Research on the Bactericidal and Antiseptic Effect of Highly Efficient Compounded Thiabendazole

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Abstract: The extensive use of traditional chemical pesticides can have an impact on human health. On the other hand, biopesticides have the advantages of having fewer side effects on the human body and being generally more beneficial to health. In order to reduce the use of the chemical pesticide thiabendazole, cinnamaldehyde, an active plant extract, is compounded with thiabendazole to form a new type of low - toxicity biopesticide fungicide and preservative, and its fungicidal and preservative effects are studied. A large number of antibacterial experiments show that when the ratio of thiabendazole to cinnamaldehyde is 5 mg/L:0.05 g/L, it has a good inhibitory effect on Mucor and yeast, and when the ratio is 5 mg/L:0.15 g/L, it has a better inhibitory effect on Aspergillus niger. Through the analysis of the antibacterial experiments, the composite concentration ratio of thiabendazole:cinnamaldehyde of 5 mg/L:0.15 g/L is selected, and based on this ratio, the experimental agent CAT (cinnamaldehyde and thiabendazole) is prepared. After compounding, greenhouse experiments are carried out. A large number of greenhouse experiment results show that the inhibitory effect of the compounded agent is not lower than that of the thiabendazole agent used alone. The research results provide a research basis for reducing the use of thiabendazole and developing new - type, high - efficiency and low - toxicity biopesticide preservatives. Through greenhouse experiments and cooperation with enterprises, the research also proves that this experimental agent is suitable for industrial production.

Keywords: Biopesticides; Highly Effective and Low Toxicity; Broad-spectrum Antibacterial Activity; Formulation

DOI: 10.57237/j.jaf.2024.04.003

1 Introduction

On a global scale, yield loss due to plant pathogens ranges from 10% to 16%. At the same time, an upsurge in the human population and the future demand for food and food security has been the topmost concern for scientists and agriculturists [1]. Continuous rise in application of pesticides in the agro-ecosystems in order to ensure food supply to the ever-growing population is of greater concern to the human health and the environment [2]. With the rise of the global sustainable development trend, green experimental pharmaceuticalion or green manufacturing has become an inevitable direction for future industrial development. Compared to chemical pesticides, biopesti-

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Received: 9 October 2024; Accepted: 19 November 2024; Published Online: 5 December 2024 http://www.agrforestry.com

cides have the advantages of strong selectivity, no pollution, lower likelihood of developing resistance, minimal disruption to the ecological environment, and wide availability of raw materials, making them promising for broad application prospects. Developing biopesticides and exploring new areas of development is currently the focus of pesticide researchers. They are expected to play a crucial supporting role in the field of bioagriculture and gradually evolve into a strategic emerging industry. This shift not only aligns with the principles of green chemistry and environmental protection but also responds to the global need for sustainable agriculture and food security. As the world population grows and the demand for safe and environmentally friendly agricultural practices increases, biopesticides are poised to become a cornerstone of the agricultural industry, contributing to a more sustainable and resilient food experimental pharmaceuticalion system [3].

The spoilage rate of fruits and vegetables in China is indeed high, with the latest statistics indicating that the loss rate of fruits and vegetables in China is approximately between 20% and 30%, resulting in significant economic losses [4]. triabendazole, a chemical preservative belonging to the benzimidazole class of fungicides, is a highly effective, low-toxicity, and broad-spectrum systemic fungicide widely used for the control of diseases and pests in fruits and vegetables. Although this pesticide provides excellent protection and curative effects for crops, excessive residues can pose health risks to consumers [5]. Isolated cinnamaldehyde has been shown to effectively inhibit the growth of an array of microorganisms such as bacteria, moulds, and yeasts, as well as having been reported to inhibit toxin experimental pharmaceuticalion by micro-organisms [6]. Cinnamaldehyde has been reported to inhibit bacteria, yeasts, and filamentous molds via the inhibition of ATPases, cell wall biosynthesis, and alteration of membrane structure and integrity [7]. Conventional chemical fungicides are commonly used for controlling these fungal diseases, yet pose challenges such as drug residue and environmental pollution [8]. As a crucial measure to develop sustainable agriculture, replacing chemical pesticides with bio-pesticides has attracted significant attention from the business and academic circles. However, bio-pesticides have the characteristics of high price, slow medicinal properties, and poor stability in the environment, thus resulting in a low level of farmer acceptance [9].

