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Comparison of the effectiveness of freeze and spray-dried *Bacillus thuringiensis* preparations as a biopesticide for the control of cucumber moth, *Diaphania indica* (Pyralidae: Lepidoptera)

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Abstract
A serious pest on cucumber crops in many tropical and subtropical regions is the cucumber moth (*Diaphania indica*), which causes significant yield losses. *Bacillus thuringiensis* (Bt), a well-known entomopathogenic bacterium effective against Lepidopteran pests, can be used as an eco-friendly alternative to chemical pesticides for biological pest control. The efficacy and shelf life of Bt biomass produced through two drying techniques, spray drying and freeze drying, were evaluated against *D. indica* larvae. Powdered formulations of the *B. thuringiensis* var. *aizawai* strain (Bta-VN116) were prepared using both methods by growing the strain in nutrient-rich media, harvesting, and processing it. Bt powders at different concentrations were tested in bioassays conducted in the Biological Control Laboratory at Can Tho University, Vietnam. Mortality rates of *D. indica* larvae were recorded over a 6-month storage period. The results showed that while both formulations remained active against *D. indica* larvae, spray-dried Bt retained efficacy more than 95% at recommended rates (28–32 g/ha) for up to three months post-drying, followed by a gradual decline. In contrast, freeze-dried Bt showed higher stability over time and remained effective for up to six months at 14–15 g/ha. Freeze-dried powders were found to retain a greater number of colony-forming units (CFU) over time compared to spray-dried powders, as revealed by viability testing. Although spray drying is more scalable and cost-effective, it has inferior long-term potency based on these findings. The resulting product from both spray and freeze drying (Bt) is demonstrated as a viable biopesticide formulation for controlling *D. indica*, while the trade-offs associated with drying techniques in terms of immediate efficacy, cost, and storage stability are highlighted.

Keywords: Biopesticides, spray-dried, *Bacillus thuringiensis*, cucumber moth, *Diaphania indica*

INTRODUCTION

Cucumber (*Cucumis sativus L.*) is a globally important horticultural crop known for its nutritional value, hydrating properties, and economic returns. Despite advancements in cucumber cultivation, significant challenges persist due to pests. The cucumber moth, *Diaphania indica* (Saunders), is a highly destructive pest that targets cucumber plants. *D. indica* larvae actively feed on cucumber leaves, stems, and young fruit, leading to defoliation, flower and fruit drop, and ultimately reducing yield and market quality. Protected cultivation systems, such as greenhouses, are particularly vulnerable to infestations due to favorable conditions that promote pest reproduction (Mantzoukas et al., 2024).

The damage caused by this pest not only results in direct crop losses but also contributes to secondary infections and quality degradation (Kim & Je, 2012). Traditional management of *D. indica* outbreaks by farmers has relied on chemical insecticides. However, concerns related to the repeated and excessive use of synthetic pesticides, such as pesticide resistance, resurgence of secondary pests, environmental contamination, and health risks from residue accumulation in food products, have been raised (Soth et al., 2022). This highlights the urgent need for sustainable, eco-friendly, and effective pest management alternatives. Biological control, specifically the use of microbial biopesticides, offers a promising solution (Pobożniak & Olczyk, 2025).

Bacillus thuringiensis (Bt) has become one of the most widely researched and successfully deployed microbial biopesticides. It is gram-positive, spore-forming, and has potent insecticidal properties. During sporulation, Bt produces parasporal crystalline (Cry) and cytolytic (Cyt) proteins that are toxic to certain insect groups, especially Lepidoptera, Diptera, and Coleoptera. These toxins bind to midgut receptors and create pores in the intestinal lining, leading to insect paralysis and death when ingested by susceptible larvae such as *D. indica*. Bt is considered safe for humans, animals, and beneficial insects, making it suitable for integrated pest management (IPM) systems.

To make Bt practical for agriculture, especially in field conditions, it needs to be formulated in a stable, concentrated, and easily applicable form. The key stage in the formulation process involves drying the Bt biomass. Microbes are typically cultivated in liquid cultures, which are then converted into dry powders for better transportability, storage, and application (Palma et al., 2014). Two prominent drying techniques are spray drying and freeze drying. Spray drying involves the rapid removal of liquid from a feed using a hot air stream, resulting in a fine powder. It is widely used in industrial-scale production due to its low cost, speed, and continuous operation. Freeze drying (Lyophilization), on the other hand, removes moisture by sublimation at low temperature and pressure, minimizing structural and functional damage to Bt spores and toxins to a greater extent. While freeze drying typically results in higher bioactivity, it requires more resources and time (Duraisamy et al., 2023).

