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Eco-Biochemical Approaches to Sandfly Vector Reduction: Mechanisms, Potential, and Application Strategies

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Abstract: Leishmaniasis remains a critical global health challenge, yet traditional management of its sandfly vectors is increasingly hindered by insecticide resistance and the environmental toxicity of chemical agents. This review provides a novel synthesis of eco-biochemical alternatives, shifting focus from general microbial biocontrol to the specific molecular mechanisms of entomopathogenic chitinases, proteases, and lipases. The scope encompasses a detailed examination of cuticle degradation pathways and the mid-gut colonization process, identifying how these enzymes facilitate host invasion and systemic infection. Key insights highlight the synergistic "enzyme cocktail" effect, where the simultaneous action of multiple enzymes significantly enhances larval mortality and disrupts adult physiology. Furthermore, the review evaluates advanced application strategies, including CRISPR-mediated genetic enhancement, nanoparticle encapsulation for controlled release, and successful low-cost trap-based deployment in field settings. By bridging biochemical theory with practical delivery tactics, this work establishes a comprehensive framework for sustainable, high-specificity sandfly vector reduction in global health programs.

Keywords: entomopathogenic enzymes; sandfly; mechanisms; potential; application; biocontrol agents.

1. Introduction

Phlebotomine sandflies are primary vectors of sandfly-borne leishmaniasis, caused by protozoan parasites of the genus *Leishmania*, and the disease represents a significant global health challenge [1]. This disease affects an estimated 700,000–1 million new cases annually, recording severe manifestations such as visceral, mucocutaneous, and cutaneous leishmaniasis [2]. Some regions, such as South Asia, East Africa, and Latin America, are impoverished communities that are disproportionately impacted, particularly by visceral leishmaniasis, which is fatal if untreated [2]. According to Rocklöv and Dubrow [3], an effective vector management strategy is required due to industrialization and climate change, which are contributing to the surging sandfly population.

Most chemical insecticides used contain organophosphates, pyrethroids, and synthetic organochloride insecticides (e.g., DDT) as major components to suppress sandflies [4]. However, the prolonged use of these synthetic chemicals results in the development of insecticide resistance among sandfly populations [4]. It also causes environmental contamination with lingering effects that unintentionally harm non-target organisms such as insects, wildlife, and human health [5]. Moreover, continuous use is required with chemical insecticides, increasing control costs and logistical challenges, especially in resource-limited areas [5].

Due to their capacity to produce bioactive substances and extracellular enzymes, fungi have numerous biotechnological and environmental uses, such as biodegradation, bioremediation, biofuel production, pharmaceutical development, and biological pest management [6–8]. As a result, the use of environment-friendly biological methods based on naturally occurring, highly host-specific microorganisms has emerged as a viable and effective approach for reducing the impact of sandfly infestations on the environment and managing their populations and their impact on humans [6]. Many other organisms can also be used as biological agents. Microsporidia are pathogenic protists that infect a variety of insect species, including silkworms and honey bees, and have significant potential as an insect control method [7]. Similarly, entomopathogenic nematodes (*Steinernema spp*., *Heterorhabditis spp*.) can infect insects and create septicemia in the host, which ultimately will result in the death of the host [9].

While biocontrol strategies have attracted attention as alternatives to chemical insecticides, most existing reviews focus either on general biological control methods or on isolated microbial agents in vector management [9]. This review is to systematically synthesize and integrate current knowledge on entomopathogenic enzymes, specifically protease, chitinase, and lipase, as distinct bioactive compounds for sandfly control. This review differs from existing literature in three key aspects: it focuses on the specific enzymatic mechanisms by which fungal and microbial metabolites disrupt sandfly physiology, it bridges the gap between laboratory-level enzyme characterization and field-applicable biocontrol strategies, and it evaluates the scalability, cost-effectiveness, and environmental sustainability of enzyme-based approaches in resource-limited settings endemic to leishmaniasis. This review aims to position entomopathogenic enzymes as a viable complementary tool within integrated vector management (IVM) programs.

