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Biological Control of Insect Pests Using Entomopathogenic Enzymes: Opportunities and Challenges

Aina Maisarah binti Mohammad Mahathir [1], Tony Hadibarata [1,*], Muhammad Noor Hazwan bin Jusoh [1], Inn Shi Tan [2] Yie Hua Tan [3]

[1] Department of Environmental Engineering, Curtin University Malaysia, CDT 250, Miri 98009, Malaysia; Maisarah@postgrad.curtin.edu.my (A.M.B.M.M.); hadibarata@curtin.edu.my (T.H.); mn.hazwan@curtin.edu.my (M.N.H.B.J.);
[2] Department of Chemical & Energy Engineering, Curtin University Malaysia, CDT 250, Miri 98009, Malaysia; tan.s@curtin.edu.my;
[3] Petroleum and Chemical Engineering Programme, Universiti Teknologi Brunei, Jalan Tungku Link Gadong BE1410, Brunei Darussalam; yiehua.tan@utb.edu.bn;
* Correspondence: hadibarata@curtin.edu.my;

Scopus Author ID 16233109100

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Abstract: Interest in using entomopathogenic enzymes as eco-friendly alternatives to chemical pesticides has grown with the increasing demand for sustainable pest management. These enzymes, such as proteases, chitinases, and lipases, selectively target the structural and physiological features of the insect pest, degrading protective barriers and disrupting vital processes. When used appropriately, ~~the~~ bacterial enzymes also offer additional benefits. This review examines the significance of entomopathogenic enzymes in pest control, highlighting recent technological developments to improve enzyme production, stability, and delivery. Advances in microbial fermentation, genetic engineering, and nanoencapsulation have reduced cost reduction and enhanced enzyme resilience for outdoor applications. The integration of these enzymes with other biocontrol agents, such as microbial insecticides and natural predators, and their compatibility within an Integrated Pest Management (IPM) framework are also discussed. However, challenges include high production costs, environmental degradation, specificity limitations, and regulatory hurdles. Further research is needed to address these issues, making enzyme-based strategies more viable for large-scale applications. We identified research gaps, including the need for cost-effective production techniques, stabilized enzymes for application under diverse field conditions, and streamlined regulatory requirements. As biotechnology advances, developing custom-made enzymes for specific pests will expand the potential for safer and more effective pest control methods. Entomopathogenic enzymes represent a promising shift towards sustainable agriculture, offering targeted and environmentally friendly safe pest management alternatives.

Keywords: fungal enzymes; biological pest control; eco-friendly pest solutions; enzyme-based biocontrol; enzyme applications; microbial metabolites.

1. Introduction

Insect pests have become so widespread that increased incidence across the globe is affecting several sectors, particularly agriculture, public health, and ecological balance. Globally, pest populations can severely affect food security, human health, and biodiversity as human populations grow and agriculture activities intensify [1]. Traditionally, chemical pesticides have been used effectively to control pests, but their use comes at a significant cost to the environment and human health [1]. Consequently, biological control strategies are gaining attention as sustainable alternatives, with entomopathogenic enzymes emerging as microorganism-specific agents of biological origin [2]. This review discusses entomopathogenic enzymes as potential biopesticides, their classification based on their action mechanisms, a survey of practical studies of these enzymes, and challenges related to their use.

1.1. Global challenges posed by insect pests in agriculture and public health.

Insect pests plague agriculture, causing substantial major economic losses that significantly impact food production and national economies. Globally, insect pests are estimated to account for annual crop yield losses of 20 to 40%, resulting in billions of dollars in damages every year [3]. These losses strain food supply chains, particularly in areas already reeling from food insecurity. Beyond direct losses, crop damage indirectly affects labor markets and agriculture-related industries. In addition to agricultural impacts, insect pests pose significant threats to public health [3]. They act as vectors for infectious diseases such as malaria, dengue fever, and Zika virus, which are transmitted through the saliva of host organisms. These diseases collectively kill hundreds of thousands of people annually, primarily in warm climate regions [3]. Invasive pest species threaten biodiversity, particularly in fragile ecosystems, which can cause cascading effects on native plants and animal populations [4]. Over the past two decades, large-scale declines in ash in North America have been caused by invasive pests, such as the emerald ash borer, with lasting ecological consequences [5]. These challenges underscore the urgent need for effective pest management strategies that do not exacerbate risks to environmental or human health risks [6].

Figure 1. Total economic cost per continent and the three costliest invasive terrestrial invertebrates for each region. Different color gradients used to represent the total financial burden per continent. This figure was recreated based on an open-access article [7].