Therefore, this experiment involves combining cinnamaldehyde with triabendazole to achieve a preservative effect, while simultaneously reducing the required amount of triabendazole. This ensures that fruits, vegetables, and other agricultural experimental pharmaceuticals can be preserved in a healthier manner, benefiting both the natural ecological environment and human health, thus making it more appealing to people. This approach secures the development of green agriculture and aligns with China's policy of adhering to a green development path. By promoting the use of a safer and more sustainable preservative strategy, it supports the overall goal of environmental protection and human well-being, reflecting a commitment to sustainable agricultural practices.

2 Materials and Methods

2.1 Materials

2.1.1 Chemicals and Reagents

Triabendazole, cinnamaldehyde, Potato Dextrose Agar (PDA) medium;

2.1.2 Test Organisms

Mucor (Mucorales), yeast (Saccharomycetes), and black mold (Cladosporium).

2.2 Methods

2.2.1 Preparation of PDA Agar Medium

(1) Cleaning and peeling: Wash the potatoes, peel them and cut them into small pieces.

(2) Cooking the potatoes: Put the cut potato pieces into a pot, add 1000 milliliters of distilled water, and boil for 20 - 30 minutes until the potatoes become soft.

(3) Filtration: Use gauze or filter paper to filter the potato pieces and collect the filtrate.

(4) Adding glucose and agar: Pour the filtrate back into the pot, add glucose and agar, and stir gently until the agar is completely dissolved.

(5) Adjusting the pH value: Use pH test paper or a pH meter to measure the pH value of the solution. If necessary, use dilute hydrochloric acid or sodium hydroxide solution to adjust the pH value to 5.6 - 5.8.

(6) Sub - packaging: Divide the solution into petri

dishes or test tubes, and the volume of each container is about 100 milliliters.

(7) Sterilization: Put the containers filled with the solution into an autoclave and sterilize at 121° C for 20 minutes.

(8) Cooling and solidifying: After sterilization, take the containers out of the autoclave and let them cool to room temperature until the agar solidifies.

2.2.2 Preparation of PDA Containing Individual and Combined Agents

(9) Preparation of Cinnamaldehyde Solution: Mix 0.1 ml of 95% cinnamaldehyde with 100 ml of ethanol to create a cinnamaldehyde solution with a concentration of 1 g/L.

(10) Preparation of triabendazole Solution [10]: Weigh 0. 102 g of triabendazole and dissolve it in 50 ml of methanol. Then, add 200 ml of distilled water to make a triabendazole solution with a concentration of 400 mg/L.

(11) Preparation of triabendazole Solutions at Different Concentrations: From the prepared triabendazole stock solution, create solutions with concentrations of 1 mg/L, 5 mg/L, 10 mg/L, 25 mg/L, and 125 mg/L.

(12) Preparation of Cinnamaldehyde Solutions at Different Concentrations: Prepare cinnamaldehyde solutions with concentrations of 0. 01 g/L, 0. 05 g/L, 0. 1 g/L, 0. 3 g/L, and 0. 5 g/L.

(13) Preparation of Combined triabendazole and Cinnamaldehyde Solutions: Mix the above-mentioned concentrations of triabendazole and cinnamaldehyde at ratios of 5 mg/L: 0. 005 g/L, 5 mg/L: 0. 025 g/L, 5 mg/L: 0. 05 g/L, 5 mg/L: 0. 15 g/L, and 5 mg/L: 0. 25 g/L for experimentation.

