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Effectiveness of biological products of *Verticillium lecanii* to against of whiteflies *Bemia tabaci* (Homoptera: Aleyrodoidea)

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ABSTRACT*Aim:* *This study investigates the effectiveness of the entomopathogenic fungus Verticillium lecanii as a biopesticide against the whitefly species Bemisia tabaci, which is known for damaging vegetables in agricultural settings.* ***Methods:*** *The research involved collecting adult B. tabaci from a pesticide-free eggplant garden and rearing them on clean eggplant plants in controlled laboratory conditions. The effectiveness of both fresh and dried forms of Verticillium lecanii was evaluated through a randomized experiment with multiple dosages, supplemented with Tween 80. Efficacy was determined by counting the number of surviving larvae every day for ten days and analyzing them according to the Abbott formula and ANOVA.* ***Results:*** *The results demonstrated that various concentrations of Verticillium lecanii, both in fresh and dried forms, significantly*

Keywords: *Bemisa tabaci, Entomopathogenic fungi, Verticillium lecanii, bioproduct, biopesticide.*

experiment with multiple dosages, supplemented with Tween 80. Efficacy was determined by counting the number of surviving larvae every day for ten days and analyzing them according to the Abbott formula and ANOVA. Results: The results demonstrated that various concentrations of Verticillium lecanii, both in fresh and dried forms, significantly reduced the survival rate of B. tabaci larvae in both laboratory and greenhouse settings. Whiteflies can be effectively controlled by applying the fungal bioproduct, according to the findings. Conclusion: This research presents a viable biological control alternative for managing B. tabaci populations in vegetable crops, thereby reducing reliance on chemical pesticides and promoting sustainable agricultural practices. Implications: By highlighting the efficacy of Verticillium lecanii, this study contributes to the growing body of research on biopesticides and their role in integrated pest management, offering insights for future studies and applications in agricultural pest control.

INTRODUCTION

Whiteflies, such as *Bemisia tabaci*, are among the top pests attacking many vegetable crops worldwide. These insects weaken the leaves by sucking out essential nutrients, making photosynthesis harder and reducing the plant's overall vitality. The direct harm caused by *B. tabaci*, along with its ability to carry viruses, exacerbates the challenges faced by growers (Cuthbertson & Walters, 2005). Due to their rapid reproduction and development of resistance to many pesticides, traditional farming methods struggle to control this pest (Abubakar et al., 2022).

With the increasing demand for sustainable and environmentally safe pest control, entomopathogenic fungi are being recognized as valuable biological control agents (Koller et al., 2023). *Verticillium lecanii* shows great potential for controlling whitefly pests. Upon contact, the fungus penetrates the whitefly's cuticle, grows inside the insect, and ultimately causes its death. This organism is suitable for integrated pest management (IPM) as it poses fewer risks to the environment and non-target species compared to chemical pesticides (Keerio et al., 2020).

Recently, there has been a growing interest in using biopesticides derived from *Verticillium lecanii*. These products offer a natural way to control whitefly populations, allowing farmers to reduce their reliance on synthetic chemicals (Alavanja & Bonner, 2012). This shift towards more sustainable farming practices benefits both the environment and sustainability (Asiamah et al., 2020).

This study aims to assess various substrates and concentrations to produce *Verticillium lecanii* to identify the most effective formulation for whitefly control. Through controlled experiments conducted in both laboratory and greenhouse settings, the research seeks to optimize the growth conditions and application methods of the fungal biopesticide. Ultimately, the goal is to enhance the field-level efficacy of *V. lecanii*, enabling its broader adoption as a reliable tool in sustainable crop protection and integrated pest management strategies.

Material and methods

Preparation of whitefly, host plant and fungus source

Source of whitefly: Adult whitefly B. tabaci was collected from an eggplant garden that had not been sprayed with pesticides for at least 7 days in Binh Tan, Vinh Long, then released into a cage measuring 17 x 14 x 14cm (length x width x height) at the greenhouse of the Plant Protection Faculty, College of Agriculture, Can Tho University. Whiteflies were raised on previously prepared clean eggplant plants (about 20 days), monitored until there were 20 second-instar whitefly larvae on the plants, then the experiment was conducted. *Verticillium lecanii* fungus source was provided from Biological Control Laboratory, Faculty of Plant Protection, College of Agriculture, Can Tho University. This fungus was cultured on SDAY3 medium (Xuân, T. T., & Tuấn, L. A, 2016): Yeast extract (1g), D-glucose (40g), KH2PO4 (1g), MgSO4 (0.5g), Peptone (10g), Agar (20g), Distilled water (1000mL).