2. Methodology for Literature Section

A systematic search was conducted across the following electronic databases from 2020 to 2025: PubMed/MEDLINE, Web of Science, Scopus, Google Scholar, and CAB Abstracts. PubMed/MEDLINE was selected for its comprehensive coverage of biomedical literature on vector control and parasitic diseases. Web of Science was utilized for search capabilities and citation tracking to identify seminal and recent publications. Scopus was incorporated to ensure broader coverage of life sciences and applied research beyond traditional biomedical indexing. Google Scholar was consulted for literature and supplementary sources, including conference proceedings and institutional repositories. CSB Abstracts was included to capture literature from veterinary and agricultural entomology fields, which frequently contain data on insect physiology and control strategies applicable to sandfly vectors.

The search strategy combined controlled vocabulary, including the core search string applied across all databases, was (entomopathogenic AND (enzyme OR protease OR chitinase

OR lipase)) AND (sandfly OR Phlebotomine). To capture related literature, supplementary searches were conducted using the following strings: (sandfly biocontrol OR "vector control") AND (fungi OR fungal OR microbial); (leishmaniasis vector) AND (enzyme* OR biocontrol OR biopesticide); and (Leishmania) AND (entomopathogenic OR biological control). To ensure relevance and applicability, the following exclusion filters were applied: non-English-language publications were excluded; studies published more than 20 years prior to the search were excluded to prioritize contemporary evidence; and literature focused solely on mammalian toxicology without implications for vector control was excluded.

Studies were included in the review if they comprised primary research or systematic reviews examining entomopathogenic microorganisms, including fungi, bacteria, and nematodes with potential applications in vector control. Included studies reported enzymatic mechanisms, specifically proteases, chitinases, lipases, or other proteolytic activities that target arthropod cuticles or disrupt critical physiological systems. Additionally, studies were included if they investigated sandfly or related dipteran vectors (Culicidae, Simuliidae) and demonstrated enzyme-mediated mortality or fitness reduction. Field trials, laboratory assays, and epidemiological studies evaluating biocontrol efficacy were incorporated, as were articles addressing integrated vector management (IVM) programs that incorporate biological control strategies.

Studies were excluded if they addressed chemical insecticide resistance without discussing or proposing biocontrol alternatives. Research focused exclusively on Leishmania parasite biology or transmission mechanisms without direct relevance to vector control interventions was excluded. Additionally, in silico studies and theoretical models lacking experimental validation were not included, as this review prioritized evidence-based findings. Literature discussing only whole-organism parasites without characterizing or isolating specific enzymatic mechanisms was excluded to maintain focus on enzyme-based approaches. Finally, non-peer-reviewed opinion pieces and editorials were excluded to ensure methodological rigor and reliability.

3. Entomopathogenic Bacteria and Fungi in Sandfly Control

An environmentally friendly alternative uses entomopathogenic fungi and bacteria to naturally infect sandflies, causing their mortality through enzymatic degradation and systemic infection [9]. Fungi such as *Beauveria bassiana* and *Metarhizium anisopliae*, and bacteria such as *Bacillus sphaericus* and *Bacillus thuringiensis* (Bt) are among the widely studied agents for microbial-derived biocontrol [10]. According to Sabbahi *et al*. [10], they are proven effective against sandflies. These entomopathogenic organisms employ mechanisms primarily targeting larval, pupal, and adult stages of sandflies in breeding habitats.

Beauveria bassiana and *Metarhizium anisopliae*, soil-dwelling ascomycete fungi, initiate infection by adhering to the sandfly cuticle via conidia (spores), a process mediated by hydrophobic surface proteins and mucilaginous sheaths that facilitate intimate contact with the host exoskeleton [10]. The germination process is not simply a mechanical breach; rather, fungal hyphae employ a sophisticated, multi-enzyme cascade to penetrate the cuticle. This degradative process is initiated by the sequential secretion of protease isoforms, including serine proteases, aspartic proteases, and metalloproteases, which target structural proteins such as alpha-helical sclerotized proteins and resilin, the elastomeric protein conferring mechanical flexibility to the arthropod exoskeleton [10]. Concurrently, chitinase enzymes (including endochitinases and exochitinases) hydrolyze the β-1,4-glycosidic bonds within chitin polymer