2.2.3 Antibacterial Test

To study the inhibitory effect of cinnamaldehyde on pathogenic fungi and calculate its bacteriostatic rate. The growth of bacteria around the sample was observed and the diameter of the inhibition circle was measured [11].

(i) triabendazole Inhibition Tests on Mucor:

Conduct three sets of inhibition tests using triabendazole at concentrations of 1 mg/L, 5 mg/L, 10 mg/L, 25 mg/L, and 125 mg/L on Mucor. Measure the diameter of the Mucor colony on the first, third, and fifth days. Calculate the inhibition rates for the three sets of experiments and find their average. Create a bar chart of the inhibition rates. Determine that the best inhibition rate for triabendazole is achieved at the minimum concentration of 10 mg/L.

(ii) Cinnamaldehyde Inhibition Tests on Mucor:

Perform three sets of inhibition tests using cinnamaldehyde at concentrations of 0.01 g/L, 0.05 g/L, 0.1 g/L, 0.3 g/L, and 0.5 g/L on Mucor. Measure the diameter of the Mucor colony on the first, third, and fifth days. Calculate the inhibition rates for the three sets of experiments and find their average. Create a bar chart of the inhibition rates.

(iii) Combined triabendazole and Cinnamaldehyde Inhibition Tests:

Use half the concentration of triabendazole that showed the best inhibitory effect (i.e., 5 mg/L) combined with specific ratios of cinnamaldehyde to conduct inhibition tests. Perform three sets of inhibition tests using the above concentrations of triabendazole and cinnamaldehyde, at ratios of 5 mg/L: 0.005 g/L, 5 mg/L: 0.025 g/L, 5 mg/L: 0.05 g/L, 5 mg/L: 0.15 g/L, and 5 mg/L: 0.25 g/L on Mucor, black mold, and yeast. Measure the diameter of the Mucor, black mold, and yeast colonies on the first, third, and fifth days. Calculate the inhibition rates for the three sets of experiments and find their average. Create bar charts of the inhibition rates.

2.3 Greenhouse Experimental Testing

2.3.1 Experimental Objective

The objective is to utilize a new, highly effective, and low-toxicity biopesticide to mitigate the impact of fungi that pose a threat during the preservation of fruits and vegetables, thereby achieving sterilization and preservation effects. This will help reduce adverse effects on human health and extend shelf life. The team is actively responding to the National "14th Five-Year Plan" for Green Agricultural Development, protecting ecological safety, and innovating the variety of biopesticides.

2.3.2 Experimental Subject

The subjects for the experiment are: Green beans (Jiandou), Grapes (Ti Zi).

2.3.3 Experimental Design and Execution

(i) Turn on the ultraviolet light in the clean bench for half an hour.

(ii) Wash and dry the freshly purchased grapes and green beans; divide them into three portions.

(iii) Prepare the Agent on the Clean Bench

Cinnamaldehyde Solution: Mix 0.1 ml of 95% cinnamaldehyde with 100ml of ethanol to make a 1g/L cinnamaldehyde solution.

Triabendazole Pesticide Preparation: Weigh 0.102g of triabendazole and dissolve it in 250ml of methanol to make a 400mg/L triabendazole solution. Transfer 3. 75ml of the 400mg/L triabendazole and 5ml of the 1g/L cinnamaldehyde into a bottle, add 300ml of distilled water, and mix to create a combined reagent. Transfer the combined reagent into small spray bottles. Spray an equal amount of the combined reagent and triabendazole onto the corresponding fruits and vegetables, while leaving one portion untreated as a control group.

(iv) Place the fruits and vegetables in the greenhouse for cultivation, dividing them into three groups: control, combined, and triabendazole. Conduct each group's experiment three times. After one week, observe the results and calculate the good fruit rate and weight loss rate, taking the average value. This setup allows for the assessment of the biopesticide's efficacy under controlled conditions, providing valuable insights into its potential for agricultural applications.

