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Sustainable Production and Application of Bt Biopesticides

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Abstract *Bacillus thuringiensis* (Bt) is a bacterium widely used in biological control of pests. Bt biopesticides are considered important tools for combating agricultural pests due to their ability to produce crystalline proteins with insecticidal activity. Compared with traditional chemical pesticides, Bt biopesticides have higher specificity and are mainly targeted at specific pest groups, while having less impact on humans and other non-target organisms. The widespread use of chemical pesticides has not only led to the increase of pest resistance, but also caused serious impacts on the environment and human health. Therefore, the development and optimization of Bt biopesticides can not only reduce the use of chemicals, but also help maintain ecological balance and improve the quality and yield of crops. This study comprehensively analyzes the production technology, genetic engineering improvement methods, and application strategies of Bt biopesticides to improve their efficiency and application range. By exploring the fermentation process, formulation stability, industrial production, and strain enhancement characteristics of Bt biopesticides through genetic engineering, this study aims to provide a comprehensive improvement plan to provide a scientific basis for future research directions and policy formulation.
Keywords Bt biopesticides; Genetic engineering; Integrated pest management; Environmental impact; Sustainable agriculture

1 Introduction

Bacillus thuringiensis (Bt) is a gram-positive bacterium widely recognized for its insecticidal properties, primarily due to the production of crystal (Cry) proteins during sporulation. These proteins are toxic to a variety of insect pests, making Bt a valuable biopesticide in sustainable agriculture (Wafa et al., 2020; Kumar et al., 2021). Bt biopesticides have been utilized for decades, not only for their effectiveness in pest control but also for their environmental safety compared to chemical pesticides (Ballardo et al., 2017). The bacterium's insecticidal proteins, including Cry and Cyt proteins, target specific insect species, reducing the risk to non-target organisms and minimizing environmental impact (Kumar et al., 2021; Li et al., 2022).

The sustainable production and application of Bt biopesticides are crucial for several reasons. Firstly, the overuse of chemical pesticides has led to significant environmental issues, including soil and water pollution, and the development of pest resistance (Wafa et al., 2020). Bt biopesticides offer a more environmentally friendly alternative, reducing the reliance on harmful chemicals and promoting ecological balance. Additionally, sustainable production methods, such as solid-state fermentation using biowaste digestate (Rosas-García, 2009), can enhance the economic viability and environmental benefits of Bt biopesticides (Li et a.., 2011). Furthermore, integrating Bt biopesticides with other pest management strategies, such as chemical insecticides, can improve efficacy and delay resistance development in pest populations (Wafa et al., 2020).

This study provides a comprehensive overview of the sustainable production and application of Bt biopesticides, summarizes the historical development and current application of Bt biopesticides in agriculture; evaluates the environmental and ecological impacts of Bt biopesticides, including their effects on non-target organisms and soil microbial communities; explores the potential of Bt biopesticides in integrated pest management (IPM) strategies and their role in sustainable agriculture. By addressing these objectives, this study aims to highlight the importance of Bt biopesticides in promoting sustainable agricultural practices and identify future research directions to enhance their production and application.

2 Production Techniques of Bt Biopesticides

21 Fermentation processes

Fermentation processes are critical for the production of *Bacillus thuringiensis* (Bt) biopesticides. Solid-state fermentation (SSF) and submerged fermentation (SmF) are the two primary methods used. SSF involves the growth of microorganisms on solid materials without free-flowing water, making it suitable for utilizing agricultural and industrial wastes. For instance, biowaste digestate has been successfully used as a substrate for Bt production in SSF, demonstrating the importance of reactor configuration and temperature control in optimizing viable cell and spore counts (Rodríguez et al., 2019). Similarly, wastewater sludge has been employed as a raw material in both SSF and SmF, showing significant improvements in oxygen transfer and protease activity at larger scales (Yezza et al., 2004; Li et al., 2011). The use of vegetable wastes and soy fiber residues has also been explored, highlighting the potential for cost-effective and environmentally friendly Bt biopesticide production (Ballardo et al., 2016; Pan et al., 2021).

2.2 Formulation and stabilization

Formulation and stabilization are crucial steps to ensure the efficacy and shelf-life of Bt biopesticides. The development of compost-like materials enriched with Bt through SSF has been shown to produce stable and effective biopesticides suitable for soil amendment (Ballardo et al., 2017; Mattedi et al., 2023). The addition of specific carbon and nitrogen sources, as well as metal ions, can enhance the insecticidal activity of Bt, as demonstrated in studies using vegetable wastes (Pan et al., 2021). Moreover, the transformation of heavy metals in wastewater sludge during SSF reduces their bioavailability and environmental risks, further stabilizing the biopesticide product. These strategies not only improve the stability and effectiveness of Bt biopesticides but also contribute to sustainable waste management practices.

2.3 Scale-up and industrial production

Scaling up the production of Bt biopesticides from laboratory to industrial scale involves addressing several challenges, including maintaining consistent oxygen transfer, nutrient availability, and process control. Studies have shown that scaling up from shake flasks to larger fermentors can significantly improve viable cell and spore counts, as well as protease activity, due to better oxygen transfer (Yezza et al., 2004). The use of box-type SSF equipment has enabled kg-scale production of Bt biopesticides, providing technical support for large-scale industrial production (Chen, 2009). Additionally, the optimization of fermentation conditions, such as temperature, pH, and aeration, is essential for maximizing toxin protein yield and entomotoxicity potential (Wafa et al., 2020). Future research should focus on reducing production costs, modifying fermentation processes, and developing efficient Bt strains through genetic methods to further enhance industrial-scale production.