chains, fragmenting the rigid chitinous matrix that comprises the exoskeletal scaffold [10]. This dual enzymatic attack is strategically coordinated, while proteases disrupt protein-protein cross-linking and resilin elasticity, chitinases depolymerize the structural polymer itself. Additionally, lipases degrade the lipid-rich epicuticle, the outermost waxy layer composed of long-chain hydrocarbons and lipids that normally serves as a desiccation and immunological barrier against microbial penetration. Once hyphal penetration breaches the cuticle and reaches the hemocoel (insect blood cavity), Metarhizium anisopliae secretes cyclic peptides termed destruxins cyclodepsipeptides that function as non-selective ion channel toxins [11]. These secondary metabolites disrupt calcium (Ca^{2+}) and potassium (K^{+}) homeostasis in the insect nervous system and musculature, causing paralysis, loss of postural control, and cessation of feeding behavior [11]. Destruxins additionally suppress the insect's cellular immune response by impairing hemocyte (insect immune cell) phagocytic function and encapsulation reactions, thereby facilitating continued fungal proliferation within the hemocoel [11]. Published studies report highly variable proportions of protease, chitinase, and lipase activity across different B. bassiana and M. anisopliae strains, ranging from approximately 10-60% of total secreted enzymatic activity, yet mechanistic explanations for these strain-level differences remain unexplored [12]. Furthermore, while Onsongo *et al*. [12] documented approximately 70% larval mortality within 15 days under controlled laboratory conditions (28°C, 80% relative humidity), this finding has not been consistently replicated across different sandfly species, geographic isolates, or field settings. The discrepancy between laboratory and field efficacy is likely driven by temperature fluctuations, UV radiation degradation of conidia, and variable soil moisture, highlighting a critical gap. Field-based kinetic studies characterizing the persistence and enzymatic activity of fungal inocula in natural breeding habitats remain largely absent from the literature [12].

In contrast to fungal cuticular penetration, *Bacillus sphaericus* and *Bacillus thuringiensis israelensis* (Bti) employ a fundamentally different mechanism of toxin delivery via the larval midgut epithelium. When sandfly larvae inhabiting moist breeding sites ingest bacterial cells or spores, Cry toxins (crystal proteins), proteinaceous inclusion bodies produced during bacterial sporulation, enter the larval digestive tract [13]. The alkaline pH of the insect midgut (typically pH 9-11) solubilizes these crystalline inclusions, releasing Cry protoxins that are subsequently proteolytically activated by midgut serine proteases into smaller, catalytically active toxin fragments (approximately 60-70 kDa for Cry1A toxins) [13]. The molecular mechanism of Cry toxin pathogenesis involves a multi-step process that differs markedly from fungal enzymatics. Activated cry fragments possess a three-domain structure (Domain I: alpha-helical pore-forming region; Domain II: beta-sheet receptor-binding domain; Domain III: lectin-like domain with potential immunomodulatory functions) [13].

Domain II of the Cry toxin binds with high affinity to specific cadherin-like and alkaline phosphatase receptors on the apical surface of midgut epithelial cells. This specificity confers the remarkable selectivity of Bti for dipteran larvae while minimizing toxicity to non-target aquatic invertebrates and vertebrates [14]. Following receptor binding, Domain I inserts into the midgut epithelial cell membrane, oligomerizing to form large cation-selective pores (approximately 1-2 nanometers in diameter) [14]. This pore formation disrupts the osmotic gradient maintained across the epithelial barrier, leading to uncontrolled influx of sodium (Na^{+}) and calcium (Ca^{2+}) ions and efflux of potassium (K^{+}), resulting in cell lysis and barrier dysfunction, a process termed osmotic lysis or septicemia [14]. Secondary effects include

midgut bacterial translocation, physiological dysregulation, and larval death, typically occurring 24-72 hours post-exposure [14].