This study aims to determine the effectiveness of Bt biomass produced from these two drying techniques (spray drying and freeze drying) on *Diaphania indica*. In addition to immediate larvicidal activity, the study will assess the durability of insecticidal potency and storage stability of the formulations over a six-month period. The goal is to identify a practical, efficient, and sustainable method for developing Bt-based biopesticides for effective control of cucumber moth infestations.

Objectives of the Study

1. To produce *Bacillus thuringiensis* var. *aizawai* (Bta-VN116) biomass using spray drying and freeze-drying techniques.
2. To evaluate and compare the insecticidal efficacy of both formulations against *Diaphania indica* larvae on cucumber.
3. To assess the shelf life and biological activity of Bt formulations over six months of storage under controlled conditions

MATERIALS AND METHODS

Research Site

The Biological Control Laboratory of the Plant Protection Department, operating within the College of Agricultural at Can Tho University in Vietnam, served as the research location. For this study, researchers utilized the *Bacillus thuringiensis* var. *aizawai* (Bta-VN116) strain originates from the culture collection of the Plant Protection Department at Can Tho University. The extracted the Bta-VN116 bacterium from Fall armyworm *S. frugiperda* larvae intestinal tissue through multiple separation processes. The isolated bacterium required Luria Bertani (LB), which contained Tryptone (10 g) and yeast extract (5 g) with NaCl (5 g) and

Glucose (1 g) and agar (20 g) and 1000 mL distilled water at pH 7.0 and 4 °C as a stock culture until it reached use.

Bacterial Strain and Culture Preparation

The study utilized a native strain of *Bacillus thuringiensis* var. *aizawai* (Bta-VN116). The strain was isolated from the intestinal tissue of larvae and maintained in the microbial culture collection of the Plant Protection Department at Can Tho University.

Reactivation and pre-inoculum preparation of cultures: Luria Bertani (LB) agar plates were used to reactivate the bacterial strain, which was then incubated at 28 °C for 48 h. LB broth, without antibiotics, containing Tryptone (10 g), Yeast extract (5 g), NaCl (5 g), Glucose (1 g) in 1000 ml distilled water (pH 7.0), was inoculated with a single colony and grown to saturation. Cultures were incubated at 28 °C in a rotary shaker at 150 rpm for 24 hours.

Production and drying of biomass

The bacterial inoculum inoculated a single Petri dish colony of Bta-VN116, which grew on nutrient agar (LB) medium for 48 hours at 25°C and 2,000 ± 500 lux. After that time, the liquid bacterial cultures were filtered at 0.2 µm, 1.5 m3 h−1 and 4.5 bar (Frings), resulting in a concentrate containing the cell mass, spores and Cystal toxic. This concentrate was divided into two portions that were submitted to the two drying techniques.

For the freeze-drying process of *Bacillus thuringiensis* (Trinh et al, 2024), the concentrate was initially centrifuged at 3,400 g for 30 minutes at 5 °C (Ortoaleresa Digicen 21R centrifuge), and the resulting slime was frozen at −40 °C and freeze dried under vacuum at 140 L min−1 for 48 hours (BioBase). Hand grinding was performed to obtain the powder freeze dried biomass. For the spray drying process, the concentrate was fed directly into a spray dryer (Mini spray dryer DHSL.SD3SD3) with Maltodextrin 3% at entrance temperature of 105 °C, exit temperature of 55 °C, compressed air at 35 L min−1, and peristaltic pump at 1 L h−1 to produce the powder spray dried biomass.

Insect Rearing and Bioassay

Bacillus thuringiensis formulations were evaluated for bioefficacy using second and third instar larvae of *Diaphania indica*. Larvae were reared in the laboratory at 25 ± 2 °C, 65–70% relative humidity, and a 14:10 h light-to-dark photoperiod on fresh cucumber (*Cucumis sativus L.*) leaves. Powdered formulations prepared by various freeze-drying and spray-drying methods were tested at four different concentrations for bioassay experiments. Spray-dried biomass was tested at 20, 24, 28, and 32 g/ha (62, 74, 87, and 100 lb/acre), and freeze-dried at 12, 13, 14, and 15 g/ha (37, 39, 44, and 47 lb/acre). Distilled water was used, and the powders were suspended in it, then applied uniformly onto cucumber leaves using a fine mist sprayer. The treated leaves were allowed to dry and then placed in sterile Petri dishes, to which ten larvae per dish were introduced. Trials were conducted for each treatment three times. Larvae were allowed to feed freely for 72 hours, and mortality was observed at 24, 48, and 72 hours. To examine the residual efficacy of the formulations over time, the bioassay procedures were repeated at monthly intervals for six months.