3 Genetic Engineering for Enhanced Bt Strains

3.1 Gene cloning and expression

Gene cloning and expression techniques have been pivotal in enhancing the efficacy of *Bacillus thuringiensis* (Bt) biopesticides. By isolating and inserting specific Bt genes into various host organisms, researchers have been able to produce strains with improved insecticidal properties (Zhou et al., 2020). For instance, the construction of Bt recombinant engineered strains through genetic engineering has become a major focus, allowing for the expression of Bt proteins that are structurally and functionally different from naturally occurring Bt prototoxins. This approach not only enhances the insecticidal activity but also addresses the environmental persistence of Bt proteins, which is crucial for sustainable agricultural practices (Li et al., 2022; Ortiz et al., 2023).

3.2 CRISPR and genome editing

The advent of CRISPR/Cas9 genome editing technology has revolutionized the development of enhanced Bt strains. This precise genome editing tool allows for targeted modifications that can significantly improve the resistance management and efficacy of Bt biopesticides. For example, CRISPR/Cas9-mediated knockout of specific genes in the diamondback moth, *Plutella xylostella*, has demonstrated high levels of resistance to Bt Cry1Ac toxin, providing in vivo evidence for the role of these genes in Bt toxin resistance (Guo et al., 2019).

Additionally, CRISPR/Cas9 has been employed in plant genome editing to develop crops with improved traits, including enhanced resistance to pests, which complements the use of Bt biopesticides (Chen et al., 2019).

3.3 Development of multi-trait Bt strains

Developing multi-trait Bt strains involves combining multiple insecticidal genes to create a single strain capable of targeting a broader spectrum of pests or overcoming resistance in specific insect populations. This strategy is exemplified by the isolation and characterization of novel Bt strains, such as the AB1 strain from Sri Lanka, which exhibits toxicity towards Dipel-resistant *Plutella xylostella* (Shanmugam et al., 2020). The AB1 strain produces a variety of Cry proteins, including Cry1Ca and Cry1Da, which contribute to its effectiveness against resistant pest populations (Baragamaarachchi et al., 2019). Such multi-trait strains are crucial for maintaining the long-term sustainability and effectiveness of Bt biopesticides in agricultural pest management.

By leveraging advanced genetic engineering techniques, including gene cloning, CRISPR/Cas9 genome editing, and the development of multi-trait Bt strains, researchers are making significant strides in enhancing the efficacy and sustainability of Bt biopesticides. These innovations not only improve pest control but also contribute to the broader goals of sustainable agriculture and environmental protection.

4 Environmental Impact and Safety Assessment

4.1 Impact on non-target organisms

The environmental impact of Bt biopesticides on non-target organisms has been a significant area of research. Studies have shown that while Bt biopesticides are generally considered safe, they can have varying effects on different non-target species. For instance, the ecotoxicological effects of *Lavandula luisieri* hydrolate on soil organisms revealed acute toxicity to the plant *Allium cepa* and slight toxicity to the earthworm *Eisenia fetida*, along with significant impacts on soil bacterial communities (Pino-Otín et al., 2019). Similarly, the hydrolate from *Artemisia absinthium* showed acute toxicity to non-target soil organisms, including earthworms and plants, and altered microbial community functions (Fournier et al., 2020). Additionally, the impact of Bt crops on non-target plant-associated insects and soil organisms has been extensively reviewed, indicating no significant harmful effects in approved GM events, although continuous risk assessments are necessary (Yaqoob et al., 2016).

4.2 Soil and water health

The application of Bt biopesticides and Bt crops can influence soil and water health through their interactions with soil microorganisms and aquatic organisms. Bt proteins released into the soil from transgenic plants or biopesticides can affect soil microbial diversity and activity. For example, Bt proteins have been shown to alter soil microbial communities, impacting nutrient cycling and soil health (Figure 1) (Li et al., 2022). Furthermore, the ecotoxicity of *Artemisia absinthium hydrolate* on aquatic organisms demonstrated acute toxicity to species such as *Daphnia magna* and *Vibrio fisheri*, highlighting potential risks to water ecosystems (Pino-Otín et al., 2019). The impact of biopesticides on soil microbial endpoints compared to conventional crops has also been systematically reviewed, emphasizing the need for comprehensive assessments to understand their long-term effects on soil biodiversity and ecosystem services (Kostov et al., 2014).

4.3 Ecotoxicological studies

Ecotoxicological studies are crucial for understanding the broader environmental implications of Bt biopesticides. Research on the environmental behaviors of Bt insecticidal proteins has provided insights into their adsorption, retention, and degradation in soils, as well as their potential ecological risks. Additionally, studies on the impact of commercial Bt products on marine organisms, such as marine bivalves, have shown significant physiological and ecological responses, including altered feeding rates and energy budgets, indicating the need for careful consideration of Bt biopesticides' effects beyond terrestrial ecosystems (Manachini et al., 2013). The comparative impact of synthetic pesticides and biopesticides on soil microbial communities has also been investigated, revealing that both types of pesticides can decrease the complexity of soil microbial networks, although their specific effects on microbial community composition and function may differ (Fournier et al., 2020).