Critical distinction in mechanism: Unlike fungal enzymes that catalytically degrade polymeric substrates (chitin, collagen, lipids) through repeated cleavage events, Cry toxins function as non-enzymatic pore-forming toxins that act through physical insertion and structure-induced cell destruction [14]. This mechanistic difference has significant implications, whereby bacterial toxins do not require enzymatic substrate availability or cofactor status, rendering them more stable and persistent under variable environmental conditions. Indeed, Hong *et al*. [15] demonstrated that Bti spores maintain toxigenic potential in water for 4-6 weeks, substantially longer than the 7-10 days of persistence typically observed for fungal conidia in aquatic environments [15].

4. Enzyme-mediated Pathogenicity (Chitinases, Proteases, Lipases) in Entomopathogens

Approaches using entomopathogenic fungi and bacteria are principally mediated by extracellular enzymes that promote host invasion, nutrient acquisition, and systemic infection [16]. Enzymes such as proteases, chitinases, and lipases can degrade insect tissues and disrupt key physiological functions, thereby amplifying their pathogenicity and highlighting their potential as biocontrol agents against sandfly infestations [16]. These entomopathogens release the enzymes during sepsis, targeting the cuticle, gut, and internal tissues, leading to sandfly mortality [16].

Chitinases are known to hydrolyze chitin, a major structural component of the insect exoskeleton and the intestinal matrix [17]. In specific fungi such as Beauveria bassiana and Metarhizium anisopliae, chitinases possess CHI1 and CHI2 isoforms capable of penetrating the hypha through the cuticle, creating pathways for better establishment [18]. Once penetrated, the intestinal barrier is disrupted, allowing other enzymes and toxins to access the hemolymph [18]. Research on Phlebotomus spp. indicates that chitinase overexpression in M. anisopliae strains causes a substantial increase in larval mortality, ranging from 40% to 60%, due to the disruption of the sandfly's protective layers, resulting in desiccation and starvation [19].

The illustration below is a simple cross-section of a sandfly cuticle, demonstrating its three main layers from exterior to interior (Figure 1). A blue downward-pointing arrow denotes the secretion of enzymes onto the surface, while the purple circular symbols represent chitinase molecules. This illustration shows chitinase penetrating the middle and inner cuticle layers, hydrolyzing chitin polymers, and gradually degrading the cuticle's structure. This highlights the interaction between secreted enzymes and cuticular components during pathogen-induced infection.

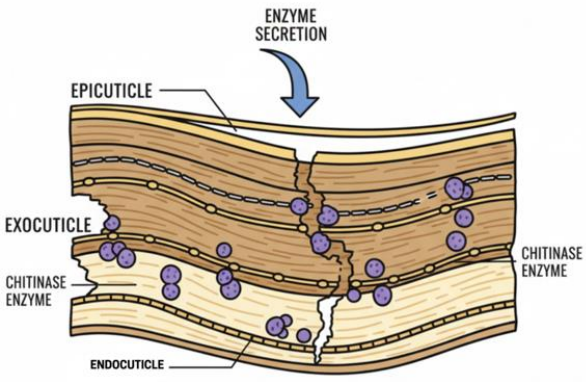

Figure 1. Cuticle degradation by chitinase.

As proteolytic enzymes, proteases such as subtilisins and metalloproteases break down proteins in the host cuticle and tissues, also providing nutrients for fungal growth while eliciting immune responses that weaken the insect [20]. In *B. bassiana*, proteases like Pr1 facilitate cuticle degradation, with combined effects when combined with chitinases [21]. For bacterial pathogens such as Bacillus thuringiensis, proteases aid in activating Cry toxins post-ingestion, thereby enhancing gut perforation in sandfly larvae [21]. A biocontrol approach using a high-protease-content formulation can effectively reduce adult sandfly emergence by specifically targeting the protein-rich tissues of the pupal stage [21]. Figure 2 shows a sandfly's exoskeleton, and the distinct layers are labeled from the outside cuticle toward the interior. The scattered green clusters within the layers represent protease enzymes. The arrows indicate the movement of enzymatic breakdown. The white cracks appearing across the layers visualize the loss of structural components caused by protease activity. The diagram depicts the movement of proteases as they infiltrate the layers and degrade protein components, leading to the progressive disintegration of the sandfly's exoskeleton.