Evaluation of the effectiveness of biological products Verticillium sp. in fresh and dry forms against whitefly under laboratory conditions and green house

Method of implementation: The experiment was arranged in a completely randomized manner with 5 treatments including 4 treatments with different dosages of the preparation and a control treatment (Table 1), each treatment was supplemented with 0.5% Tween 80. Each treatment was carried out with 4 repetitions, each repetition corresponding to 20 whitefly larvae. In addition, to help preserve the preparation better and increase its applicability, the experiment evaluated the effectiveness of the dry preparation (the Verticillium sp. preparation was just produced in fresh form on rice substrate and dried under 35oC conditions for 3 days) and then tested the effectiveness of the dry fungal preparation against whitefly larvae under laboratory conditions.

Table 1: Bioproduct of *Verticillium lecanii* fresh and dry

Fresh of Bioproduct	**Dried of bioproduct**	**Dose (g/L)**
ingridients		
Verticillium lecanii fresh	*Verticillium lecanii* dried	6.25 g/L
Verticillium lecanii fresh	*Verticillium lecanii* dried	7.81 g/L

Verticillium lecanii fresh	*Verticillium lecanii* dried	9.38 g/L
Verticillium lecanii fresh	*Verticillium lecanii* dried	10.94 g/L
Control (water)	Control (water)	-

Recording criteria: Record the number of surviving whiteflies at 2, 4, 6, 8 and 10 days after treatment.

Table 1 reviews the investigation into how the bioproduct made from *Verticillium lecanii* fares against whitefly larvae in different forms and conditions. Researchers conducted the study using a randomized design, offering five treatments to plants: four using different amounts of the bioproduct (6.25, 7.81, 9.38, and 10.94 g/L) and one containing only water. Each treatment contained 0.5% Tween 80 to achieve consistent results. The fungus was tested in both freshly cultured forms on rice substrate and after being dried by heating the freshly prepared fungus at 35°C for three days. The procedure was repeated four times for each treatment, and each replicate included 20 whitefly larvae. The number of surviving whiteflies was recorded five times at 2, 4, 6, 8, and 10 days after applying the test solutions. With this model, it was possible to assess dosage effects and explore the suitability of the dried preparation for field use as a biopesticide.

The effectiveness of the fungus was calculated using the Abbott formula (1925):

$$\boldsymbol{Effectiveness}\,(\%) = (\boldsymbol{C} - \boldsymbol{T}) \,/\, \boldsymbol{C} \times \mathbf{100}$$

Where:

C = the number of live whiteflies in the **control group**,

T = the number of live whiteflies in the **treated group**.

This formula measures the percentage reduction in the pest population due to the treatment, accounting for natural mortality observed in the control.

Statistical Analysis

The data was first assembled and organized in Microsoft Excel 2010, which supported easy entry, grouping, and preliminary examination of the data. For further statistical analysis, the data was analyzed using SPSS version 26, as it is a popular statistical tool in scientific research. A statistical method called Analysis of Variance (ANOVA) was performed to establish if there was a difference between the various treatments. This technique allows for determining if mean values are different among many groups by examining the variability within and across them. Duncan's Multiple Range Test (DMRT) was applied immediately after the ANOVA to compare the mean values of each treatment. This helps in identifying the precise differences among the tested fungal treatments, enabling accurate judgment on their effects.

Results

The effectiveness of a biological agent made from *Verticillium lecanii* was examined in both a laboratory and a greenhouse setting against *Bemisia tabaci*. The study aimed to assess the efficacy of the bioproduct in fresh and dried forms at various concentrations. The survivability of whitefly larvae was monitored and recorded at the end of the designated treatment period. The results were analyzed in detail, comparing the levels of mortality induced by each bioproduct and their responses in different environments. The findings of this study can provide valuable insights for the biopesticide industry and future research on utilizing *Verticillium lecanii* in integrated pest management.

