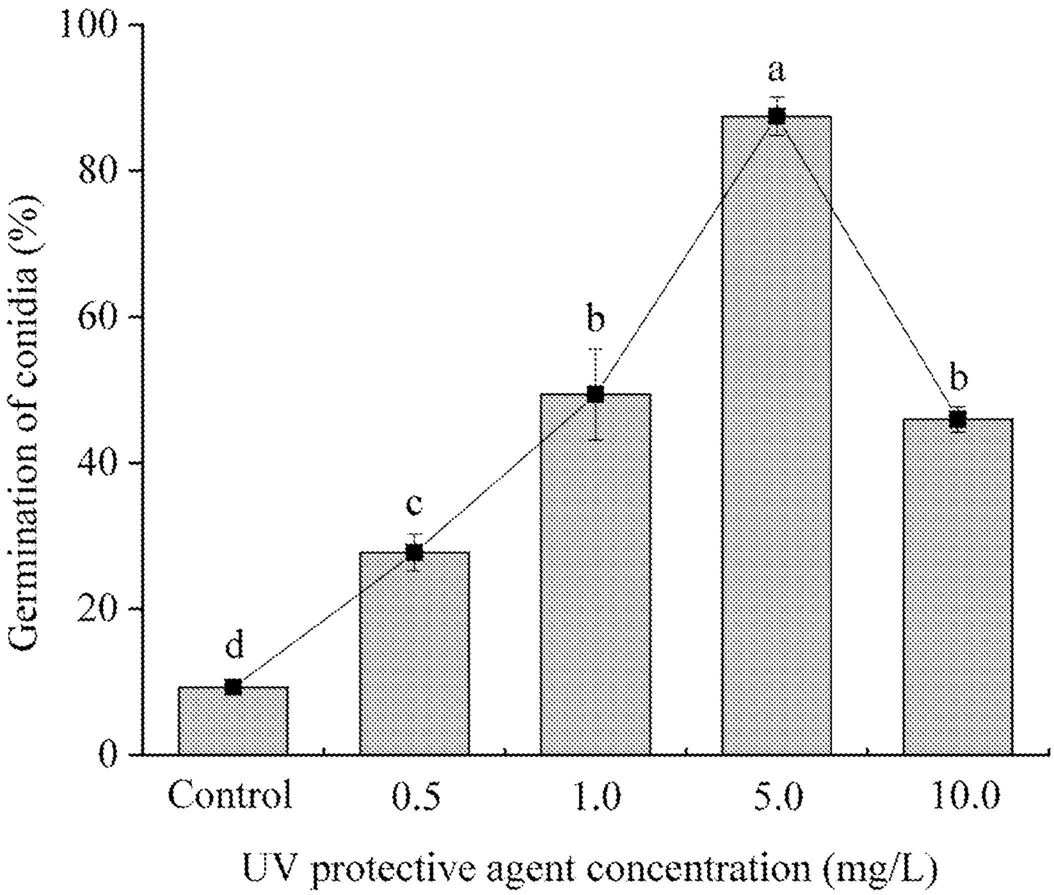
Germination of conidia (%)
100
80
60
40
20
0
a
b
c
d
b
Control
0.5
1.0
5.0
10.0
UV protective agent concentration (mg/L)

Figure 2