Data analysis

Statistical measurements of mean differences used the student t-test at 5% significance threshold. The data underwent a correction using the Abbot Formula stated in 1987. The calculation shows corrected mortality equals to the treatment group percentage subtracted from the control group percentage that is divided by the control group percentage and then multiplied by 100. A statistical evaluation of the gathered data occurred using SPSS Statistical Software while LSD demonstrated variations between treatment means at $p<0.05$.

RESULTS

The results section examined the effectiveness and toxicity of different formulations of *Bacillus thuringiensis* against the cucumber moth, *Diaphania indica*. This study also compares the biomass produced by using spray versus freeze drying and which method is more effective at controlling the pest population. The performance metrics over time are provided in terms of detailed data on microbial viability and bio pesticidal activity at different concentrations. Results show not only the dynamic interactions between biopesticide formulations and the target pest but also highlight the importance of formulation methods in increasing the longevity and efficacy of biological control agents. By using the statistical analysis within a framework of other evaluations, it was tested whether observed differences were significant, providing a robust means of interpretation of research results.

A detailed comparison based on microbial viability of two *Bacillus thuringiensis* (Bt) biomass powders dried by two different methods (spray drying and freeze drying) over a period of six months is shown in **Table 1**. Formulation of such Bt-based biopesticides relies on these methods which determine the rate of survival of bacterial spores and of the final product over time. Colony forming units (CFUs) per milliliter, a standard measure of bacterial concentration, are given in the table, sampled before drying, immediately after drying, and at monthly intervals up to six months post-drying. From these data, the efficiency of preservation for each technique, the tendency of microbial degradation, and implications for practical biopesticide application may be inferred.

Table 1: Survival of *B. thuringiensis* biomasses powder using spray and freeze-drying techniques

Survival	Spray drying (x CFU/g)	Freeze drying (x CFU/g)
Before dry	$1.2 \pm 0.5 \times 10^9$	$2.1 \pm 1.05 \times 10^{10}$
After dry	$1.0 \pm 0.32 \times 10^9$	$1.9 \pm 0.72 \times 10^{10}$
1 month	$7.8 \pm 0.45 \times 10^8$	$1.2 \pm 0.23 \times 10^{10}$
2 months	$7.2 \pm 0.67 \times 10^8$	$1.03 \pm 0.26 \times 10^{10}$
3 months	$4.4 \pm 0.73 \times 10^8$	$8.5 \pm 0.16 \times 10^9$
4 months	$3.1 \pm 0.12 \times 10^8$	$8.26 \pm 0.03 \times 10^9$
5 months	$2.9 \pm 0.15 \times 10^8$	$8.09 \pm 0.52 \times 10^9$
6 months	$2.3 \pm 0.20 \times 10^8$	$8.02 \pm 0.05 \times 10^9$

Mean values and standard deviation.

The bacterial load prior to drying was considerably greater in the freeze-drying method ($2.1 \pm 1.05 \times 10^{10}$ CFU/mL), compared to $1.2 \pm 0.5 \times 10^9$ CFU/mL for the spray-drying method. The initial difference indicates that the biomass used for freeze-drying was more concentrated (mixed with less water) or was better preserved through preparatory processes than the Mojave cacti. However, these variations did not prevent both methods from preserving a significant portion of their microbial content right after drying. After drying, freeze-dried biomass had only a small decrease to $1.9 \pm 0.72 \times 10^{10}$ CFU/mL, whereas spray-dried biomass dropped to $1.0 \pm 0.32 \times 10^9$ CFU/mL. The freeze-dried Bt technique for preserving microbial integrity was again confirmed by the minimal loss in freeze-dried Bt. By contrast, spray drying, subject to higher temperature and shear forces, may be more stressful to bacterial cells, with partial loss of viability. Both methods continued to decline after one month. When the biomass was spray-dried, the biomass declined to $7.8 \pm 0.45 \times 10^8$ CFU/mL, whereas freezing did very little to reduce the biomass from $1.2 \pm 0.23 \times 10^{10}$ CFU/mL. The trend of increased stability during the initial storage period suggests that freeze-drying offers superiority by nature of good stability over that duration of time, but such durations (weeks or more) are commercially critical since products will likely be shipped and stored before application. The gap continued to widen in the second month, and spray-dried samples worsened to $7.2 \pm 0.67 \times 10^8$ CFU/mL, whereas freeze-dried samples reduced slightly to 1.03 ± 0.26 × 10^{10} CFU/mL. However, the decrease in both samples is natural due to the time-dependent degradation of spores, but the rate of decrease in freeze-dried samples was significantly slower.