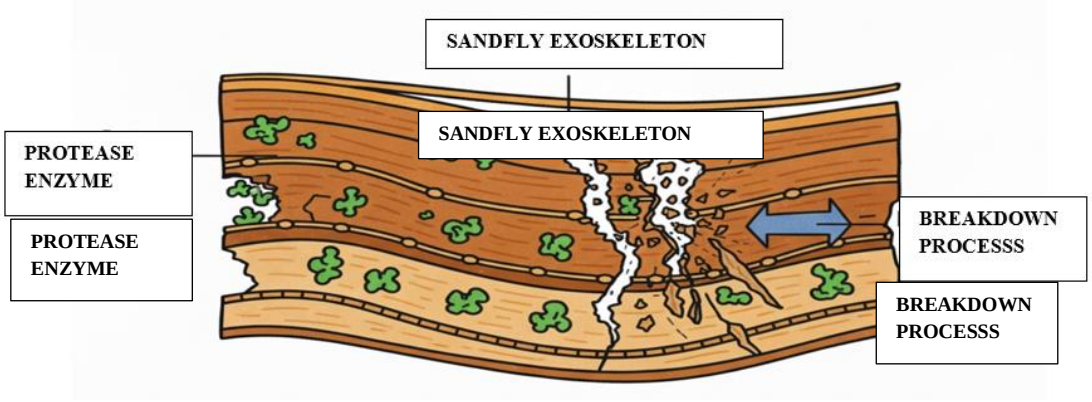

Figure 2. Protease-mediated exoskeleton degradation for sandfly biocontrol.

Lipases are enzymes that hydrolyze lipids by breaking down triglycerides into glycerol and free fatty acids, targeting the waxy epicuticle and internal fat bodies of sandflies [22].

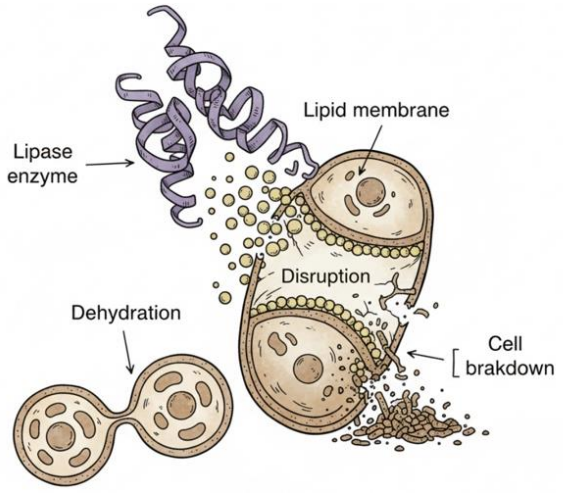

Figure 3. Lipase degrades the lipids.

In *M. anisopliae*, lipases disrupt lipid metabolism, causing energy depletion and immunosuppression. Combined with chitinases and proteases, they form an "enzyme cocktail" that accelerates infection. For instance, lipase-deficient mutants of *B. bassiana* exhibit 50% lower virulence against *Lutzomyia longipalpis*. In bacteria, lipases from *Bacillus sphaericus* contribute to larval gut damage, amplifying toxin effects in moist breeding sites.

Figure 3 shows how a lipase enzyme disrupts a cell. A purple squiggly line represents the lipase enzyme as it approaches and binds to the phospholipid bilayer of the cell membrane. The bilayer lipid sheet surrounding the cell interior with various organelles represents the membrane. The enzyme line represents the interaction of lipase penetrating the lipid layer. This interaction creates pores and disrupts the membrane, causing cell lysis and release of intracellular material from the infected host.