1.2. The concept of biological control and the role of entomopathogenic enzymes in pest management.

Biological control is an alternative to synthetic pesticides using natural enemies, such as predators, pathogens, or parasites of the pest species, to regulate harmful organisms while preserving local flora and fauna [8]. Unlike synthetic pesticides, biological control offers more specific effects, minimizing non-target effects, reducing environmental pollution, and protecting beneficial organisms [8]. Entomopathogenic microorganisms, such as fungi, bacteria, and viruses, can infect and kill insect pests, a phenomenon that forms the foundation of biological control, a promising field [9]. Because agents lack the undesirable externalities of chemical pesticides, such as resistance development and environmental degradation, they are valuable tools for regulating pest populations [9].

Entomopathogenic enzymes play a crucial role as biological control agents. Entomopathogenic microorganisms, such as fungi and bacteria, rely on specific enzymes to infect and kill their insect hosts [10]. Proteases, chitinases, and lipases are key examples of enzymes breaking down the insect's protective barriers and overcoming defenses [11]. For instance, proteases remove proteins within an insect's body, weakening its defense mechanisms, while chitinases break down chitin in the insect exoskeleton, allowing microbial entry [12]. The specificity of these enzymes to target pests and the lack of risk to non-target species make these enzyme-friendly biotechnology with little or minimal risk [13. Additionally, entomopathogenic enzymes are natural and biodegradable, preventing environmental accumulation typical of synthetic pesticides [14]. These enzymes are also compatible with Integrated Pest Management (IPM) programs, which combine multiple control strategies into a comprehensive and effective approach [15]. The biological control strategies based on enzymes can reduce the need for pesticides and hence reduce the ecological impact introduced by traditional pest management.

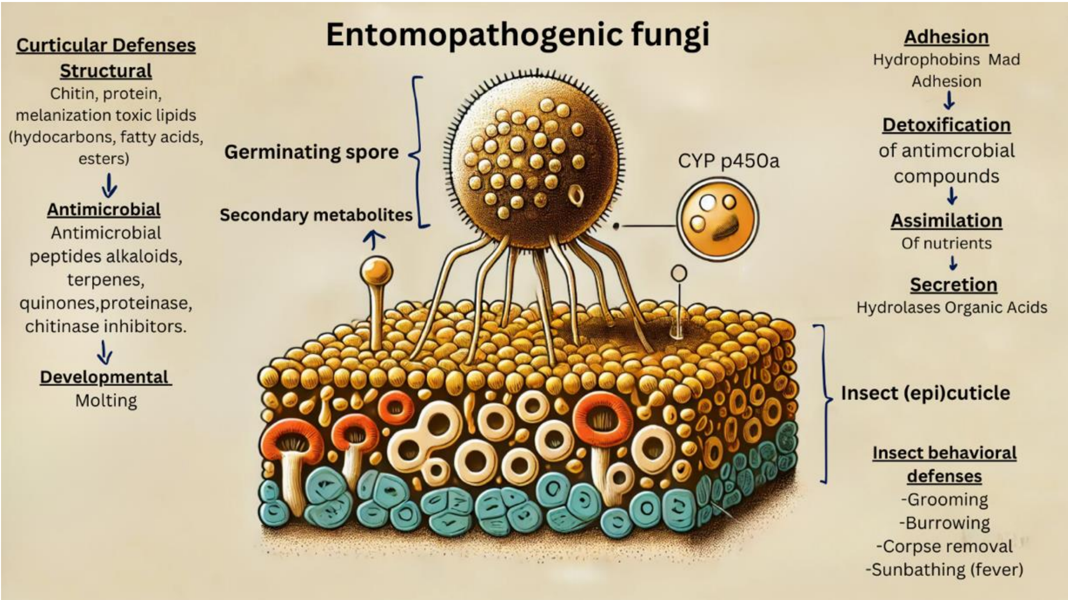

Figure 2. The interaction between entomopathogenic fungi against insect (epi)cuticle where fungal spores adhere to the insect cuticle, followed by germination and penetration. This figure was recreated based on an open-access article [16].

2. Entomopathogenic Enzymes: Types and Mechanisms

Entomopathogenic fungi and bacteria have evolved many sophisticated mechanisms to infect and kill their insect pest natural enemies [17]. These microorganisms secrete various enzymes, primarily proteases, chitinases, and lipase, which are necessary to break down insects' structural and physiological barriers. These enzymes enable fungi and bacteria to infect and sustain themselves within host insects, further acting on insects' different structural and biochemical constituents, killing them [18].

The insect cuticle is a multi-layered structure made up of chitin and protein, and it also acts as a first line of defense against any infection [19]. Enzymes that can break down this protein are called proteases through proteolysis, where the protein is broken down to a single amino acid [20]. Specific proteases, such as serine proteases and metalloproteases, are known to break down the protein and allow the pathogen to pass through the cuticle and reach the internal organ of the insect [21]. Serine protease works by cleaving the peptide bond in the protein sequence. At the same time, metalloprotease cuts the protein by using metal ions and cutting the type IV collagen, a critical component of the basal membrane structure [22].