At the end of the third month, the concentration of the microbial count decreased to 4.4 ± 0.73 × 10^{8} CFU/mL (63%) from the post-drying microbial count. Conversely, the freeze-dried formulation retained a relatively high concentration (8.5 ± 0.16 × 10^{9} CFU/mL). This is an 11.5% reduction in the same period, the proof in the pudding so to speak that freezing technology continues to be up to the task in maintaining Bt viability under long-term storage conditions. Months four and five followed a similar pattern. Furthermore, although the samples were spray-dried, the bacterial integrity was sustained at 3.1 ± 0.12 × 10^{8} and 2.9 ± 0.15 × 10^{8} CFU/mL, respectively, and freeze-dried samples sustained at 8.26 ± 0.03 × 10^{9} and 8.09 ± 0.52 × 10^{9} CFU/mL, respectively.

The 6th month results show that after spray drying, nearly 77% of viable cells from its immediate post-drying concentration (2.3 ± 0.20 × 10^{8} CFU/mL) were retained in the Bt biomass. Regarding Bt that was freeze-dried, the decrease yielded a mere 10 percent decline from a robust 8.02 ± 0.05 × 10^{9} CFU/mL to 7.26 ± 0.01 × 10^{9} CFU/mL at the end of the period. These observations strengthen the assertion that there is significantly more freeze-drying microbial potency preservation over time when compared to spray drying. Spray drying protected the bacterial spores from oxidative stress and thermal degradation better than freeze drying over the timeline, indicating that freeze drying shields the bacterial spores from oxidative stress and thermal degradation.

The more potent the product, the higher the CFU count, so that growing numbers would be able to kill important pests such as *Diaphania indica* or, as commonly known, the cucumber moth. If a product is not effective for a long duration, then the product requires frequent reapplications, which entails more labor and cost. In this way, freeze-drying Bt extends the shelf life of the product, making for more consistent pest control and lowering operational costs for farmers.