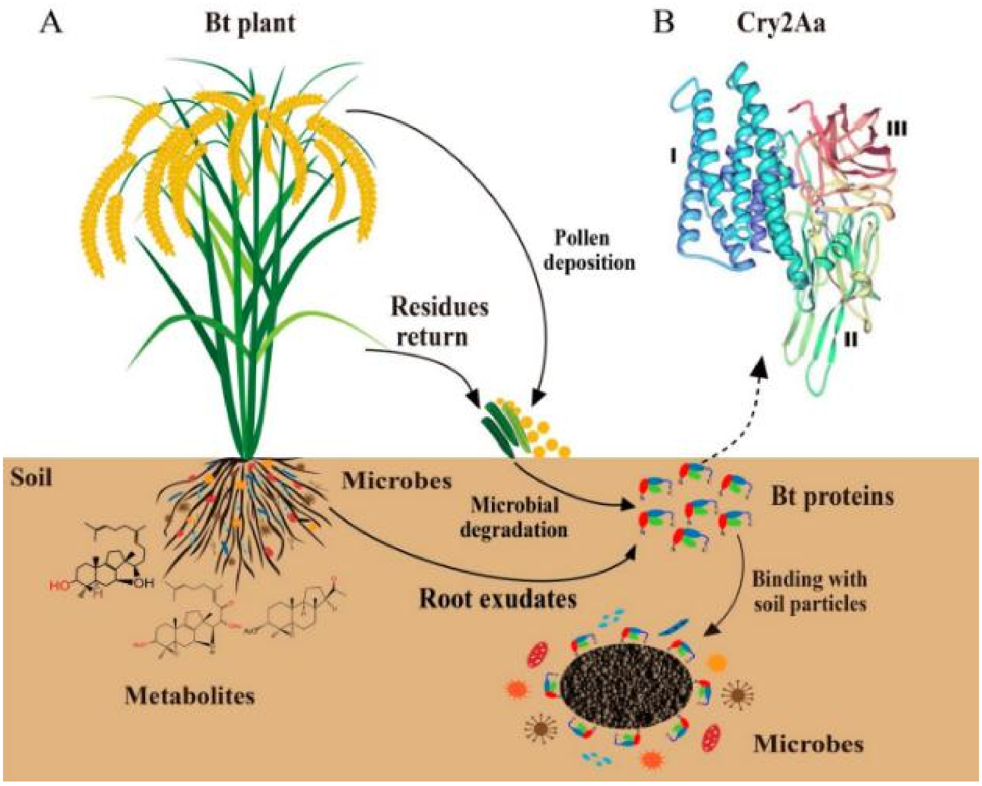

Figure 1 Environmental behaviors of Bt protein (A) and its three-dimensional structures (B) (Adopted from Li et al., 2022)

Li et al. (2022) showed the behavior of Bt protein in the environment and its three-dimensional structure. Bt plants introduce Bt protein into the soil through pollen deposition and residue regression. Bt protein binds to soil particles and is degraded by root exudates and microorganisms. Root exudates release metabolites that affect the microbial community in the soil. The three-dimensional structure of Bt protein (Cry2Aa) includes multiple functional regions that contribute to its binding and toxicity to target pests. This dynamic process shows that Bt plants not only have advantages in controlling pests, but also the persistence and degradation behavior of their metabolites and proteins in the soil need further study to evaluate their long-term impact on soil ecosystems.

5 Application Methods and Strategies

5.1 Field application techniques

Field application techniques for Bt biopesticides are critical for maximizing their efficacy and sustainability. Traditional methods include foliar sprays, soil applications, and seed treatments. Foliar sprays are the most common, allowing for direct application to the plant surfaces where pests feed. Soil applications target root-feeding pests, while seed treatments provide early protection against pests during the vulnerable seedling stage. The effectiveness of these techniques can be influenced by environmental factors such as weather conditions and pest behavior. For instance, the use of transgenic Bt crops, which express Bt toxins throughout the plant, has been shown to reduce the need for additional insecticide applications and enhance pest control efficiency (Naranjo, 2011; Carrière et al., 2015; Kang et al., 2021).

5.2 Integrated pest management (IPM)

Integrated Pest Management (IPM) is a holistic approach that combines multiple strategies to manage pest populations in an environmentally and economically sustainable manner. Bt biopesticides play a significant role in IPM programs by providing a biological control option that can be integrated with other methods such as crop rotation, use of natural predators, and chemical controls. The integration of Bt crops with IPM practices has been shown to reduce pest populations and insecticide use, thereby promoting ecological balance and reducing the risk of pest resistance (Table 1) (Naranjo, 2011; Martinez and Caprio, 2016; Anderson et al., 2019; Baker et al., 2020). For example, the use of Bt cotton has led to significant reductions in insecticide applications and has been a cornerstone in IPM strategies for cotton production (Manjunath, 2023).

5.3 Resistance management strategies

Resistance management is crucial for maintaining the long-term efficacy of Bt biopesticides. Strategies to manage resistance include the use of refuges, crop rotation, and the development of pyramided Bt crops that express multiple Bt toxins. Refuges, areas where non-Bt crops are planted, help maintain a population of susceptible pests that can dilute the resistance genes in the pest population. Crop rotation disrupts pest life cycles and reduces the selection pressure for resistance. Pyramided Bt crops, which produce multiple Bt toxins, are designed to delay resistance by targeting pests with different modes of action (Glaser and Matten,, 2003; Raymond et al., 2007). Additionally, integrating Bt with other biotechnological approaches, such as RNA interference (RNAi), can provide new avenues for resistance management (Zhu et al., 2016; Kang et al., 2021).

Table 1 Average time (years) to corn rootworm resistance or extinction simulating a hypothetical non-high dose Bt pyramid with a 5% refuge and additional management tactics (Adopted from Martinez and Caprio, 2016)

Strategy	Block			RIB		
	Mean	SEM	Sample size	Mean	SEM	Sample size
Bt+refuge	21 7b	0.354	100	222b	0.262	100
Bt+refuge+SAI	21 7b	0.301	100	21 7b	0.265	100
20% increased refuge	25.5c	0.327	100	28.5c	0.361	100
Crop rotation	5.0a	0.025	100	5.1a	0.022	100

6 Economic and Social Considerations

6.1 Cost-benefit analysis

The economic viability of Bt biopesticides is a critical factor influencing their adoption. Studies have shown that Bt crops, such as Bt cotton and Bt maize, can lead to significant economic benefits for farmers. For instance, Bt cotton adoption in China resulted in savings of approximately 8.46 billion US dollars on pesticide use over a 15-year period, with additional benefits from increased yields and reduced labor costs (Qiao and Huang, 2018). Similarly, a meta-analysis of GM crops, including Bt varieties, indicated that these crops generally lead to higher economic performance due to yield increases and reductions in pesticide application costs, despite higher seed costs. However, the economic benefits can vary significantly between regions, influenced by factors such as pest pressure and pest management practices (Finger et al., 2011).