3 Results

3.1 The Analysis of the Antimicrobial Effects of Combined Preservatives

This study aimed to develop an eco-friendly, low-toxicity, and efficient bio-preservative by optimizing the ratio of cinnamaldehyde, a plant-derived active compound, and triabendazole, a chemical pesticide. Extensive individual drug inhibition experiments revealed that triabendazole is effective against Mucor at concentrations of 10mg/L, 25mg/L, and 125mg/L, whereas cinnamaldehyde exhibits bactericidal effects at 0.3g/L and 0.5g/L. From figures 1-6, we identified a 5mg/L triabendazole to 0.15g/L cinnamaldehyde ratio as most effective against fungi, including Mucor and Aspergillus niger. This optimal ratio was used to formulate our experimental pharmaceutical CAT (Cinnamaldehyde and Triabendazole).

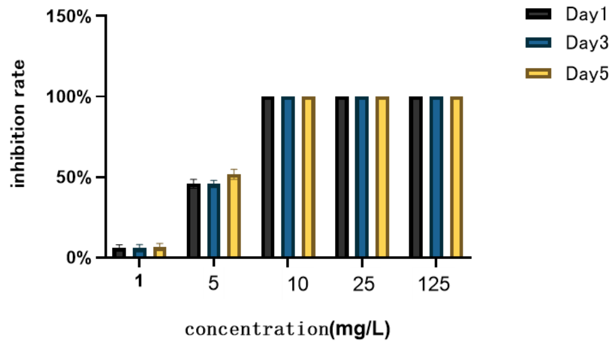

Figure 1 Bar chart showing the inhibition rate of triabendazole on Mucor

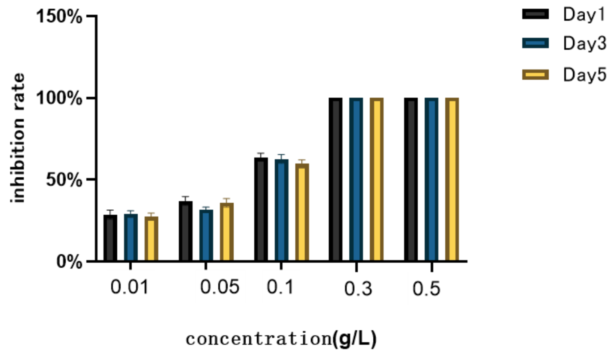

Figure 2 Bar graph showing theantibacterial rates of cinnamaldehydea

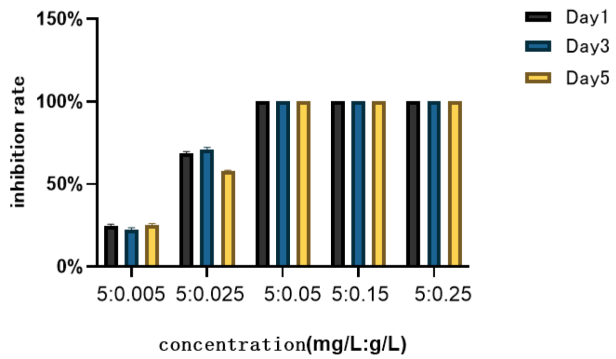

Figure 3 Bar chart of the inhibition rates of Methylthiophanate and Cinnamaldehyde synergistically against Mucor

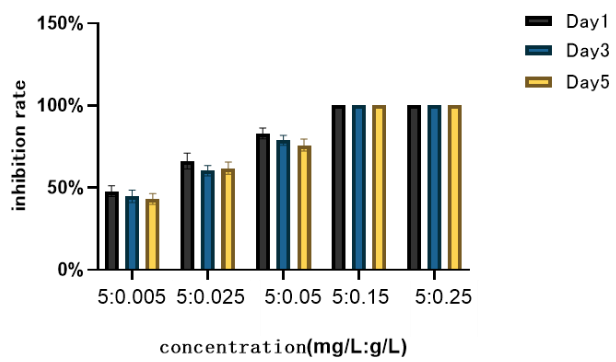

Figure 4 Bar chart of the inhibitory rates of triabendazole and cinnamaldehyde mixture against black mold