According to [23], proteases suppress the insect's immune defenses. For example, serine protease breaks down hemolymph proteins necessary for the insect's immune response [23]. Insects have very responsive immune responses whenever they encounter pathogens in their immune systems [24]. However, protein enzymes can break down the protein in the cuticle to reduce the defense measures and allow rapid protease multiplication [25].

Next, the chitin-specific enzymes called chitinase can also digest the insect's cuticle [26]. Most of the insect's cuticle is made from chitin, making it an easy target for entomopathogenic enzymes [26]. For example, bacteria like *Bacillus thuringiensis* and fungi named *Metarhizium anisopliae* use chitinase to digest the chitin, allowing other enzymes and pathogens to enter the insect's body [27].

According to [27], chitinase is a main effector in degrading an insect's cuticle as the enzyme weakens the first line of defense of the insect and allows the other enzymes, such as lipase and protease, to penetrate the internal body of the insect. This is also how chitinase-associated pathogens escape the insect immune system by digestion of chitin microfibrils in the insect gut or hemocoel [28]. Chitinases are extremely specific since chitin is found in only some fungi and arthropods, which minimizes the off-target effects and offers additional potential as biopesticides [28].

Lipases (lipid-degrading enzymes) are crucial for disrupting the structural integrity of cell membranes and accessing energy reserves in the insect body [29]. *Beauveria bassiana* produces lipases that hydrolyze lipids in the lipid-rich layers of the insect cuticle and inner tissues, aiding pathogen entry and nutrient acquisition [30]. Lipases serve a dual function in infection: facilitating penetration and supporting the pathogen’s nutritional needs.

Firstly, they aid in degrading the lipid part of the protection layer (cuticle) by aiding enzymes in penetrating it. Then, lipases destroy cellular processes by depriving essential lipids in insect tissues. It weakens the insect immune system by killing cells, making the latter more susceptible to the destruction of cell membranes and the death of a cell [31]. Moreover, lipases modulate lipid signaling pathways essential for effector suppression and thus are critical elements of innate immunity [32].

2.1. Recent advances in understanding enzyme specificity.

The specificity of entomopathogenic enzymes is strongly influenced by genetic factors that regulate enzyme production and activity. Multiple studies regarding entomopathogenic genomes show that enzyme specificity is influenced by genetic variability [33]. For example, the enzyme's ability to degrade the insect's cuticle can be increased through the specific mutation of the protease genes [33]. Another example is the chitinase enzyme that only binds to insect-specific chitin structures and only acts on its target [34, 35]; protease becomes more specific through substrate preferences that match the proteins in an insect's cuticle. These adaptations are critical for developing effective and environmentally friendly biopesticides without negatively impacting non-target insect populations [36].

Environmental factors, such as temperature, pH, and humidity, can all influence the specificity and activity of enzymes [37]. Field applications often expose these enzymes to fluctuating environmental conditions, prompting research into engineering enzymes that remain stable and active under diverse circumstances [38, 39]. They have shown that techniques such as enzyme encapsulation and stabilizing additives can provide some protection for enzymes under diverse conditions so that they remain efficacious. With these recent advances in understanding enzyme specificity, new, more targeted, and efficient biocontrol agents are in the lead. Genetic and biochemical modification of entomopathogenic enzymes refines the distinct genetic and biochemical characteristics of these enzymes [40]. These innovations hold promise for pest management solutions that reduce environmental risks while reducing adverse effects on non-target insect populations.

3. Applications in Pest Management

The use of entomopathogenic enzymes is emerging as an environmentally friendly replacement for chemical pesticides in pest management, and they have managed to progress in controlling pests with only a slight environmental impact. The effectiveness of different entomopathogenic enzymes in controlling a wide range of pest species has been summarized in Table 1.

Table 1. The effectiveness of the entomopathogenic enzymes.

Pest Target	Enzyme Source	Enzyme Type(s)	Effectiveness	Reference
Cotton pest (*Helicoverpa armigera*)	*Beauveria bassiana*	Protease	70% mortality in two weeks	[41]
Termites	*Metarhizium anisopliae*	Chitinase	90% reduction in four weeks	[42]
Red flour beetle (*Tribolium castaneum)*	*Bacillus thuringiensis*	Lipase	85% decrease in infestations	[43]
Aedes aegypti larvae	*Metarhizium*spp.	Chitinase, Lipase	95% mortality	[44]
Soybean pest (*Spodoptera litura*)	Not specified	Protease	60% population reduction	[45]
Bark beetles (*Dendroctonus ponderosae)*	Not specified	Chitinase	75% reduction in one month	46]
Thrips in horticultural crops	Mixed sources	Chitinase, Lipase	80% reduction in greenhouse trials	[47]
Maize stem borer	*Beauveria bassiana*	Not specified	60% reduction in infestation	[48]
Brown planthopper (*Nilaparvata lugens*)	Not specified	Protease	Reduction to ~30% ± 10% of initial population in two weeks	[49]
Aphids	Not specified	Chitinase, Horticultural oils	65% reduction	[50]
Grapevine moth (*Lobesia botrana)*	Not specified	Chitinase	68% reduction in pest population	[51]
Fruit fly (*Bactrocera dorsalis)*	Not specified	Chitinase	70% reduction in population and fruit damage	[52]

3.1. Successful applications of entomopathogenic enzymes.

Entomopathogenic enzymes have applications in many industries, such as agriculture, forestry, public health, and stored product protection. In agriculture, such treatment methods based on enzymes have been widely used as they prevent the expansion of pest species that damage crops and thus contribute to sustainable farming approaches. The various applications of entomopathogenic enzymes across agriculture, forestry, public health, and post-harvest protection sectors are presented in Table 2.