5. Comparative Analysis and Knowledge Gaps

Both fungal and bacterial biocontrol agents achieve larval mortality through mechanisms fundamentally distinct from chemical insecticide neurotoxicity, which typically operates through neuroreceptor antagonism or acetylcholinesterase inhibition, creating a low probability of cross-resistance development with existing synthetic pesticides [23]. Both agents also demonstrate high species specificity, although this specificity varies considerably between agents, with Cry toxins showing narrower specificity than fungal enzymes. This differential specificity reduces non-target impacts compared to broad-spectrum chemical insecticides, representing a significant ecological advantage in integrated vector management programs. However, critical contradictions exist between laboratory findings and field observations that warrant detailed examination. Laboratory studies consistently demonstrate greater than 70% mortality for fungal biocontrol agents. Yet, field trials conducted in endemic sandfly breeding habitats report substantially lower suppression of adult emergence, typically ranging from 20 to 50%, a discrepancy largely attributed to environmental degradation of conidia under fluctuating temperature, humidity, and UV radiation conditions [23]. In contrast, Bti achieves more consistent field efficacy, with larval suppression rates of 40-80% across diverse ecological settings, yet mechanistic explanations for this superior environmental persistence remain speculative rather than empirically demonstrated [24]. This paradox suggests that the biological properties conferring environmental stability in Bti, such as spore coat composition, metabolic dormancy, or toxin sequestration mechanisms, have not been comprehensively characterized in comparative studies with fungal inocula.

A second critical unresolved question concerns the translation of enzymatic substrate availability from controlled laboratory conditions to in vivo physiological environments. Published studies characterizing protease, chitinase, and lipase activity employ standardized in vitro conditions with optimized pH ranging from 6 to 8 and incubation at 37°C, yet sandfly hemolymph maintains a narrower pH range of 7.2 to 7.6, and tissue temperature fluctuates with ambient environmental conditions that often deviate substantially from laboratory parameters [24]. Whether enzyme kinetics and catalytic efficiency determined under standardized conditions accurately predict in vivo enzymatic performance in the hemocoel remains unclear, suggesting that apparent laboratory efficacy may overestimate real-world pathogenic potential [24].

A third area of contradiction concerns immune evasion mechanisms and their capacity to fully suppress sandfly defenses. While destruxins produced by *Metarhizium anisopliae* effectively suppress insect immune responses in model organisms, recent molecular evidence indicates that certain sandfly species, particularly *Phlebotomus* species endemic to regions with high leishmaniasis transmission, possess inducible antimicrobial peptide synthesis pathways, including defensins and cecropins that may partially counter fungal immunosuppression [24]. This phenomenon of potential immune resistance or compensatory immune responses has not been adequately addressed in current biocontrol literature, which predominantly assumes

fungal immunosuppression is unidirectional and complete rather than subject to countervailing insect defense mechanisms.

Several critical knowledge gaps require investigation to advance biocontrol development. First, enzymatic activity kinetics must be characterized not in isolated laboratory conditions but in natural soil and water matrices with variable pH, temperature, and organic matter content that reflect actual breeding habitat conditions. Second, comparative transcriptomic and proteomic analysis of successful versus failed fungal penetration attempts is needed to identify rate-limiting enzymatic steps and strain-level differences in enzyme expression patterns. Third, field-based pharmacokinetic studies quantifying toxin persistence, bioavailability, and dose-response relationships in endemic transmission settings are essential for predicting real-world efficacy and optimizing inoculum concentrations. Fourth, longitudinal studies examining potential cross-resistance or compensatory immune responses following repeated exposure to fungal or bacterial biocontrol agents would clarify whether repeated applications risk reducing long-term effectiveness through insect population adaptation.