6.2 Market demand and adoption

The market demand for Bt biopesticides is influenced by several factors, including the cost of production, regulatory frameworks, and farmer awareness. The high production costs of Bt biopesticides, primarily due to expensive synthetic media and formulation processes, pose a challenge to their widespread adoption (Brar et al., 2006). However, alternative production methods using local media, such as vegetable waste, have shown promise in reducing costs while maintaining efficacy (Pan et al., 2021). The adoption of Bt crops has been substantial in countries like India and China, where Bt cotton has generated significant welfare gains for farmers (Qaim et al., 2008). Nonetheless, the market structure and regulatory environment play crucial roles in technology access and benefit distribution, affecting the overall adoption rates (Qaim et al., 2008).

6.3 Socioeconomic impacts

The socioeconomic impacts of Bt biopesticides extend beyond direct economic benefits to include health and environmental considerations. The reduction in pesticide use associated with Bt crop adoption has led to improved health outcomes for farmers and reduced environmental pollution (Fletcher et al., 2020). However, there are also concerns about the potential impacts on non-target organisms and biodiversity, which necessitate careful risk assessment and management (Kaur, 2012; Manachini et al., 2013). Additionally, the deviation between farmers' willingness and actual behavior in adopting biopesticides highlights the importance of education and market structure in promoting sustainable practices (Guo et al., 2021). Addressing these socioeconomic factors is essential for the long-term sustainability and acceptance of Bt biopesticides.

7 Policy and Regulatory Frameworks

7.1 International guidelines and standards

The international regulatory landscape for biopesticides, including *Bacillus thuringiensis* (Bt) biopesticides, is shaped by various guidelines and standards aimed at ensuring safety and efficacy. The European Pesticide Regulation (EC) No. 1107/2009 is a significant framework that encourages the use of less harmful active substances, including biopesticides, while maintaining stringent safety standards (Villaverde et al., 2014). This regulation underscores the importance of zonal evaluations and cut-off criteria to balance the need for effective pest control with environmental and human health considerations. Additionally, international agreements such as the Cartagena Protocol on Biosafety (CPB) play a crucial role in regulating the transboundary movement of genetically modified organisms, including Bt crops, to ensure biosafety and minimize potential risks (Raybould and Quemada, 2010).

7.2 National regulatory approvals

National regulatory frameworks vary significantly across countries, reflecting different levels of scientific capacity and regulatory stringency. In many developing countries, the establishment of functional regulatory systems is challenging, which can hinder the deployment of Bt biopesticides and other transgenic crops (Raybould and Quemada, 2010). For instance, the regulatory process in the United States involves rigorous pre-market notification, risk assessment, and approval procedures, followed by labelling and post-market monitoring to ensure compliance with safety standards (Grillo et al., 2020). Similarly, the European Union's regulatory framework requires comprehensive risk assessments and adherence to strict guidelines for the approval and use of biopesticides (Fletcher et al., 2020). These national regulations are designed to ensure that biopesticides are safe for human health and the environment while providing effective pest control solutions (Grillo et al., 2020).

7.3 Compliance and enforcement

Compliance and enforcement are critical components of the regulatory framework for Bt biopesticides. Effective enforcement mechanisms are necessary to ensure that manufacturers and users adhere to regulatory requirements, including proper labelling, usage instructions, and post-market surveillance (Grillo et al., 2020). In the European Union, compliance is monitored through a combination of national and EU-level inspections and audits to ensure that biopesticides are used safely and effectively (Rodríguez et al., 2019). In the United States, the Environmental Protection Agency (EPA) oversees the compliance of biopesticides with federal regulations, conducting regular inspections and taking enforcement actions against non-compliance. Additionally, the development of robust quality control measures and policy support is essential to maintain the integrity and efficacy of biopesticides in the market (Keswani et al., 2016).

8 Case Studies of Successful Bt Biopesticide Use

8.1 Agricultural applications

Bacillus thuringiensis (Bt) has been extensively utilized in agriculture due to its potent insecticidal properties, primarily attributed to the production of Cry proteins. These proteins target specific insect pests, making Bt an environmentally friendly alternative to chemical pesticides. For instance, Bt has been successfully incorporated into transgenic crops such as Bt cotton, potato, and maize, which express Cry proteins to protect against *lepidopteran* and *coleopteran* pests (Sanchis and Bourguet, 2011; Kumar et al., 2021; Li et al., 2022). Additionally, Bt-based biopesticides have been employed in the management of pests in vegetable legumes and brassicas in Asia and Africa, significantly reducing the reliance on chemical pesticides and mitigating their associated health and environmental risks (Ansari et al., 2012; Srinivasan et al., 2019) (Figure 2).

8.2 Public health programs

Bt biopesticides have also found applications in public health programs, particularly in controlling disease vectors. Bt israelensis (Bti) is a strain specifically used to target mosquito larvae, which are vectors for diseases such as malaria and dengue fever. The use of Bt in these programs has proven effective in reducing mosquito populations without harming non-target organisms, thereby offering a safer alternative to chemical insecticides (Sanchis and

Bourguet, 2011). The specificity of Bt toxins to certain insect species ensures minimal impact on human health and the environment, making it a valuable tool in public health initiatives (Manachini et al., 2013).