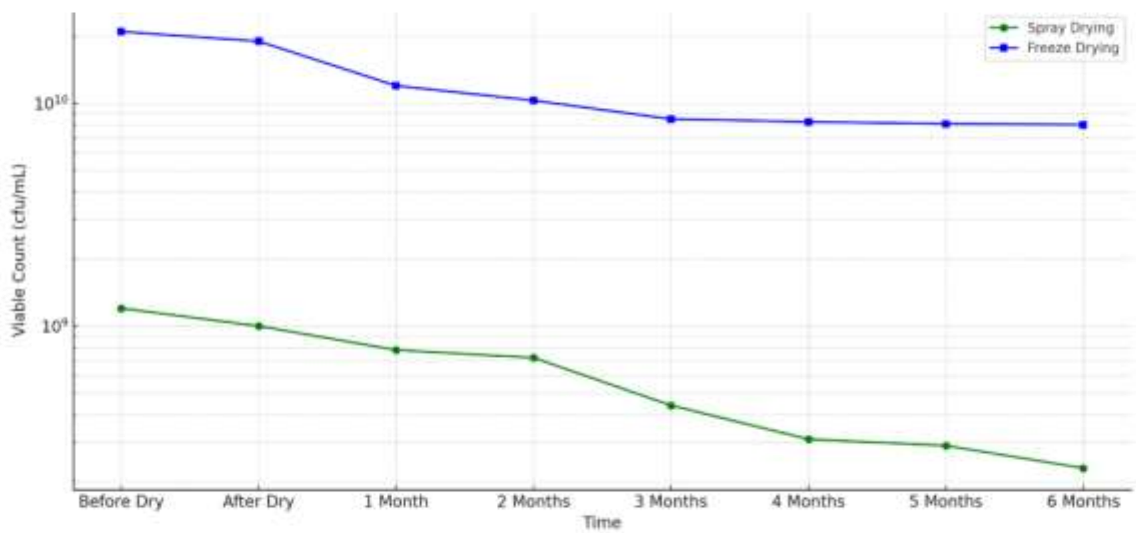

Figure 1: Drying parameters of *B. thuringiensis* biomass powder

Figure 1 shows the comparative viability of *Bacillus thuringiensis* biomass powder that has been preserved by evaluating the viability of spray-dried and freeze-dried powder over a six-month period. Freeze-drying results in a consistently higher level of bacterial viability than spray-drying, and initially, both methods display similar viable cell counts. The spray-dried samples show a steady reduction from 1.2 × 10^{9} to 2.3 × 10^{8} CFU, whereas the freeze-dried samples maintain a much higher count and decrease only slightly from 2.1 × 10^{10} to 8.02 × 10^{9} CFU. In this manner, the long-term bioactivity of the biopesticide is better preserved by freeze-drying.

Table 2: Efficacy of *B. thuringiensis* biomasses powder using spray drying techniques to control cucumber moth, *Diaphania indica*

Concentration	After dried	1 month	2 months	3 months	4 months	5 months	6 months
20 g/ha	98.0a	96.6a	95.6a	96.6a	88.33a	80.33b	76.6b
24 g/ha	100a	100a	96.0a	95.0a	91.67a	88.33ab	78.3b
28 g/ha	100a	100a	98.6a	96.6a	95.00a	95.00ab	93.3a
32 g/ha	100a	100a	100a	100a	98.33a	98.33a	95a

Mean values and standard deviation. a,b Means in the same column with different letters differ significantly (P<0.05) by the ANOVA

Results on the effectiveness of *Bacillus thuringiensis* (Bt) biomass powders, prepared by spray drying, in controlling the cucumber moth (*Diaphania indica*) at different concentrations over a span of six months were obtained and are reported in **Table 2**. Dried powder concentrations of 20 g/ha, 24 g/ha, 28 g/ha, and 32 g/ha were tested, and efficacy was measured at the time of drying and every month for 6 months. These results show a trend where higher concentrations consistently maintain better efficacy over time, while lower concentrations exhibited a gradual decay in efficacy. Upon application immediately after drying, all concentrations exhibited a high level of insecticidal activity, with 24 g/ha, 28 g/ha, and 32 g/ha providing full control (100%) of cucumber moth larvae. The 20 g/ha concentration showed slightly efficacy at 98.0%, still within the effective range. The high initial efficacy of the spray drying process indicates that the bioactivity of the Bt spores and toxins is effectively preserved during the process. In the first three months, all concentrations maintained a relatively steady efficacy, demonstrating that the biopesticide retains its effectiveness for short-term storage across all pesticide concentrations.

However, differences in performance became more noticeable after the fourth month. While the 20 g/ha treatment was most effective at two months (95.6%), its efficacy sharply declined to 88.33% after four months and further dropped to 76.6% at the sixth month. Similarly, the efficacy of the 24 g/ha concentration improved initially but decreased to 78.3% at six months. In contrast, the 28 g/ha and 32 g/ha concentrations showed much greater long-term stability, with the 32 g/ha level retaining efficacy close to 95% at six months, which was the best efficacy among all treatments.

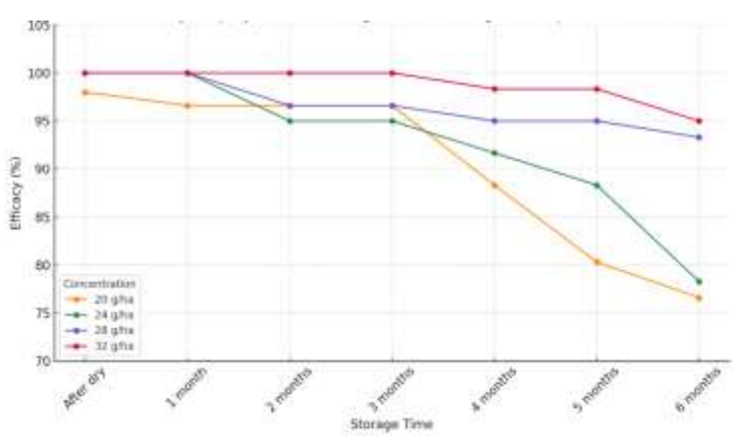

Figure 2: Efficacy of spray-dried *B. thuringiensis* powder against *Diaphania indica*