Efficacy of bioproducts against whitefly under laboratory conditions

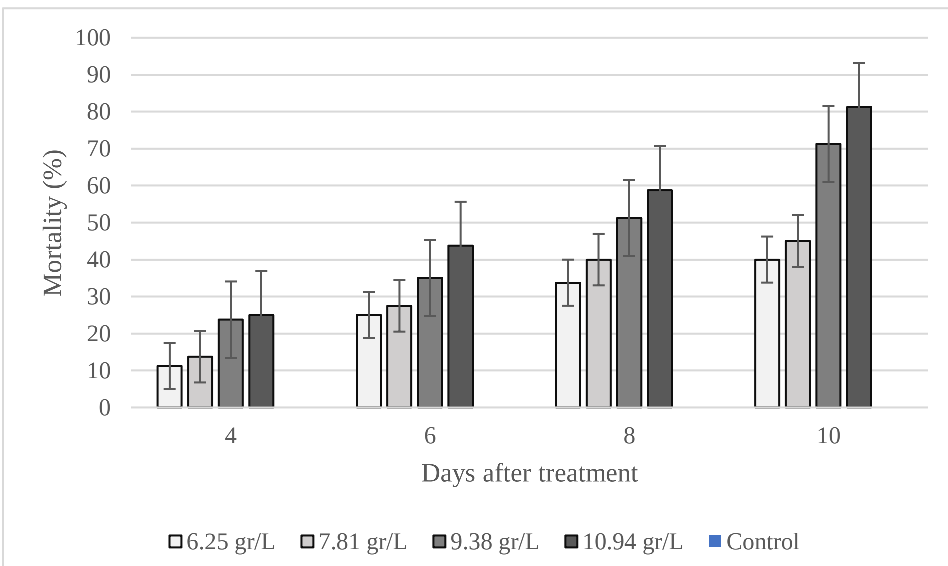

Fig 1. Fresh bioproduct against whitefly under laboratory conditions

Figure 1 represents how many whitefly larvae died after being treated with different levels of *V. lecanii* bioproduct in the laboratory, assessed at the end of each 4-, 6-, 8- and 10-day interval. Four dosages of fungal treatment and a control (water) were compared using citizen science data. The data made it obvious that there is a stronger risk of mortality as both dose and duration increase. Elevated fungal levels at any time interval were usually linked to higher death rates among bees. Twelve days into the test, the mixture with the highest dose killed more than 80% of the bacteria and the lowest concentration resulted in approximately 50% mortality. The results throughout the study clearly revealed that *V. lecanii* helped keep mortality low for members of the control group. With error bars, as concentrations go up, so does the variability in the mortality rates across the three treatments. From the results, it can be seen that higher dosages of *V. lecanii* seem to be the most reliable way to use it as a biocontrol agent against whiteflies.