Table 2. Application fields of the entomopathogenic enzymes.

Application Field	Target Pest/Use	Enzyme Type(s)	Effectiveness	Purpose/Outcome	Reference
Agriculture	Bollworms in cotton fields	Protease	Effective control	Reduces bollworm population, supports sustainable agriculture	[12,41]
Agriculture	Stem borers in maize fields	Not specified	Significant efficacy	Reduces stem borer population, reduces the need for chemical pesticides	[48]
Forestry	Bark beetles (*Dendroctonus ponderosae*)	Chitinase, Protease	High efficacy in reducing beetle population	Conserves biodiversity, minimizes tree mortality	[46]
Agriculture	Planthoppers in rice fields	Protease	~70% population reduction	Lowers pest population with sustainable impact	[49]
Stored Product Protection	Beetles and weevils in stored grains	Lipase, Chitinase	Effective protection of stored grains	Maintains grain quality, reduces post-harvest losses	[19,51]
Public Health	Mosquito larvae (e.g., Aedes aegypti)	Not specified	High mortality rates in mosquito larvae	Eco-friendly alternative to larvicides reduces vector-borne disease risk	[53]

3.2. IPM.

IPM is a multi-pronged approach that combines natural enemies, cultural practices, physical barriers, and chemical controls to suppress the pest population. A primary feature of IPM programs is using environmentally compatible, specific biocontrol agents based on enzymes [54]. Enzymes are also unlike broad-spectrum chemical pesticides and have a minimal ecological impact in that they target specific pest species and degrade naturally, leaving minimal residue where they occur [55]. Incorporating enzymes into sustainable IPM promotes the conservation and biodiversity of beneficial organisms [56].

Combining enzymes with IPM frameworks significantly enhances the efficacy of both chemical and non-chemical pest control strategies [56]. For instance, enzyme treatments combined with reduced quantities of chemical pesticides can manage pests such as the brown planthopper in rice fields similarly, with much less environmental damage and still with a degree of control [57]. Much like enzyme sprays used with physical barriers, like insect-proof netting and trap crops, thrips in greenhouses have been successfully controlled [58]. Environmental compatibility and suitability of entomopathogenic enzymes for IPM are due to their site-specificity [59]. Without affecting non-target species, these enzymes target pest-specific structures, such as chitin and cuticular proteins [60]. This selectivity is important to maintain ecological balance and sustainable pest management practices [60].

Enzyme-based agents also address the growing issue of pesticide resistance and are highly compatible with current IPM strategies [61]. The attribute of this feature turns it into a very good strategy for managing resistant pest populations and confirming long-term pest control sustainability. Additionally, using fewer chemical pesticides and enzyme-based IPM approaches works to lower residues of harmful chemicals in our foods and the environment, thereby protecting human health [61].

The general action of enzymes is also limited to the desired target, reducing damage to beneficial insects, like pollinators and natural enemies, which are key to sustaining ecosystem balance. Preservation of these beneficial populations allows for using enzyme-based treatments, which increase biodiversity and facilitate natural pest control processes while establishing a self-regulating system that minimizes the need for frequent interventions [62]. This ecological harmony and enzyme-based biocontrol agents are ideally suited to the central principle of IPM. While many advantages exist, there are still challenges in scaling up enzyme-based IPM programs. This self-regulating system minimizes the need for frequent interventions, aligning with the core principles of IPM [62]. Despite these challenges many advantages, enzyme-based IPM programs face challenges in scaling up. High production costs, formulation optimization, and regulatory hurdles remain significant obstacles to widespread adoption [63]. With continued advancements and increasingly streamlined regulatory pathways, we anticipate that enzyme-based IPM strategies will grow to have an ever-increasing agricultural and environmental role in sustainable agriculture [63].