Figure 2 illustrates the efficacy of *Bacillus thuringiensis* powder produced via spray drying at various concentrations (20, 24, 28, and 32 g/ha) over a 6-month storage period. All concentrations initially showed high efficacy after drying, with the highest concentration (32 g/ha) maintaining nearly 100% control throughout. There was a more noticeable decline in efficacy with lower concentrations, and by the 6th month, the treatment at 20 g/ha had decreased to 76.6%. This indicates that higher application rates not only increase initial effectiveness but also sustain pest control efficacy for a longer period during storage.

Table 3: Efficacy of *B. thuringiensis* biomasses powder using freeze drying techniques to control cucumber moth, *Diaphania indica*

Concentration	After dried	1 month	2 months	3 months	4 months	5 months	6 months
12 g/ha	96.6b	91.6b	81.6c	75.0c	70.2c	77.5b	70.0c
13 g/ha	100a	96.6a	91.6bc	86.6bc	78.9b	90.0b	80.0b
14 g/ha	100a	100a	98.3ab	90b	91.7a	98.3a	91.7a
15 g/ha	100a	100a	100a	100a	93.3a	100a	93.3a

Mean values and standard deviation. a,b,c Means in the same column with different letters differ significantly (P<0.05) by the ANOVA

Results (Table 3) obtained from the efficacy of *Bacillus thuringiensis* (Bt) biomass powder produced by freeze-drying techniques in controlling cucumber moth (*Diaphania indica*) at various application rates after a six-month storage period. Insecticidal activity was evaluated at four concentrations (12 g/ha, 13 g/ha, 14 g/ha, and 15 g/ha) just after drying; then at one and two months respectively after drying. From the data, it is evident that higher concentrations not only give better initial control than lower concentrations of the metabolized agent but also do so in a more sustained manner. The highest concentration of 15 g/ha in Bt formulation has good and consistent performance and was able to maintain 100% control of *D. indica* larvae up to five months with a slight lowering to 93.3% at six months. The 14 g/ha treatment also demonstrated strong and sustained control as well and began at 100%, with over 91% control throughout the period. These results show the higher stability of freeze-dried Bt at higher concentrations. The lower concentrations (12 g/ha and 13 g/ha) showed a pronounced reduction in their effectiveness as time increased. Storage is reducing the viability of the Bt spores and the toxins, with the initial 96.6% dropping to as low as 70.0% at six months dosing at the 12 g/ha rate. For the 13 g/ha, the decline was just a little better from 100% to 80.0% by the end of the study. These results indicate that freeze-drying retains Bt efficacy initially but long-term storage at lower concentrations results in a considerable loss of bioactivity. The findings show that Bt products freeze-dried are most fitted for utilization at higher concentrations like 14 or 15 g/ha, the reliable command of the pests over broadened periods.