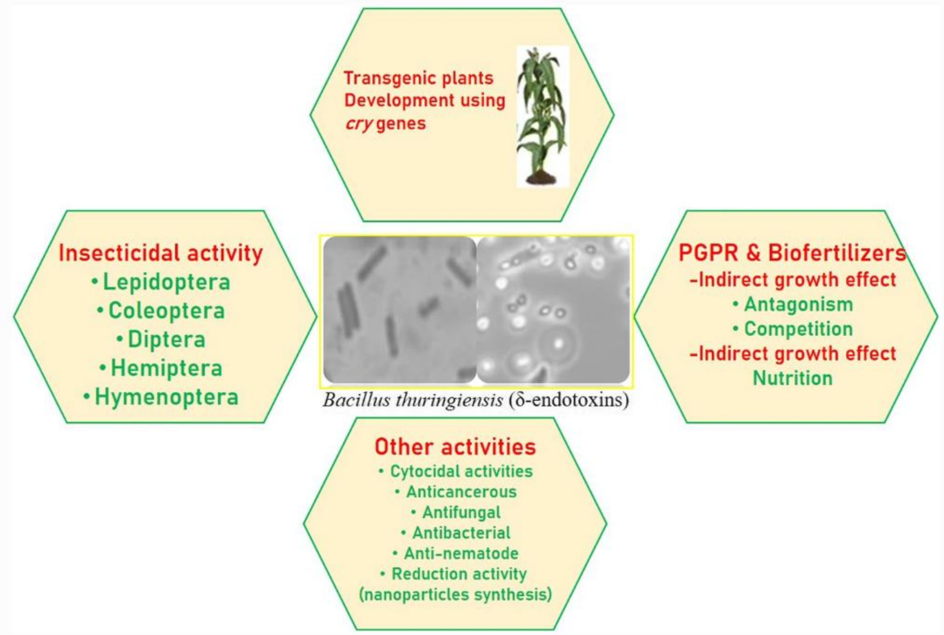

Figure 2 Application of *Bacillus thuringiensis* in sustainable agriculture (Adopted from Kumar et al., 2021)

8.3 Environmental conservation projects

In environmental conservation, Bt biopesticides have been employed to protect native plant species and ecosystems from invasive insect pests. For example, Bt has been used to control gypsy moth populations in North American forests, preventing defoliation and preserving forest health (Manachini et al., 2013). Additionally, Bt-based products have been tested for their effects on non-target marine organisms, highlighting the need for careful consideration of ecological impacts. Studies have shown that while Bt can affect marine bivalves under high exposure scenarios, its overall environmental footprint remains lower compared to chemical pesticides (Seenivasagan and Babalola, 2021). This underscores the importance of Bt in integrated pest management strategies aimed at conserving biodiversity and maintaining ecological balance (Pascoli et al., 2019; Fenibo et al., 2022).

By leveraging the specificity and efficacy of Bt biopesticides, these case studies demonstrate the potential for sustainable pest management across various sectors, contributing to agricultural productivity, public health, and environmental conservation.

9 Challenges and Future Directions

9.1 Technical and practical challenges

The production and application of *Bacillus thuringiensis* (Bt) biopesticides face several technical and practical challenges. One significant issue is the high production cost, which limits the widespread adoption of Bt biopesticides. This is primarily due to the expensive synthetic media used in the fermentation process, as well as the costs associated with harvesting and formulation (Brar et al., 2016; Pan et al., 2021). Additionally, the stability of Bt formulations under field conditions is a concern, as factors such as ultraviolet radiation, rain, pH, and temperature can degrade the pesticidal proteins, reducing their efficacy (Oliveira et al., 2021). Another challenge is the need for frequent applications due to the instability of Bt under sunlight, which increases the overall cost and labor involved in pest control (Ortiz and Sansinenea, 2023).

9.2 Research gaps and opportunities

Despite the extensive research on Bt biopesticides, several gaps remain that present opportunities for future investigation. One area that requires further exploration is the optimization of bioprocess parameters, including nutritional requirements, culture media, and fermentation technologies, to enhance the growth, sporulation, and toxin formation yields of Bt (Wafa et al., 2020). Additionally, there is a need for more systematic risk assessment studies to evaluate the environmental and health impacts of new Bt formulations, particularly those involving micro and nanoparticulate systems (Kumar et al., 2021). Research on the use of alternative raw materials, such as agricultural by-products and wastewater sludge, for Bt production could also help reduce costs and promote sustainable practices (Aranda et al., 2000). Furthermore, the development of regulatory frameworks and risk mitigation strategies is essential to facilitate the widespread deployment of innovative Bt-based products (Fletcher et al., 2020).

9.3 Innovations and future trends

Innovations in the production and application of Bt biopesticides are crucial for overcoming existing challenges and enhancing their effectiveness. Advances in micro/nanotechnology have led to the development of more efficient Bt formulations, such as microencapsulations and microgranules, which can improve the stability and residual entomotoxicity of Bt under adverse environmental conditions. The use of genetically modified crops expressing Bt toxins offers another promising approach to increase crop yields and reduce the need for chemical pesticides (Ortiz and Sansinenea, 2023). Additionally, the integration of RNA interference (RNAi) technology with Bt biopesticides could provide a narrow-spectrum alternative to chemical-based control measures, targeting pests with high accuracy and specificity (Fletcher et al., 2020). Future research should focus on reducing the cost of culture media components, modifying fermentation processes, and developing efficient Bt strains through genetic methods to enhance the overall efficiency and sustainability of Bt biopesticides.