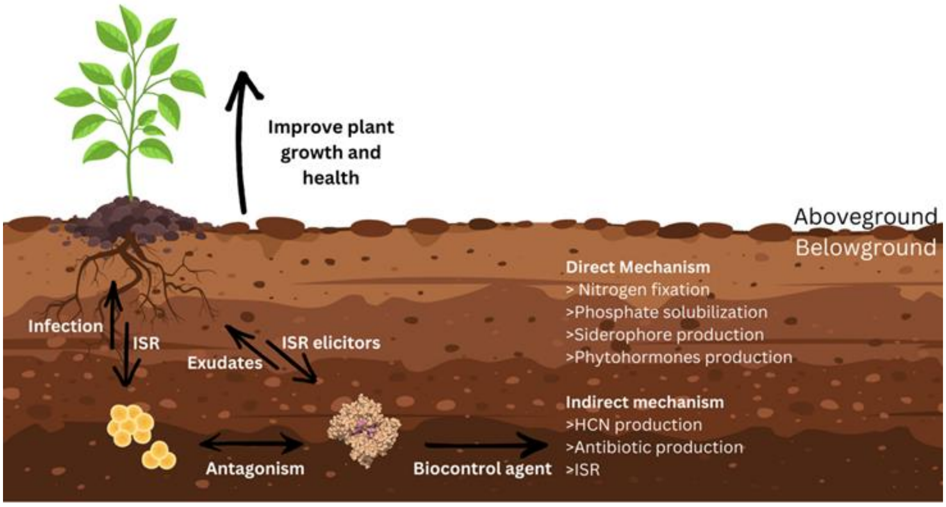

Figure 3. The biological control interactions. The illustration depicts the biological control interactions between biocontrol agents, antagonism, and plant growth and health improvement. This figure was recreated based on an open-access article [64].

4. Technological Innovations and Formulations

Table 3 highlights recent innovations in entomopathogenic enzymes, focusing on enhancing their stability, compatibility, and efficiency with integrated pest management (IPM) strategies.

Table 3. The innovations of entomopathogenic enzymes.

Aspect	Key Points	Examples	References
Advancements in Enzyme Production	- Enhanced efficiency and yield through microbial fermentation and genetic engineering.	- Microbial strains: *B. thuringiensis*, *B.Bassiana*, *M.anisopliae* engineered for producing high yields of proteases, chitinases, and lipases.	[64]
	- Optimization of fermentation conditions (e.g., pH, temperature, nutrient concentration).		[65]
Genetic Engineering	- Genome modification for more specific, stable, and active enzymes.	- Hybrid enzymes: Chitinase-lipase and protease-chitinase combinations.	[66]
	- Development of hybrid enzymes with multiple functional domains for targeting multiple pest structures.	- Genetic modifications enable enzyme functionality across varying temperatures for broader applications.	[67]

Aspect	Key Points	Examples	References
	- Enhanced enzyme stability under diverse environmental conditions.		[67]
Delivery Mechanisms	- Traditional liquid formulations are prone to degradation by UV radiation and temperature fluctuations.	- Microencapsulation for moisture/pH-triggered enzyme release.	[68]
	- Advanced encapsulation techniques protect enzymes and enhance stability.	- Nanoencapsulation using nanomaterials for targeted delivery.	[69]
	- Controlled release ensures prolonged activity and reduced reapplication.	- Liposomal encapsulation shields enzymes and increases bioavailability.	[69]
Nanotechnology in Formulation	- Nanoparticles are enzyme carriers that protect against degradation and allow controlled release.	- Nanomaterial-based encapsulation.	[70]
	- Nano-clays improve enzyme stability and persistence in field applications.	- Nano-clay-enhanced formulations for soil and crop applications.	[71]
	- Solid formulations (e.g., powders, granules) enhance compatibility with pest management practices.	- Powder and granule forms for easy application.	[71]
Role in Integrated Pest Management (IPM)	- Reduction in production costs.	- Enzyme-based pest control integrated into IPM strategies to replace traditional chemical pesticides.	[72]
	- Increased enzyme stability and improved delivery mechanisms.		[72]
	- Compatibility with other pest management methods promotes sustainable pest control practices.		[72]

4.1. Stability, storage, and compatibility advancements.

Recent advancements in entomopathogenic enzyme technology focus on improving their stability, delivery, and integration into pest management frameworks, as summarized in Table 4 below.

Table 4. The advancements of the entomopathogenic enzymes.

Strategy	Method	Benefits	Challenges	References
Use of Stabilizing Agents	Addition of stabilizers like glycerol, sorbitol, polyethylene glycol (PEG), and polyvinyl alcohol (PVA)	-**Structural Integrity**: Prevents enzyme denaturation by binding to enzyme molecules, thus maintaining their structure.	- **Increased Cost**: Some stabilizers, especially polymers, can raise formulation costs.	[73]
		- **Thermal and UV Stability**: Stabilizers such as glycerol have been shown to improve enzyme resilience against high temperatures.	- **Toxicity Concerns**: Certain stabilizers may pose environmental or application toxicity issues.	[74]
		- **Extended Shelf Life**: Prolongs the enzyme's usable period, reducing the frequency of purchases and replacements.	- **Impact on Activity**: Over-stabilization may reduce enzymatic activity or efficacy under certain conditions.	[75]
Controlled Release Carriers	Use of biodegradable carriers like alginate and chitosan, along with advanced carriers such as nanoparticles, liposomes, and microcapsules	- **Extended Pest Control**: Carriers such as alginate slowly dissolve in water, releasing enzymes gradually and maintaining pest control over extended periods.	- **Complex Manufacturing**: Carrier-based formulations often require specialized production processes, increasing complexity and cost.	[76]