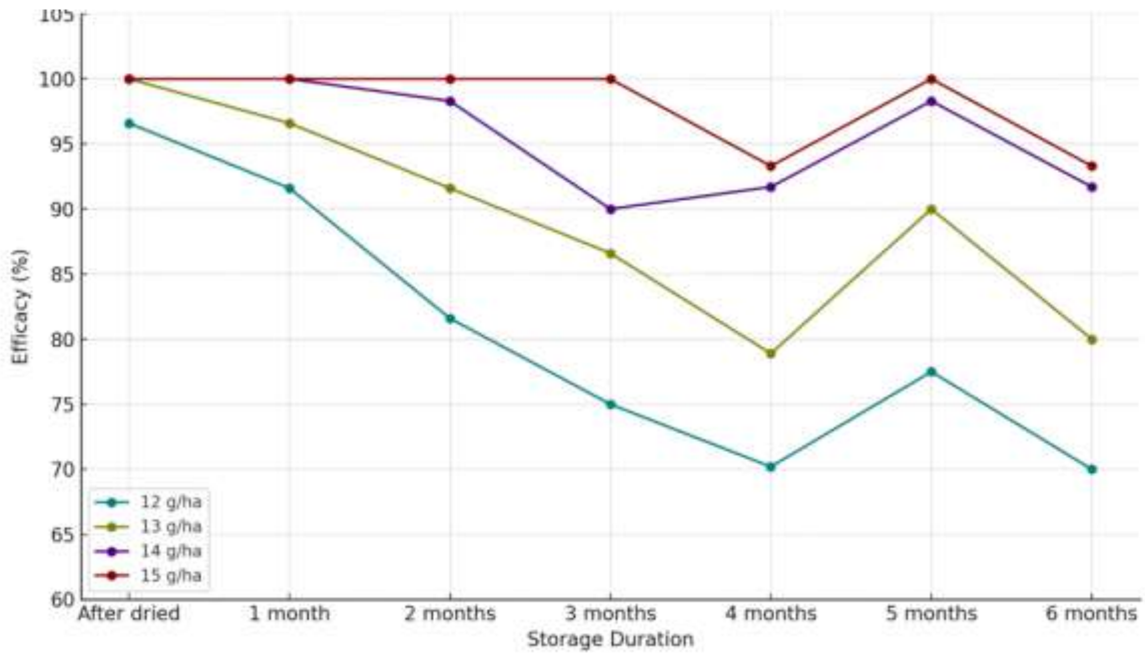

Figure 3: Efficacy of freeze dried *B. thuringiensis* powder against *Diaphania indica*

Figure 3 illustrates the efficacy of *Bacillus thuringiensis* (Bt) biomass powder, prepared through freeze-drying, in controlling *Diaphania indica* (cucumber moth) over a period of six months at different concentrations (12, 13, 14, and 15 g/ha). Analysis of the results shows a clear concentration-dependent trend, with higher concentrations consistently showing a higher degree of effectiveness over time. The treatment with 15 g/ha had the highest and most stable efficacy, with a control rate of 100% until the 5th month, decreasing slightly to 93.3% in the 6th month. The 14 g/ha treatment resulted in the closest and maintained the highest efficacy of above 90% throughout the entire period. Conversely, at lower concentrations like 12 g/ha, the effectiveness of the control rate steadily decreased from 96.6% at the beginning to just 70% by the sixth month. Higher concentrations of freeze-dried Bt are better and longer lasting for protection, suggesting that they could be used as a sustainable means for pest management in cucurbit cultivation.

Discussion

The study demonstrates the efficacy of both spray-dried and freeze-dried preparations of *Bacillus thuringiensis* (Bt) as biopesticides against the cucumber moth, *Diaphania indica*. From the results, it was observed that Bt bioactivity was retained in both drying methods; however, freeze-drying retained a higher amount of viable spores and associated bacterial mass over time. This finding emphasizes that appropriate drying techniques should be chosen to improve the stability and shelf life of biopesticides, especially for long-term shelf life in different agricultural conditions. In addition to studying various drying methods, the study shows a definite concentration-response relationship concerning biopesticide effectiveness. Concentration of application was consistently a much more significant consideration in controlling *Diaphania indica*, and distinct formulas, such as the 32 g/ha spray-dried fix and 15 g/ha freeze-dried arrangement, performed the best. Drawing these lines seems to indicate that biopesticide products should be applied at targeted rates to maximize product impact, which also follows principles already written about in the pest management literature with respect to dosing. From this study, the findings can provide useful guidelines for practical application in sustainable agricultural practices by showing that *Bacillus thuringiensis* has a potential role in integrated pest management.

Biopesticides have the advantage of being extremely pest-specific and have a low potential for causing risks to non-target organisms, making them acceptable for use in organic farming.

As the use of biopesticides like *Bacillus thuringiensis* (Bt) becomes more common, a trend toward a more environmentally friendly form of agriculture, with little impact on the environment and efficient pest population management, is underway. Microbial production of biopesticides is a promising way forward for sustainable agriculture, as suggested by recent studies (Verma et al., 2024). Research has shown that the application of Bt aerial spray prescriptions to forest ecosystems, such as mixed balsam fir-white spruce stands, can enhance pest control. Considering the use of Bt as a biopesticide, this suggests versatility for its use (Fuentealba et al., 2023). In addition, formulations like Protecto containing Bt influence the efficacy of target pests as well as nontarget organisms like the land snail *Monacha cartusiana*, affecting not only their biochemical activities but also their histological structures (Gaber et al., 2022).