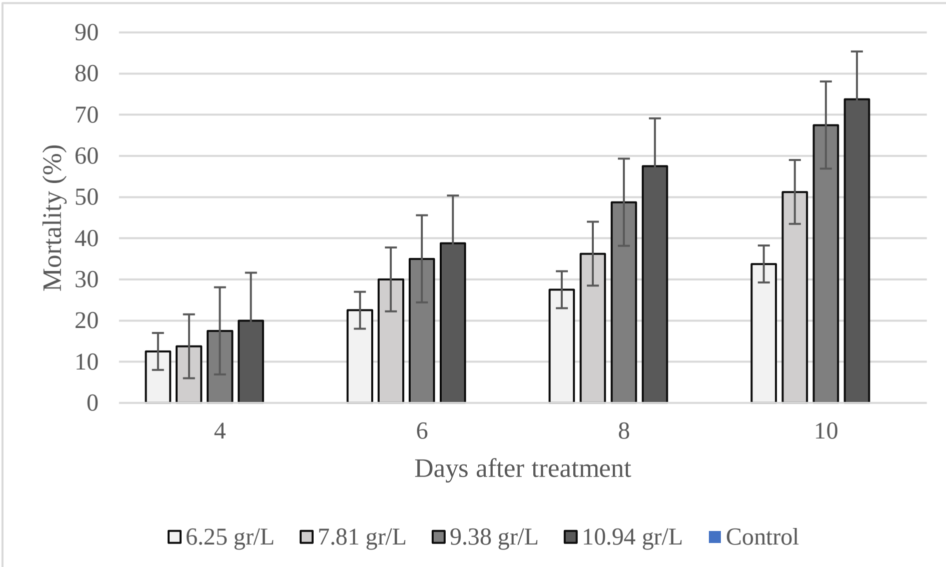

Fig 2. Dried bioproduct against whitefly under laboratory conditions

Figure 2 displays the death rate of whitefly larvae fed dried *Verticillium lecanii* bioproducts in a laboratory at 4, 6, 8, and 10 days following the treatment. It compares the effects of four dried bioproduct concentrations (6.25, 7.81, 9.38, and 10.94 grams per liter) to a solution that was treated with only water. An analysis of the results reveals that mortality increases with time for all fungal treatments, mostly due to higher doses. On day 10, the dose of 10.94 g/L delivered the highest number of deaths, equaling about 75%, while 9.38 g/L was only slightly lower. However, at the lowest level of pesticide (6.25 g/L), there was much less death, which shows how sensitive the results were to the dose. It was clear from the control group that the observed improvements were thanks to the fungal treatment. The results on the graph suggest that dried *V. lecanii* retains effectiveness, while higher concentrations help reduce the number of whiteflies, showing it may have value as a biocontrol agent for pests.

Efficacy of bioproducts against whitefly under greenhouse

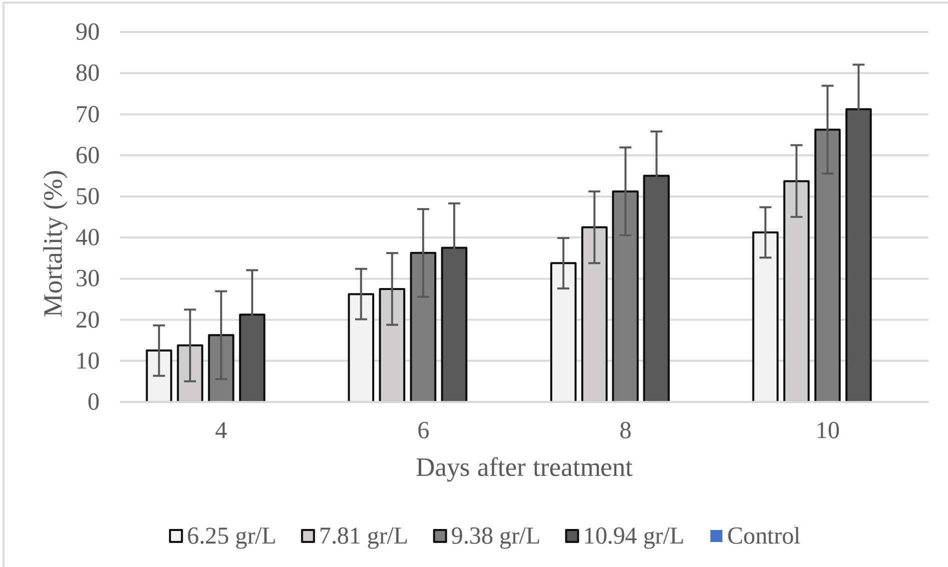

Fig 3. Fresh bioproduct against whitefly under greenhouse

Fresh *Verticillium lecanii* bio-products were used to treat whitefly larvae in greenhouse experiments. Figure 3 shows a comparison of the mortality rates for whitefly larvae at four treatment concentrations (6.25 g/L, 7.81 g/L, 9.38 g/L, and 10.94 g/L) and for the control. Observations were recorded at four, six, eight, and ten days after treatment. The results indicate that higher exposures and longer applications to whiteflies result in increased mortality. All treatment groups exhibited an increase in death rates over time, with the 10.94 grams per liter concentration consistently leading to the highest number of deaths, reaching about 70% on day 10. Compared to the control, both the highest-dose treatment and the middle-dose treatment showed significant increases in death rates, while the lowest-dose treatment resulted in the lowest mortality. Water tank mortality remained minimal throughout the experiment, confirming that the effect was due to *V. lecanii*. The data suggest that using *V. lecanii* at higher concentrations yielded the best results in controlling whitefly larvae in our greenhouse trials. Incorporating this biological tool could be a sustainable approach in integrated pest management.