10 Concluding Remarks

The systematic review of the literature on the sustainable production and application of *Bacillus thuringiensis* (Bt) biopesticides reveals several critical insights. Bt has been extensively studied and utilized due to its high specificity and environmental safety compared to chemical pesticides. Research has focused on optimizing bioprocess parameters, including nutritional requirements, culture media, and fermentation technologies, to enhance Bt production efficiency. Additionally, innovative approaches such as solid-state fermentation using biowaste digestate have shown promise in producing cost-effective Bt biopesticides. The environmental behavior of Bt proteins and their potential ecological impacts have also been examined, highlighting the need for biosafety evaluations. Furthermore, Bt's role extends beyond pest control to include applications as a biofertilizer and in the development of transgenic plants.

Sustainable practices in the production and application of Bt biopesticides are crucial for several reasons. Firstly, they help mitigate the adverse effects of chemical pesticides, such as soil, water, and food source pollution, and the emergence of pest resistance. By utilizing biowaste and optimizing fermentation processes, the production costs of Bt biopesticides can be reduced, making them more accessible and economically viable Moreover, sustainable practices ensure that Bt biopesticides have minimal unintended impacts on non-target organisms and ecosystems, thereby preserving biodiversity and ecological balance. The integration of Bt biopesticides into sustainable agriculture practices can significantly contribute to reducing the reliance on chemical pesticides and promoting environmental health.

Future research and development in the field of Bt biopesticides should focus on several key areas. Firstly, there is a need for further optimization of bioprocess parameters to enhance Bt production yields and reduce costs. Exploring alternative substrates and fermentation technologies, such as solid-state fermentation with biowaste, can provide cost-effective and sustainable production methods. Additionally, comprehensive biosafety evaluations are essential to assess the long-term environmental impacts of Bt proteins and their interactions with soil microbial communities. Research should also investigate the development of Bt-based bioformulations with improved stability and efficacy under field conditions. Finally, interdisciplinary approaches that integrate bioinformatics, genetic engineering, and ecological studies can help design Bt biopesticides with enhanced specificity and reduced non-target effects.

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Conflict of Interest Disclosure
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References
Anderson J., Ellsworth P.C., Faria J., Head G., Owen M., Pilcher C., Shelton A.M., and Meissle M., 2019, Genetically engineered crops: importance of diversified integrated pest management for agricultural sustainability, Frontiers in Bioengineering and Biotechnology, 7: 24. https://doi.org/10.3389/fbioe.2019.00024

Ansari M.S., Ahmad N., and Hasan F., 2012, Potential of biopesticides in sustainable agriculture, 1: 529-595. https://doi.org/10.1007/978-94-007-1591-2_17

Aranda E., Lorence A., and Trejo M., 2000, Rural production of *Bacillus thuringiensis* by solid state fermentation, ChemInform, 32: 317-332. https://doi.org/10.1007/978-94-017-1429-7_17

Baker B., Green T., and Loker A., 2020, Biological control and integrated pest management in organic and conventional systems, Biological Control, 140: 04095. https://doi.org/10.1016/j.biocontrol.2019.104095

Ballardo C., Abraham J., Barrena R., Artola A., Gea T., and Sánchez A., 2016, Valorization of soy waste through SSF for the production of compost enriched with *Bacillus thuringiensis* with biopesticide properties, Journal of Environmental Management, 169: 126-131. https://doi.org/10.1016/j.jenvman.2015.12.029

Ballardo C., Barrena R., Artola A., and Sánchez A., 2017, A novel strategy for producing compost with enhanced biopesticide properties through solid-state fermentation of biowaste and inoculation with *Bacillus thuringiensis*, Waste Management, 70: 53-58. https://doi.org/10.1016/j.wasman.2017.09.041

Baragamaarachchi R.Y., Samarasekera J.K., Weerasena O., Lamour K., and Jurat-Fuentes J., 2019, Identification of a native *Bacillus thuringiensis* strain from Sri Lanka active against Dipel-resistant *Plutella xylostella*, PeerJ, 7: e7535. https://doi.org/10.7717/peerj.7535

Brar S., Verma M., Tyagi R., and Valéro J., 2006, Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides, Process Biochemistry, 41: 323-342. https://doi.org/10.1016/J.PROCBIO.2005.07.015

Carrière Y., Crickmore N., and Tabashnik B., 2015, Optimizing pyramided transgenic Bt crops for sustainable pest management, Nature Biotechnology, 33: 161-168. https://doi.org/10.1038/nbt.3099

Chen K.L., Wang Y.P., Zhang R., Zhang H.W., and Gao C.X., 2019, CRISPR/Cas genome editing and precision plant breeding in agriculture, Annual Review of Plant Biology, 70(1): 667-697. https://doi.org/10.1146/annurev-arplant-050718-100049

Chen L.W., 2009, Study on production of Bt biopesticide by box-type solid-state fermentation, Chemistry and Bioengineering, 3-244.