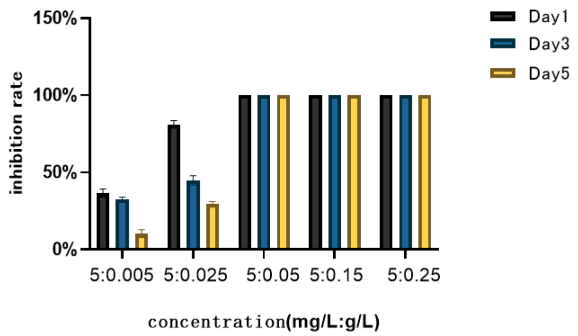

Figure 5 Bar chart of the antifungal efficacy of thiophanate methyl and cinnamaldehyde against yeast

Figure 1 shows that triabendazole exhibits good antifungal effects against Mucor at concentrations of 10mg/L, 25mg/L, and 125mg/L. Figure 2 indicates that cinnamaldehyde extract has good bactericidal effects at concentrations of 0.3g/L and 0.5g/L. Figure 3 demonstrates that the combination of triabendazole and cinnamaldehyde, at compounded ratios of 5:0.05mg/L:g/L, 5:0.15mg/L:g/L, and 5:0.25mg/L:g/L, has good antifungal effects against Mucor. Figure 4 shows that triabendazole and cinnamaldehyde, at compounded ratios of 5:0.15mg/L:g/L and 5:0.25mg/L:g/L, have good antifungal effects against Aspergillus niger (Black Mold). Figure 5 reveals that triabendazole and cinnamaldehyde, at compounded ratios of 5:0.05mg/L:g/L, 5:0.15mg/L:g/L, and 5:0.25mg/L:g/L, have good antifungal effects against yeast. These results indicate that triabendazole and cinnamaldehyde have good antifungal effects against Mucor, Black Mold, and yeast at various concentrations. Specifically, the optimal compounded ratio is 5:0.05mg/L:g/L, achieving a compounded effect of 50%, demonstrating a synergistic effect.

3.2 Greenhouse Experimental Effect Analysis

Figures 6a and b depict the control group, showing decay in the cowpeas and grapes, with fungal growth observed on the grape branches, leading to branch decay. Figures 6c and d represent the compounded group, while Figures 6e and f illustrate the group treated with 10mg/L of thiophanate-methyl. In these two groups, apart from some fungal growth at the ends of the grape branches, no rotting was observed in the grapes. The cowpeas, while somewhat yellowed, generally exhibited good condition with less decay compared to the control group.

These results suggest that both the combined group and the triabendazole group can significantly improve the disease resistance of cowpeas and grapes, reducing the plants' rot and yellowing. This may be because the components in these treatment groups inhibit the growth of pathogens, protecting the plants from disease damage. For agricultural experimental pharmaceuticalion and plant protection, these findings hold significant practical implications, guiding farmers in taking appropriate disease prevention measures when growing cowpeas and grapes, thereby improving crop yields and quality.