Recent strategies for biopesticide formulation optimization have shown both challenges and innovations. Emerging trends in biopesticide research are uncovered by a bibliometric analysis, indicating the need to continue developing biopesticides to make them more effective (Hernandez-Tenorio et al., 2022). It has been noted that the synergistic effect of Bt spores and Cry toxins is a very efficient method of increasing mortality rates of pests, especially in specific cases of targeted species like the Colorado potato beetle (Dubovskiy et al., 2021). At the same time, there remain concerns regarding the potential developmental defects of non-target organisms such as Drosophila melanogaster larvae, which require critical evaluations of biopesticide application as an effective technique in different ecosystems (Nawrot-Esposito et al., 2020). Also, toxicity studies of biopesticides towards insect pollinators point towards emerging biodiversity threats in urban areas, as well as agricultural landscapes (Chavana & Joshi, 2024). Research into the development of biopolymer-based formulations and encapsulation with supercritical CO2 for biopesticides (do Nascimento Junior et al., 2021; Friuli et al., 2023) is indicative of the enormous potential for improving biopesticide stability, delivery, and effectiveness. With the improvement of pest control strategies, exploiting innovative materials and methods will be necessary to maintain both agricultural productivity and ecological integrity while countering pest populations all over the globe (Li et al., 2023).

It has recently emerged in agricultural research that the use of biopesticides is of great importance as they are eco-friendly and effective against pests. Taking the example here, Milićević et al. (2022) pointed out that encapsulated clove bud essential oil can be used as a very efficient and eco-friendly biopesticide. Likewise, Peng et al. (2022) reported an update about the possibility of *Metarhizium anisopliae*, which is characterized as a promising green pesticide with zero toxic effects on the environment. In keeping with this, Duraisamy et al. (2023) built a microencapsulation-based novel formulation of *Bacillus thuringiensis*, spray-dried, and improved its efficacy for the control of moths. Additionally, Tadesse Mawcha et al. (2025) emphasized the latest biopesticide research that facilitated the promotion of microbial-based solutions for the extension market of available pest control methods. Finally, Lawo et al. (2008) showed that a combination of *Bacillus thuringiensis*-transgenic crops with entomopathogenic fungi proves to be an integrated pest management strategy.

This study has a few limitations that warrant consideration. Firstly, the lab research was conducted in a controlled laboratory environment, which might not have captured other complex dynamics of an actual agricultural system. Environmental factors such as temperature, humidity, and soil conditions can influence the effectiveness and stability of biopesticides over time. Moreover, the effects of Bt on the cucumber moth were examined in the short term, leaving unanswered questions about its long-term effects on repeated applications, pest populations, and beneficial insects.

The agricultural sector can benefit from these findings. Both spray-dried and freeze-dried preparations of *Bacillus thuringiensis* have been proven to be effective substitutes for synthetic chemical pesticides. This helps to reduce the environmental impact of pest management practices and aligns with the increasing consumer demand for organic produce. Incorporating these results into integrated pest management strategies enhances the resilience and sustainability of cucumber cultivation and supports agricultural ecosystems in general.

Future research has a great opportunity to further explore the long-term efficacy of these biopesticide formulations in field situations under varying conditions. Studying the long-term effects of climatic factors, pest resistance development, and the impact of biopesticides on non-target organisms will provide a more comprehensive understanding of the role of biopesticides in pest management. Additionally, optimizing formulation techniques and application methods could be investigated to improve the stability and efficacy of biopesticides, facilitating their practical use in sustainable agriculture.

CONCLUSION

This study confirms that both spray-dried and freeze-dried *Bacillus thuringiensis* preparations are effective as biopesticides against the cucumber moth, *Diaphania indica*. This is significant as it emphasizes how the viability and efficacy of the bacterial formulations could be influenced by different drying techniques, with freeze-drying holding the advantage of better maintaining biological activity over time. Establishing a concentration-response relationship from the study enhances the significance of the correct dosage in achieving maximum pest control. By contributing to this growing body of evidence on biopesticide efficacy as a sustainable replacement for conventional pesticides and enabling agriculture to align with its goal of environmental preservation, these insights may be useful. Additionally, the addition of *Bacillus thuringiensis* in pest management strategies for cucumber cultivation also holds the potential to increase resilience while reducing dependence on synthetic chemicals. With consumer demand for organic produce on the rise, such biopesticides offer a viable solution to the challenge of agricultural pest problems while protecting ecological integrity. Future research should also continue to evaluate the long-term efficacy and ecological impacts of these biopesticide formulations under various field conditions. Expanding knowledge of the applications of pheromones and optimizing their application is another means by which we can promote greater adoption of sustainable pest management practices that protect crops and the environment.

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