The virulence of entomopathogenic fungi and bacteria fundamentally depends on the production, secretion, and in vivo efficacy of extracellular hydrolytic enzymes and secondary metabolites [24]. However, the current literature predominantly emphasizes isolated enzymatic mechanisms without adequately accounting for the complex interplay among multiple enzyme families, immunological context, and environmental persistence. Future biocontrol development requires moving beyond descriptive characterization toward systems-level understanding in which quantitative models integrate enzyme kinetics, insect immunological dynamics, and environmental degradation to predict field efficacy and optimize delivery strategies. Additionally, critical examination of strain selection represents an underexplored avenue for improving biocontrol reliability, as identification of high-enzyme-producing isolates with enhanced environmental stability could substantially improve the translation of biocontrol agents from laboratory research to resource-limited endemic settings where leishmaniasis transmission remains uncontrolled.

6. Mid-gut Colonization Pathway

One of the critical phases in the infection cycle of entomopathogens targeting host vectors is mid-gut colonization. Pathogens need to strategically establish themselves within the intestine after the initial enzyme-dependent virulence factors to overcome physical barriers and defense mechanisms [25]. Once ingested, entomopathogenic fungi and bacteria face a dynamic environment influenced by intestinal pH, digestive enzymes, and the peritrophic matrix. The overall infection success is determined by the entomopathogens' ability to germinate, survive, and proliferate in this environment.

During this process, the microbial metabolites and secreted enzymes interact directly with host tissues, disrupting gut physiology and weakening Leishmania parasites [25]. The entomopathogens create a hostile environment for parasites by disrupting nutrient absorption and protective barriers, resulting in altered immune responses. This contact increases the biocontrol potential of fungal and bacterial agents, making midgut colonization an important mechanism for pathogens to suppress the vector. Fungal species such as Metarhizium anisopliae use hyphal penetration and enzymatic degradation to disrupt intestinal structures, while bacteria such as Bacillus thuringiensis avoid antimicrobial pressure by forming biofilms. Table 1 below summarizes the main steps of the colonization pathway.

Table 1. Entomopathogen mid-gut colonization pathway in sandflies.

Pathway	Key Steps & Mechanisms	Representative Pathogen(s)	Observed Effect	Reference
Ingestion-Based Entry	Spores or conidia are ingested with food/water; germination is triggered by gut cues (pH 7–8, chitin fragments, amino acids)	*Metarhizium anisopliae*, *Bacillus thuringiensis* (Bt)	Establishes initial infection in the mid-gut lumen.	[25]
Penetration & Biofilm Formation	Hyphal enzymes breach the peritrophic matrix; Bt forms protective biofilms resisting digestive enzymes and antimicrobial peptides	*M. anisopliae* (hyphae), Bt (biofilm)	Enables deeper invasion and rapid microbial proliferation.	[26]
Enzymatic Facilitation	Proteases degrade host proteins; chitinases hydrolyze the peritrophic matrix. In *Phlebotomus papatasi*, chitinase increases fungal colonization by ~50%	*M. anisopliae* (chitinase, protease)	Accelerates colonization; may cause gut occlusion and starvation.	[27]
Host Factor Influence	Species-specific susceptibility; optimal temperature 25–30°C; high humidity enhances infection. Gut melanization may resist infection, but virulent strains can bypass it	*Lutzomyia longipalpis*	Determines infection intensity across sandfly species.	[28]

7. Physiological Impacts on Sandflies

The colonization process in sandflies initiates physiological disruptions that compromise their survival and reproductive capability while enhancing biocontrol strategies [28]. A primary effect is the alteration of the gut microbiota, leading to dysbiosis in which pathogens can modify microbiome composition, favoring pathogen-friendly species and suppressing beneficial ones [29]. This imbalance disrupts digestion by eliminating symbiotic bacteria needed for nutrient breakdown, resulting in a weakened immune system and malnutrition [29]. This increases sandfly susceptibility to infection [29].