Fenibo E., Ijoma G., Nurmahomed W., and Matambo T., 2022, The potential and green chemistry attributes of biopesticides for sustainable agriculture, Sustainability, 14(21): 14417. https://doi.org/10.3390/su142114417

Finger R., Benni N., Kaphengst T., Evans C., Herbert S., Lehmann B., Morse S., and Stupak N., 2011, A meta analysis on farm-level costs and benefits of GM crops, Sustainability, 3: 743-762. https://doi.org/10.3390/SU3050743

Fletcher S., Reeves P., Hoang B., and Mitter N., 2020, A perspective on RNAi-based biopesticides, Frontiers in Plant Science, 11: 51. https://doi.org/10.3389/fpls.2020.00051

Fournier B., Santos S., Gustavsen J., Imfeld G., Lamy F., Mitchell E., Mota M., Noll D., Planchamp C., and Heger T., 2020, Impact of a synthetic fungicide (fosetyl-Al and propamocarb-hydrochloride) and a biopesticide (*Clonostachys rosea*) on soil bacterial fungal and protist communities, The Science of the Total Environment, 738: 139635. https://doi.org/10.1016/j.scitotenv.2020.139635

Glaser J., and Matten S., 2003, Sustainability of insect resistance management strategies for transgenic Bt corn, Biotechnology Advances, 22(1-2): 45-69. https://doi.org/10.1016/J.BIOTECHADV.2003.08.016

Grillo R., Fraceto L., Amorim M., Scott-Fordsmand J., Schoonjans R., and Chaudhry Q., 2020, Ecotoxicological and regulatory aspects of environmental sustainability of nanopesticides., Journal of Hazardous Materials, 404 Pt A: 124148. https://doi.org/10.1016/j.jhazmat.2020.124148

Guo H., Sun F., Pan C., Yang B., and Li Y., 2021, The deviation of the behaviors of rice farmers from their stated willingness to apply biopesticides-a study carried out in Jilin province of China, International Journal of Environmental Research and Public Health, 18(11): 6026. https://doi.org/10.3390/ijerph18116026

Guo Z., Sun D., Kang S., Zhou J., Gong L., Qin J., Guo L., Zhu L., Bai Y., Luo L., and Zhang Y., 2019, CRISPR/Cas9-mediated knockout of both the PxABCC2 and PxABCC3 genes confers high-level resistance to *Bacillus thuringiensis* Cry1Ac toxin in the diamondback moth *Plutella xylostella* (L.), Insect Biochemistry and Molecular Biology, 107: 31-38.
https://doi.org/10.1016/j.ibmb.2019.01.009

Kang S., Sun D., Qin J.Y., Guo L.H., Zhu L., Bai Y., Wu Q.J., Wang S.L., Zhou X.G., Guo Z.J., and Zhang Y.J., 2021, Fused: a promising molecular target for an RNAi-based strategy to manage Bt resistance in *Plutella xylostella* L., Journal of Pest Science, 2021: 1-14.
https://doi.org/10.1007/S10340-021-01374-3

Kaur S., 2012, Risk assessment of Bt transgenic crops, *Bacillus thuringiensis* Biotechnology, 2012: 41-85.
https://doi.org/10.1007/978-94-007-3021-2_3.

Keswani C., Sarma B.K., and Singh H.B., 2016, Synthesis of policy support quality control and regulatory management of biopesticides in sustainable agriculture, Agriculturally Important Microorganisms: Commercialization and Regulatory Requirements in Asia, 2016: 3-12.
https://doi.org/10.1007/978-981-10-2576-1_1

Kostov K., Krogh P., Damgaard C., Sweet J., and Hendriksen N., 2014, Are soil microbial endpoints changed by Bt crops compared with conventional crops? a systematic review protocol, Environmental Evidence, 3: 1-11.
https://doi.org/10.1186/2047-2382-3-11

Kumar P., Kamle M., Borah R., Mahato D., and Sharma B., 2021, *Bacillus thuringiensis* as microbial biopesticide: uses and application for sustainable agriculture, Egyptian Journal of Biological Pest Control, 31: 1-7.
https://doi.org/10.1186/s41938-021-00440-3

Li Y.J., Wang C., Ge L., Hu C., Wu G.G., Sun Y., Song L.L., Wu X., Pan A.H., Xu Q.Q., Shi J.L., Liang J.G., and Li P., 2022, Environmental behaviors of *Bacillus thuringiensis* (Bt) insecticidal proteins and their effects on microbial ecology, Plants, 11(9): 1212.
https://doi.org/10.3390/plants11091212

Li Z., Zhou S., Wang Y., Liu Z., and Xu R., 2011, Cost-effective production of *Bacillus thuringiensis* biopesticides by solid-state fermentation using wastewater sludge: effects of heavy metals, Bioresource Technology, 109(7): 4820-4826.
https://doi.org/10.1016/j.biortech.2010.12.098

Manachini B., Arizza V., Rinaldi A., Montalto V., and Sarà G., 2013, Eco-physiological response of two marine bivalves to acute exposition to commercial Bt-based pesticide., Marine environmental Research, 83: 29-37.
https://doi.org/10.1016/j.marenvres.2012.10.006

Manjunath T., 2023, Integration of augmentative biocontrol with synthetic pesticides and other control methods for IPM-challenges and prospects, Journal of Biological Control, 36(4): 179-186.
https://doi.org/10.18311/jbc/2022/34091

Martinez J., and Caprio M., 2016, IPM use with the deployment of a non-high dose Bt pyramid and mitigation of resistance for western corn rootworm (*Diabrotica virgifera virgifera*), Environmental Entomology, 45(3): 747-761.
https://doi.org/10.1093/ee/nvw015

Mattedi A., Sabbi E., Farda B., Djebaili R., Mitra D., Ercole C., Cacchio P., Gallo M., and Pellegrini M., 2023, Solid-state fermentation: applications and future perspectives for biostimulant and biopesticides production, Microorganisms, 11(6): 1408.
https://doi.org/10.3390/microorganisms11061408

Naranjo S., 2011, Impacts of Bt transgenic cotton on integrated pest management, Journal of Agricultural and Food Chemistry, 59(11): 5842-5851.
https://doi.org/10.1021/jf102939c

Oliveira J., Fraceto L., Bravo A., and Polanczyk R., 2021, Encapsulation strategies for *Bacillus thuringiensis*: from now to the future, Journal of Agricultural and Food Chemistry, 69(16): 4564-4577.
https://doi.org/10.1021/acs.jafc.0c07118