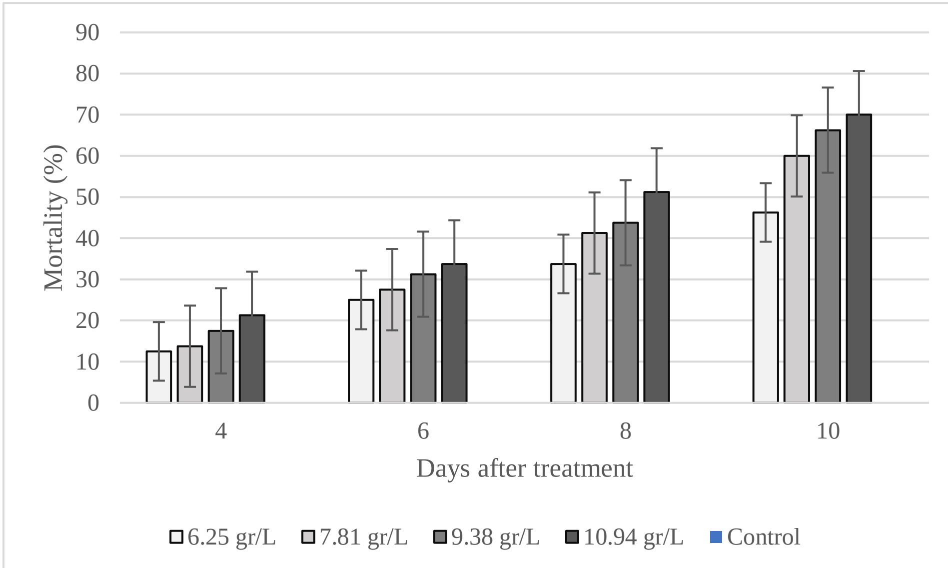

Fig 4. Dried bioproduct against whitefly under greenhouse

In the greenhouse environment, Figure 4 shows the mortality rate of whitefly larvae after using dried *Verticillium lecanii* at four rates: 6.25 g/L, 7.81 g/L, 9.38 g/L, and 10.94 g/L. A treatment with water was also part of the experiment. Mortality was counted at 4, 6, 8, and 10 days following the treatment. Whiteflies died at an increasing rate until day 10, when the most deaths were noted at all concentration levels. Between days 1 and 10, the 10.94 g/L treatment killed more than 70% of the whiteflies, which was equal to or higher than the mortality seen in the 9.38 g/L and 7.81 g/L treatments. Six and a quarter grams of *Verticillium lecanii* in a liter of water led to the lowest level of mortality in the experiment. The lack of mortality in the control group suggested that only the *V. lecanii* bioproduct and not the environment was responsible for the observed whitefly death. The results reveal that using the dried preparation of *V. lecanii* in greenhouses removes whiteflies in amounts that depend on how much is applied. Dried *V. lecanii* appears effective and reliable as a biocontrol agent in sustainable pest management in different agricultural contexts.

The study found that both products made from *Verticillium lecanii*, fresh and dried, reduced the survival of *Bemisia tabaci* larvae in both laboratory and greenhouse conditions. Results showed that as the biopesticide concentration increased, the number of surviving whitefly larvae decreased, with the most significant effect observed at the 10.94 g/L concentration in both forms. After a 10-day observation period, significant differences in mortality rates were observed among the various treatment groups compared to the control, indicating the potent impact of the biopesticide on whitefly populations. Additionally, while both types were effective, the fresh formulation appeared to be more effective in the laboratory setting. These findings highlight the added value of *Verticillium lecanii* in controlling whitefly damage in agribusiness.

Discussion

Managing *Bemisia tabaci* in agriculture is challenging because it reproduces quickly and carries plant viruses. The study assessed the use of *Verticillium lecanii* as a biopesticide against *B. tabaci* and added to the knowledge of biological control. Ongoing studies have indicated that *Verticillium lecanii* can effectively manage populations of whiteflies (Sani et al., 2020). These studies revealed that both types of *Verticillium lecanii* were effective at reducing whitefly numbers in laboratory conditions and inside greenhouses, indicating they can be used for sustainable farming.