Strategy	Method	Benefits	Challenges	References
		- **Targeted Delivery**: Nanoparticles and liposomes offer precise enzyme delivery, reducing non-target effects.	- **Environmental Impact**: Nanoparticles and certain polymers need careful evaluation for potential long-term effects on soil health and ecosystem balance.	[77]
		- **Improved Field Stability**: Microcapsules protect enzymes from UV radiation and temperature fluctuations, which are especially valuable for outdoor applications.	- **Controlled Release Adjustments**: Release rates may vary under different environmental conditions, requiring tailored formulations for specific climates or pest pressures.	[78]
		- **IPM Compatibility**: Carriers allow enzymes to be co-applied with microbial pesticides or low doses of chemical insecticides, integrating easily into IPM practices.		[79]
Enhanced Storage Techniques	Techniques like freeze-drying, temperature-controlled packaging, and desiccant-based storage systems	- **Longevity**: Freeze-drying removes moisture, preserving enzyme activity over long storage periods.	- **Specialized Storage Requirements**: Freeze-drying and temperature control may require an initial investment in equipment and specific storage conditions, which is especially challenging in rural or resource-limited settings.	[80]
		- **Field Applicability**: Temperature-controlled packaging and desiccants protect enzymes during transport and storage, allowing for reliable use even in remote areas with fluctuating conditions.	- **Rehydration Step**: Freeze-dried enzymes require rehydration, so a preparation step is added before application.	[81]
		- **Immediate Activation**: Freeze-dried enzymes can be quickly rehydrated on-site for immediate use, ensuring optimal activity at the time of application.		[82]
Integration with IPM Practices	Co-application of enzyme-based agents with other IPM methods, including microbial insecticides and low-dose pesticides	- **Synergistic Effect**: Enzyme applications enhance the effectiveness of other biocontrol methods, achieving higher pest suppression rates while reducing chemical use.	- **Compatibility Testing**: Each enzyme must be tested with other IPM agents to ensure efficacy and avoid adverse interactions.	[83]
		- **Environmental Safety**: Enzymes target specific pest physiology, supporting biodiversity by sparing non-target organisms, such as pollinators and beneficial insects.	- **Field Integration**: Practical deployment requires careful planning to balance application timing and methods among different IPM components.	[84]
		- **Reduced Chemical Dependency**: With enzymes as part of IPM, the need for chemical pesticides is reduced, supporting long-term sustainable pest management practices.		[85

4.2. Nanotechnology and biotechnology applications.

As shown in Table 5 below, various approaches like nano-encapsulation, nanocomposite films, protein engineering, and hydrogels have significantly improved the field application of these enzymes.

Table 5. The Nanotechnology and biotechnology applications of entomopathogenic enzymes.

Method	Description	Benefits	Examples	References
Nano-encapsulation	Enzymes are encased within nanoparticles, protecting them from environmental factors like UV radiation and moisture.	- **Extended Stability**: Nanoparticles shield enzymes from degradation by acting as a physical barrier.	- **Liposomes**: Biocompatible carriers from lipid bilayers for controlled release.	[86]
		- **Controlled Release**: Nanoparticles can regulate enzyme release rates for prolonged activity.	- **Chitosan Nanoparticles**: Biodegradable and adhesive, derived from crustacean shells.	[87]
		- **Targeted Delivery**: Directs enzymes to pest sites, reducing off-target effects.	- **Silica Nanoparticles**: Improved chitinase stability in pest control.	[88]
Nanocomposite films	Films applied to crop surfaces gradually release enzymes in response to environmental cues like moisture or pH changes.	- **Sustained Effect**: Ensures a consistent pest control response even in varying weather conditions.	- Applied as protective coatings on crops for consistent pest control.	[89]
		- **Reduced Wash-off**: Protects enzymes from being washed off, minimizing the need for reapplication.		[90]
Hydrogels	Hydrogels release enzymes in response to environmental changes, like moisture levels.	- **Controlled Release**: Respond to environmental triggers, providing effective delivery based on conditions.	Hydrogels with enzyme incorporation for moisture-responsive release.	[91]
		- **Reduced Application Frequency**: Minimizes the need for repeated applications.		[92]
Protein engineering	Genetic modification to enhance enzyme resistance to temperature, pH, and other stress factors.	- **Improved Thermal Stability**: Engineered enzymes maintain activity at high field temperatures.	Thermally stable enzymes used in agriculture for pest control.	[93]
		- **Increased Specificity**: Modified enzymes target specific pests, minimizing impact on non-target species.		[94]
Creation of multifunctional enzymes	Combining activities (e.g., protease and chitinase) within a single enzyme molecule for enhanced pest control.	- **Enhanced Efficacy**: Multifunctional enzymes can disrupt multiple pest defenses simultaneously.	Protease-chitinase combination enzymes for broad-spectrum pest control.	[95]
		- **Reduced Production Costs**: Fewer applications and potentially simplified production.		[96]

5. Challenges and Limitations

While the potential of entomopathogenic enzymes as eco-friendly alternatives to chemical pesticides is promising, many challenges exist to the current widespread uptake in pest management [97]. These challenges include economic, regulatory, and technical limitations and knowledge gaps that require further research and innovation.