Ortiz A., and Sansinenea E., 2023, Genetically modified plants based on *Bacillus* genes and commercial *Bacillus*-based biopesticides for sustainable agriculture, Horticulturae, 9(9): 963.
https://doi.org/10.3390/horticulturae9090963

Pan X.H., Huang T.Z., Fang Y., Rao W.H., Guo X.P., Nie D.Y, Zhang D.Y., Cao F., Guan X., and Chen Z., 2021, Effect of *Bacillus thuringiensis* biomass and insecticidal activity by cultivation with vegetable wastes, Royal Society Open Science, 8(3): 201564.
https://doi.org/10.1098/rsos.201564

Pascoli M., Jacques M., Agarrayua D., Ávila D., Lima R., and Fraceto L., 2019, Neem oil based nanopesticide as an environmentally-friendly formulation for applications in sustainable agriculture: an ecotoxicological perspective, The Science of the Total Environment, 677: 57-67.
https://doi.org/10.1016/j.scitotenv.2019.04.345

Pino-Otín M.R., Val J., Ballestero D., Navarro E., Sánchez E., González-Coloma A., and Mainar A., 2019, Ecotoxicity of a new biopesticide produced by *Lavandula luisieri* on non-target soil organisms from different trophic levels, The Science of the Total Environment, 671: 83-93.
https://doi.org/10.1016/j.scitotenv.2019.03.293

Qaim M., Pray C.E., and Zilberman D., 2008, Economic and social considerations in the adoption of Bt crops, Dordrecht: Springer Netherlands, 329-356.
https://doi.org/10.1007/978-1-4020-8373-0_12

Qiao F., and Huang J., 2018, Sustainability of the economic benefit of Bt cotton in China: results from household surveys, The Journal of Development Studies, 56: 2045-2060.
https://doi.org/10.1360/N052018-00109

Raybould A., and Quemada H., 2010, Bt crops and food security in developing countries: realised benefits sustainable use and lowering barriers to adoption, Food Security, 2: 247-259.
https://doi.org/10.1007/s12571-010-0066-3

Raymond B., Sayyed A., Hails R., and Wright D., 2007, Exploiting pathogens and their impact on fitness costs to manage the evolution of resistance to *Bacillus thuringiensis*, Journal of Applied Ecology, 44: 768-780.
https://doi.org/10.1111/J.1365-2664.2007.01285.X

Rodríguez P., Cerda A., Font X., Sanchez A., and Artola A., 2019, Valorisation of biowaste digestate through solid state fermentation to produce biopesticides from *Bacillus thuringiensis*, Waste management, 93 63-71.
https://doi.org/10.1016/J.WASMAN.2019.05.026

Rosas-García N., 2009, Biopesticide production from *Bacillus thuringiensis*: an environmentally friendly alternative, Recent patents on biotechnology, 3(1): 28-36.
https://doi.org/10.2174/187220809787172632

Sanchis V., and Bourguet D., 2011, *Bacillus thuringiensis*: applications in agriculture and insect resistance management, a review, Agronomy for Sustainable Development, 28: 11-20.
https://doi.org/10.1051/agro:2007054

Seenivasagan R., and Babalola O., 2021, Utilization of microbial consortia as biofertilizers and biopesticides for the production of feasible agricultural product, Biology, 10(11): 1111.
https://doi.org/10.3390/biology10111111

Shanmugam S., Ngo H., and Wu Y., 2020, Advanced CRISPR/Cas-based genome editing tools for microbial biofuels production: a review, Renewable Energy, 149: 1107-1119.
https://doi.org/10.1016/j.renene.2019.10.107

Srinivasan R., Sevgan S., Ekesi S., and Tamò M., 2019, Biopesticide based sustainable pest management for safer production of vegetable legumes and brassicas in Asia and Africa, Pest Management Science, 75(9): 2446-2454.
https://doi.org/10.1002/ps.5480

Villaverde J., Sevilla-Morán B., Sandín-España P., López-Goti C., and Alonso-Prados J., 2014, Biopesticides in the framework of the European Pesticide Regulation (EC) No, 1107/2009, Pest Management Science, 70(1): 2-5.
https://doi.org/10.1002/ps.3663

Wafa J., Fatma D., Luc F., and Souad R., 2020, Review on biopesticide production by *Bacillus thuringiensis* subsp, kurstaki since 1990: Focus on bioprocess parameters, Process Biochemistry, 98: 224-232.
https://doi.org/10.1016/j.procbio.2020.07.023

Yaqoob A., Shahid A., Samiullah T., Rao A., Khan M., Tahir S., Mirza S., and Husnain T., 2016, Risk assessment of Bt crops on the non-target plant-associated insects and soil organisms, Journal of The Science of Food and Agriculture, 96(8): 2613-2619.
https://doi.org/10.1002/jsfa.7661

Yezza A., Tyagi R., Valéro J., Surampalli R., and Smith J., 2004, Scale-up of biopesticide production processes using wastewater sludge as a raw material, Journal of Industrial Microbiology and Biotechnology, 31: 545-552.
https://doi.org/10.1007/s10295-004-0176-z

Zhou Y., Wu Z.Q., Zhang J., Wan Y.S., Jin W.J., Li Y.Z., and Fang X.J., 2020, Cry80Aa1, a novel *Bacillus thuringiensis* toxin with mosquitocidal activity to Culex pipiens pallens, Journal of Invertebrate Pathology, 173: 107386.
https://doi.org/10.1016/j.jip.2020.107386

Zhu F., Lavine L., O'Neal S., Lavine M., Foss C., and Walsh D., 2016, Insecticide resistance and management strategies in urban ecosystems, Insects, 7(1): 2.
https://doi.org/10.3390/insects7010002