Environmental factors have a significant impact on the success of biological control agents (Bhatt et al., 2021). According to Ou et al. (2019), successfully combining parasitoids and entomopathogenic fungi as biocontrol agents enhances joint effectiveness. Other biological agents in the field, such as *Verticillium lecanii*, may exhibit similar effects, warranting further study. Abubakar et al. (2022) further noted that utilizing different management strategies, including biological control, leads to better sustainability in agriculture. This concept is supported by our findings, as mixing applications of *Verticillium lecanii* with eco-friendly methods can better control *B. tabaci* and reduce the use of chemical pesticides.

Researchers also focused on the form of the bioproduct during their research (Islam et al., 2022). Dried *Verticillium lecanii* proved to be as effective as fresh preparations against *B. tabaci*. This finding suggests that managing storage, transporting dry mixes, and conducting operations could be simpler, potentially reducing product costs (Abdulle et al., 2021). Additionally, researchers found that *Verticillium lecanii* has effects on whitefly behavior that could enhance its performance in the field.

Koller et al. (2023) pointed out that using entomopathogens with parasitoids can have several benefits. This partnership can lead to innovative strategies for integrated pest management (IPM) by leveraging the strengths of each type of biological control. Investigating how *Verticillium lecanii* works alongside numerous beneficial insects can result in improved methods for controlling *B. tabaci* in fruit crops. The study demonstrates that *Verticillium lecanii* is effective in controlling *B. tabaci*. The results were consistent for both fresh and dried samples, supporting its use in sustainable agriculture and paving the way for further research on its synergies with other biocontrol methods. The shift towards environmentally friendly farming practices will heavily rely on bioproducts for pest management.

Verticillium lecanii, an entomopathogenic fungus, has shown promising results in controlling the whitefly, *Bemisia tabac*i, in agricultural settings. The study revealed that fresh forms of *V. lecanii* were more effective than dried forms in controlling whiteflies under controlled conditions, providing valuable insights into biological pest control. These findings align with previous research, indicating that fungal biopesticides are preferable alternatives to chemical insecticides.

The way *V. lecanii* kills whiteflies is like how other entomopathogenic fungi work. The work of Javed et al. (2023) highlights that V. lecanii provides elicitor proteins that cause plants to defend themselves against the rice leaf roller. Therefore, using these biopesticides might help combat pests and enhance crops' overall resistance to various threats (Javed et al., 2023). This study's results indicated varying degrees of efficacy between fresh and dried formulations of *V. lecanii*, supporting the findings from Wu et al. (2023), who reported on the persistent effects of *Cordyceps javanica* against *B. tabaci* in field conditions. Researchers noted that both the environment and the type of formulation played a significant role in how fungal biocontrol agents functioned. Since the results of our experiment show that dried fungi are more effective, further reviews of the production and application strategies used by other fungi are needed (Wu et al., 2023). Notably, using *Purpureocillium lilacinum*, another entomopathogenic fungus, also controls pest populations, especially chili thrips (Panyasiri et al., 2022). The study confirms that it is possible to use fungal biopesticides to better combat agricultural pests, preserve important crops, and reduce the need for chemical pesticides.

It is suggested by such interactions that we can explore many strategies for using these fungi against pests. learning about the relationship’s fungi form with their plant hosts may lead to better biocontrol and ways to improve plant growth. By using such insights, we can link crop protection to sustainable farming. Sustainable and integrated pest management plans are increasingly involving the study of fungi-based bioproducts. The proposed work on *Verticillium lecanii* shows it is an effective agent against the whitefly, *Bemisia tabaci*, in line with the increasing number of research studies listing entomopathogenic fungi as useful in farming. Through this study, the researchers contribute to knowledge that proves that diverse fungi have the potential to be strong biological control agents for pest management.