Despite several reductions in costs due to the development of microbial fermentation and genetic engineering, enzyme production at a commercial scale remains costly relative to conventional chemical pesticides [98]. This high cost restricts the affordability and utility of enzyme-based pest control solutions for large-scale agricultural applications, especially where cost efficiency is critical [99]. Another significant challenge is enzyme degradation, a particular issue in outdoor field applications where environmental conditions do not widely favor enzyme stability. Encapsulation and other formulation techniques to stabilize enzymes have been attempted, but achieving consistent enzyme activity under field conditions is a technical problem [100].

The specificity of entomopathogenic enzymes is a double-edged sword. While it reduces the likelihood of non-target impacts, it can also limit the broad-spectrum efficacy required for some pest control scenarios [101]. For example, an enzyme capable of killing one pest species might have no effect in killing a similar-looking species. However, this specificity can restrict the applicability of enzyme-based products in terms of the versatility of given products to effectively control the various populations of pests [102].

Market limitations also pose a significant barrier to adopting enzyme-based pest control solutions. [103]. The market for biological pesticides is still relatively niche since farmers and pest management professionals often gravitate towards chemical solutions because they know they are effective, cost-effective, and easy to use [104]. Increasing market acceptance requires educating stakeholders about the long-term benefits and sustainability of enzyme-based pest management.

5.1. Environmental and regulatory issues.

Before commercial use, regulatory bodies require comprehensive data on enzymes' environmental impact, toxicity, and specificity [105]. This regulatory burden slows the introduction of new enzyme products to the market, restricts their availability, and delays benefits to farmers and the environment [106].

A key environmental concern with enzyme-based pest control is its interaction with non-target organisms and overall ecosystem health [107]. Although enzymes are naturally biodegradable, there remains a requirement to eliminate, where possible, any potential detriment to beneficial insects, soil health, and aquatic ecosystems [108]. Also, if genetically modified organisms (GMOs) that produce such enzymes are released, additional regulatory and public acceptance challenges arise [109]. First, in many countries, GMOs are subject to strict regulations, as are GMOs and GMOs, further obstructing the regulatory pathway for genetically engineered enzyme-producing organisms (GEOs) [110].

Regulatory challenges include ensuring compatibility with existing IPM systems and pest control practices. Enzyme-based solutions must work harmoniously with other biological, cultural, and chemical control options to be widely adopted [111]. Regulatory agencies often evaluate new pest control products for their ability to integrate safely and effectively into comprehensive pest management plans. This requirement adds complexity to the approval process, necessitating extensive field testing and compatibility studies based on IPM integration criteria [112]. It makes regulatory approval difficult because it requires extensive field testing and compatibility studies using IPM integration criteria.

Allowing regulatory processes to streamline around enzyme-based pest control products more easily and developing a better working relationship between regulatory bodies, research, and manufacturing towards more rapid approval and acceptance of these solutions [112]. Simplifying regulatory guidelines for the particular properties of biological control agents can increase market adoption of enzyme-based products, encourage innovation, and encourage a transition to more sustainable pest management practices [112].

Table 6 below highlights the current challenges and key research gaps with strategic priorities that require immediate or secondary attention.

Table 6. Research gap and priorities in enzyme-based pest management.