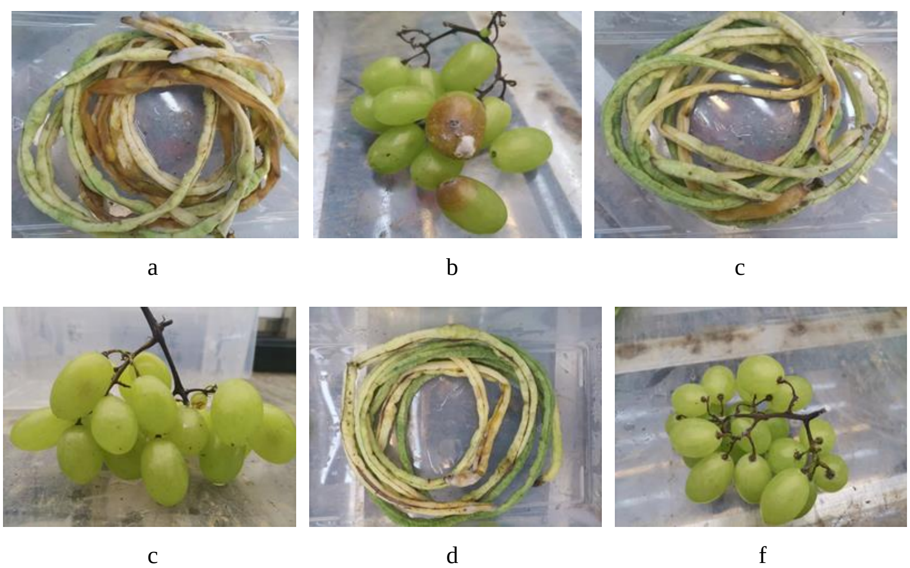

Figure 6 Observations from the greenhouse experiment

Note: a: Control group green beans; b: Control group grapes; c: Combined treatment group green beans; d: Combined treatment group grapes; e: triabendazole group green beans; f: triabendazole group grapes

3.3 Analysis of Fruit Weightlessness Rate and Good Fruit Rate in Greenhouse Experiments

3.3.1 Measuring Indicators

Weight Loss Rate due to Water Evaporation.

The weight loss rates of the control group, the combined treatment group, and the triabendazole group, as well as their average weight loss rates, from the repeated experiments.

unit of weight: g

Table 1 The weight loss rate of the control group, compound group and triabendazole group fruits

weight loss rates (%)	control group	Combined treatment group	triabendazole group
group 1	32.12%	17.93%	15.83%
group 2	34.48%	18.88%	18.38%
group 3	36.78%	15.93%	16.98%
average weight loss rates (%)	34.36%	17.58%	17.06%

This study showed the weight loss rate of the compound treatment group is significantly lower than that of the control group, indicating a remarkable preservation effect, and the compound effect is comparable to the use of thiabendazole alone (Table 1).

3.3.2 Quality Fruit Yield Percentage

In the following, the good fruit rates of the control group, the compound group, and the triabendazole group from repeated experiments, along with their average good fruit rates, are presented in English translation:

Table 2 The good fruit rate of the control group, compound group and triabendazole group fruits

good fruit rates (%)	control group	combined treatment group	triabendazole group
group 1	64.71%	90.00%	83.33%
group 2	47.37%	87.50%	88.89%
group 3	72.22%	81.25%	85.71%
average good fruit rates (%)	61.43%	86.35%	85.98%

We found that the significantly higher proportion of good-quality fruit in the combined treatment group compared to the control group indicates a notable preservation effect (Table 2).

4 Conclusion

Cinnamaldehyde is derived from cinnamon oil and can inhibit the growth of a variety of microorganisms. Its antibacterial activity has been fully documented in the literature, and it has been shown to be particularly effective against fungal pathogens. This makes cinnamaldehyde a promising disinfectant and preservative, with great potential in the treatment and prevention of fungal infections. In this experiment, the plant - derived active compound cinnamaldehyde shows excellent inhibitory effect on mold. It can strongly inhibit the growth of mold and has strong antibacterial properties, which can effectively eliminate bacteria, including Escherichia coli.

Antibacterial experiments show that when the plant - active extract cinnamaldehyde is mixed with the chemical pesticide triadimefon, the bactericidal effect is enhanced. Specifically, at a concentration ratio of 5mg/L of triadimefon to 0.15g/L of cinnamaldehyde, there is a significant inhibition of fungi such as Mucor and Aspergillus. Greenhouse experiments also confirm that the preservation effect of this mixed pesticide is similar to that of triadimefon alone. By mixing the two, the amount of triadimefon used is significantly reduced while achieving almost the same preservation effect. Considering factors such as the cost of experimental pharmaceuticals, it is determined that for biopesticides, the concentration ratio of 5mg/L of triadimefon to 0.15g/L of cinnamaldehyde meets the objectives of our team.