Studies indicate that several types of insect-killing fungi can reduce the numbers of invasive pests (Dhanapal et al., 2020). Sujithra et al. (2021) showed that the entomopathogenic fungus *Simplicillium lanosoniveum* can be effective against the invasive whitefly *Aleurodicus rugioperculatus* in coconut plantations. This goes along with our study, showing that entomopathogenic fungi are effective in agriculture, and reaching out further helps find native strains useful for different crops and problems with insect pests (Sujithra et al., 2021).

Pest control alone does not capture the true value of fungi in agroecosystems. In his book, Ruiu (2018) details how microbial biopesticides help the soil, introduce various organisms, and strengthen the livelihood of ecosystems. Such a view is in line with One Health, which points out how human, animal, and environmental health are all closely related. This means adding these fungal bioproducts to farming could be a way to benefit the environment and deal with pest problems.

Additionally, discovering that *Lecanicillium muscarium* can cope with pesticides helps shed light on the role of entomopathogenic fungi in agriculture. Accepting this tolerance can reduce conflicts between biocontrol strategies and chemicals, creating the chance to form better teamed ways of fighting pests. This is significant because farmers hope to control pests successfully without decreasing the effectiveness of natural control agents and slowing down resistance from pests. The interface between entomopathogenic fungi and their environments can be better described through studies analyzing multitrophic relations. González-Mas et al. (2019) describe how *Beauveria bassiana* fungus plausibly affects the interaction between the cotton aphid and its natural enemies, indicating that pest management is complicated. Reviewing these studies makes it clear that knowing how these relationships work improves biocontrol methods. Sharma et al. (2023) confirms the relevance of entomopathogenic fungi in sustainable agriculture after reviewing their many benefits. According to the authors, researching these biological agents in detail will help scientists improve ways to control pests.

The analysis reveals several issues that need to be considered. The research was based on experiments conducted in controlled laboratories and greenhouses, but factors such as rain, high temperatures, moisture levels, and other environmental changes in real agricultural situations can significantly impact the effectiveness of *Verticillium lecanii* on whiteflies. Additionally, since the research only examined a few concentrations and tested only the second-instar larvae of Bemisia tabaci, the overall contribution of this method to pest control was not fully understood. Moreover, as the trial lasted only a brief period, it is uncertain how long the biopesticide may pose problems for non-target organisms.

In the future, studies could address these limitations by cultivating the bioproduct in real-world settings and assessing its efficacy. Researching different materials for growing *Verticillium lecanii* and investigating its impact on all stages of whiteflies could enhance our understanding of its potential for pest control. Combining various biological or chemical treatments may also improve our approach to managing pest populations. Furthermore, examining how the biopesticide may affect other organisms and understanding its mechanism of action in causing whitefly mortality would contribute to the development of sustainable pest control techniques.

In conclusion, the findings of our study align with the broader trends in pest management research, underscoring the potential of *Verticillium lecanii* and other entomopathogenic fungi as effective biocontrol agents. Future research should focus on optimizing the application and formulation of these fungi, investigating their ecological interactions, and exploring their integration into sustainable agricultural practices.

Conclusion

The study finds that *Verticillium lecanii* is a promising biopesticide for controlling the whitefly *Bemisia tabaci* in vegetables. Laboratory and greenhouse experiments with fresh and dried *V. lecanii* formulations revealed that the number of surviving *B. tabaci* larvae decreased as the concentration increased. Its consistent success shows that the fungus reliably controls molds. *V. lecanii* is valued for its beneficial properties. The use of this biopesticide instead of chemicals helps protect the environment and beneficial insects from harm. Since *V. lecanii* can be formulated differently, it offers more opportunities to be integrated into and combined with current pest control strategies on farms. Furthermore, the research confirms that entomopathogenic fungi are becoming increasingly important in integrated pest management (IPM). As more people become concerned about the impacts of pesticides on environmental health, using *V. lecanii* to target resistant pest populations like *B. tabaci* can be highly effective. This research highlights the need for further studies on optimal application rates, persistence of effectiveness, and scalability of fungal biocontrol methods. Overall, this research demonstrates that *Verticillium lecanii* is an effective biopesticide against vegetable pests and contributes to promoting sustainable agriculture. The findings provide a solid foundation for future research and the adoption of fungal biocontrol methods, supporting efforts to manage pests in environmentally responsible ways.

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