Research Area	Description	Example / Specific Focus	Priority	Citation
Cost Reduction	Developing more efficient methods for enzyme production to lower costs and improve accessibility for large-scale applications	Optimizing fermentation conditions and exploring high-yield microbial hosts for enzyme production	Immediate Priority	[12-14]
Enzyme Stability	Enhancing enzyme resilience under environmental stresses like UV radiation, temperature, and humidity	Nano-encapsulation and hydrogels for UV protection and controlled release in outdoor conditions	Immediate Priority	[20, 21]
Regulatory Compliance	Conducting studies on non-target effects and ecosystem impact to meet regulatory standards	Trials on enzyme safety regarding beneficial insects, soil health, and biodiversity to streamline approval processes	Immediate Priority	[22,34]
Broader Pest Targeting	Engineering enzymes to be effective against a wider range of pest species	Genetic modifications to produce multifunctional enzymes capable of targeting multiple pest defenses	Secondary Priority	[38]
Environmental Resilience	Creating formulations that maintain enzyme effectiveness across diverse environmental conditions	Temperature- and moisture-responsive formulations that activate enzymes.	Immediate Priority	[47, 48]
Field Testing and Efficacy	Conducting large-scale, real-world trials to validate enzyme-based pest control in various environments	Field tests on enzyme efficacy and durability across different agricultural zones and climates	Secondary Priority	[51, 57]
Education and Market Awareness	Promoting understanding of enzyme-based pest control among farmers, stakeholders, and the general public	Awareness campaigns on the benefits and safe usage of enzyme-based biocontrol agents in integrated pest management (IPM) systems	Secondary Priority	[64, 65]
Genetic Engineering Advances	Exploring hybrid or multifunctional enzymes to increase pest control range and efficiency	Developing enzymes that combine protease and chitinase activity for enhanced pest control coverage	Immediate Priority	[69- 71]
Formulation Innovation	Developing formulations that activate in response to temperature and moisture levels for sustained pest control	Use of polymers like PEG and PVA in enzyme formulations for triggered release and extended stability in variable field conditions	Secondary Priority	[85,86]
Long-term Environmental Impact	Assessing the cumulative ecological effects of enzyme-based pest control on non-target organisms and ecosystems	Studies on the impact of long-term enzyme use on soil microbial diversity, pollinator health, and food chain dynamics	Immediate Priority	[90]
Compatibility with Biological Controls	Investigating synergy with other biocontrol agents for enhanced integrated pest management (IPM)	Combining enzyme-based treatments with microbial insecticides for increased pest suppression and IPM compatibility	Secondary Priority	[92-94]

Research Area	Description	Example / Specific Focus	Priority	Citation
Commercial Viability	Addressing adoption barriers and enhancing market potential for enzyme-based pest control	Research on cost-effective production and storage solutions to increase commercial appeal in agricultural markets	Immediate Priority	[104]
Public Acceptance	Studying public perception and acceptance of enzyme-based pest control to facilitate adoption	Surveys and studies to assess consumer attitudes and potential regulatory concerns related to enzyme-based pesticides	Secondary Priority	[109,113]

6. Future Directions and Opportunities

The future of enzyme-based pest control depends on enhancing production techniques and adopting biotechnological innovations to boost enzyme efficiency and versatility. This novel approach offers a promising alternative to conventional pesticides, ensuring decreased environmental harm, less damage to helpful insects, and improved accuracy in targeting pest species. To ensure these solutions are commercially successful and effective in diverse environmental conditions, it's essential to enhance enzyme production and formulation [113]. Advances in biotechnology, particularly in synthetic biology and metabolic engineering, have revolutionized microbial fermentation, the main technique for enzyme production [114]. Scientists can improve enzyme production and customize enzyme traits for particular pest targets by genetically altering microorganisms, as shown in research. [114]. This approach enhances the efficacy of biopesticides and contributes to making them a more cost-effective option [114].

Formulation techniques also deserve additional investigation. Creative methods such as encapsulation can shield enzymes during storage and usage, maintaining their activity for longer. This ensures that enzymes remain effective even under challenging environmental conditions [115].

7. Conclusion

Entomopathogenic enzymes serve as promising environmentally friendly substitutes for chemical pesticides in pest control. Studies utilizing enzymes that possess natural insecticidal characteristics, such as proteases, chitinases, and lipases, have resulted in the creation of pest management approaches that are environmentally friendly, leave minimal residues, and target specific pest species effectively [116]. These enzymes interfere with insect pests' structural, protective, and physiological functions, effectively controlling their populations, aligned with integrated pest management (IPM) principles [117]. Enzyme-based biocontrol solutions contribute to healthier ecosystems by safeguarding non-target species, such as pollinators and natural predators, while promoting biodiversity [117]. Nonetheless, unlocking the complete potential of enzyme-based pest control entails several significant challenges. The elevated production and enzyme instability in outdoor environments and the regulatory challenges continue to represent significant obstacles [118]. Progress in microbial fermentation, genetic modification, and formulation methods, including encapsulation and nanoencapsulation, has begun to reduce costs and enhance enzyme stability [118]. Ongoing investment in biotechnology will enable additional advancements in enzyme engineering and bioinformatics to enhance enzyme specificity, durability, and adaptability to new pest and environmental situations [119]. These advancements are crucial for the economical and environmentally safe application of enzyme-based pest control in field conditions. The future of enzyme-based pest control includes exploring the combined application of biocontrol agents, such as microbial insecticides and natural predators, to develop effective and strong pest management strategies [120]. These combined strategies can enhance the use of enzymes and promote the dissemination of these environmentally friendly techniques [120]. Entomopathogenic enzymes are set to significantly aid sustainable agriculture and ecosystem well-being as ongoing research addresses production, stability, and regulatory issues. Continued research and development will emphasize this continuous research and development and promote the shift towards an enzyme-based pest control approach that will advance sustainable pest management [120].

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Conflicts of Interest

There are no competing interests or financial and personal conflicts regarding the research presented in this paper.

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