5 Discussion

The use of natural antimicrobials instead of traditional preservation techniques, has gained popularity in recent years because consumers increasingly prefer food processed through milder preservation techniques because food processed this way have enhanced natural appeal and perceived nutritional quality. Spices and their extracts are generally recognized as safe because of their traditional uses without any documented detrimental effects [12]. One of advantages of extracts obtained from plants or plant organs used as spices or vegetable is that they can be used in the food industry without further approval as they are categorized as “Generally Recognized as Safe” by the U. S. Food and Drug Administration [13]. Plant extracts and their derived compounds demonstrate antimicrobial activity against bacteria, fungi, and viruses. Cinnamaldehyde, in particular, has garnered significant attention due to its natural origins, safety, easy availability, and broad-spectrum antimicrobial properties. The U. S. Food and Drug Administration (FDA) and the Flavor and Extract Manufacturers Association (FEMA) have recognized cinnamaldehyde as "Generally Recognized As Safe" (GRAS), and the European Commission has approved its use in food experimental pharmaceuticals. This status, combined with its antimicrobial efficacy, makes cinnamaldehyde a promising natural preservative for a variety of applications, including food preservation and healthcare experimental pharmaceuticals [14]. Cinnamaldehyde, a natural compound derived from cinnamon oil, has been demonstrated to effectively inhibit the growth of a wide range of microorganisms, including bacteria, fungi, and yeasts. Its antimicrobial activity is well-documented, and it has been shown to be particularly potent against fungal pathogens. This makes cinnamaldehyde a promising agent for disinfection and preservation, with significant potential in the treatment and prevention of infections caused by fungi. The plant-derived active compound cinnamaldehyde used in this experiment exhibits excellent inhibitory effects against mold. It strongly suppresses mold growth and has potent antimicrobial properties, effectively eliminating bacteria, including Escherichia coli.

Through antibacterial experiments, it has been observed that the plant active extract, cinnamaldehyde, when combined with the chemical pesticide triabendazole, demonstrates an enhanced fungicidal effect. Specifically, at a concentration ratio of 5mg/L triabendazole to 0.15g/L cinnamaldehyde, there is a notable inhibition of fungi such as Mucor and Aspergillus. Greenhouse experiments have also confirmed that the combined pesticide has a preservation effect similar to that of triabendazole alone. By blending the two, the usage of triabendazole is significantly reduced while achieving a preservation effect that is almost the same. Taking into account factors such as experimental pharmaceuticalion costs, our experiment has determined that a ratio of 5mg/L triabendazole to 0.15g/L cinnamaldehyde is the most suitable for our team's objectives in terms of biopesticides.

This study provides a research foundation for the future industrialization of new, highly effective, and low-toxicity bio-pesticides. The author will continue to investigate lower concentrations of triabendazole in combination with various concentrations of cinnamaldehyde, to delve deeper into the study of compounded fungicides and preservatives. Efforts are being made to uncover the mechanisms of fungicidal preservation, aiming to enhance preservation efficacy, improve targeting capabilities, while simultaneously reducing environmental pollution. By compounding cinnamaldehyde with triabendazole, a new generation of fast-acting and effective bio-pesticide preservatives will be developed, achieving a synergistic effect greater than the sum of their parts (1 + 1 > 2). Its industrialization will be beneficial for ecological balance and the development of pollution-free agriculture, contributing significantly to human health and societal progress.

Acknowledgments

This work were supported by the Serving “343” industry development project of University in Xuzhou (GX2023017), the scientific and technological transformative project of Xuzhou (KC22438), the Jiangsu Provincial Innovation and Entrepreneurship Training Project for College Students - School-Enterprise Cooperation Fund Project (202310320167H), the Jiangsu Jieqing Reserve Project (HB2016016) of Jiangsu Normal University, and the Jiangsu provincial advantage discipline construction project (PAPD). All of authors have read and agreed to the publication. The authors thank to lab members for assistance.

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