Furthermore, the altered intestinal environment forms biofilms or masses of hyphae that prevent nutrient absorption, trigger diarrhea, and disturb digestion and feeding behavior [30]. Between 3 and 7 days after infection, infected sandfly larvae experience increased mortality due to reduced feeding behavior [30]. The prolonged effects of the metabolites include a shortened lifespan in adult sandflies [31]. The presence of organic acids further disrupts intestinal function, causing regurgitation and preventing blood intake, which is important for the sandfly life cycle [31]. Paralysis also occurs when metabolites released by entomopathogens enter the hemolymph [32]. Infected sandflies also exhibit behavioral changes like reduced flight action and reduced mating ability, which lessen population density [32]. The persistence of these metabolites ensures the consistency of their effects into adulthood [33]. Although these impacts differ by species, the larval stage is generally more susceptible than the adult stage [33]. Lutzomyia species demonstrate increased susceptibility due to their aquatic reproductive habits [34]. These mechanisms highlight the significant role of entomopathogenic agents in disrupting sandfly physiology and provide insight for biocontrol strategies [34].

8. Potential Efficacy and Target Species

Enzymes such as chitinases, proteases, and lipases are potential insecticides that target insect pests by damaging their structural components, disrupting physiological processes, and inducing fatality. One commonly used enzyme is chitinase. Chitinases degrade the cuticle with their ability to hydrolyze the β-1,4-N-acetylglucosamine polymers that form the exoskeleton of insects. A study demonstrated that a purified chitinase from *Beauveria bassiana* caused the mortality of Bactrocera dorsalis larvae within 5 days, demonstrating the enzyme's insecticidal

activity [35]. Another study by Gebremariam *et al*. [36] on extracellular enzyme profiles showed that B. *bassiana* isolate AAUMB-29 contained the highest chitinase index among 92% of isolates, correlating with the rapid mortality of whitefly (*Bemisia tabaci*) nymphs. In Metarhizium anisopliae, chitinase activity contributed to 95% mortality of *Spodoptera frugiperda* larvae at 1.5×10^7 conidia mL^{-1} [37]. Native Trichoderma strains showed the highest extracellular chitinase production and induced above 80% larval mortality in the Egyptian cotton leafworm (*Spodoptera littoralis*) during oral bioassays [38]. Co-production of chitinase by *Metarhizium robertsii* enhanced *Beauveria bassiana* efficacy against *Diatraea saccharalis* larvae by 66.7% [39]. Plant-derived chitinase NkChit2b-1 from Nepenthes khasiana showed broad-spectrum antifeedant activity against beet armyworm (Spodoptera exigua) and suppressed brown planthopper (Nilaparvata lugens) feeding, proving the utility of chitinases as biopesticides [40]. Proteomic analysis of *Beauveria pseudobassiana* RGM 2184 identified three cold-adapted chitinases that effectively degraded *Lobesia botrana* cocoons under field conditions [41]. Field applications of chitinase-enriched formulations have consistently reduced pest populations in crops like maize, tomato, and wheat while maintaining non-target safety.

Next is the use of proteases secreted by entomopathogenic fungi, which can hydrolyze structural proteins of the insect cuticle and gut epithelium, thereby weakening physical barriers and enabling rapid transcuticular infection. A study by Ferreira *et al*. [42] showed that purified subtilisin-like protease (Pr1) from *Beauveria bassiana* caused 78% mortality of Aphis gossypii nymphs within 48 hours, confirming that the protease is lethal without conidia. Another study by Mubeen *et al*. [43] reported that *Metarhizium anisopliae* isolates producing ≥1.2 U mL^{-1} Pr1 achieved above 90% mortality of *Spodoptera frugiperda* larvae, while reducing the median lethal time of conidia by 35% using crude protease extracts. Native Trichoderma harzianum strains exhibited significant extracellular protease activity, resulting in over 85% mortality in Helicoverpa armigera larvae in leaf-dip assays [44]. Protease-enriched formulations from submerged fermentation of *Metarhizium robertsii* showed synergistic effects when combined with commercial B. bassiana products, improving control efficacy against *Lobesia botrana* [45]. Research into cold-adapted proteases from *Antarctic B. pseudobassiana* has shown effective pest suppression even at low temperatures (5°C), thereby broadening the potential application range of biopesticides [46].

Lipase enzymes produced by entomopathogenic fungi such as Beauveria bassiana and Metarhizium anisopliae, and by bacteria such as *Bacillus thuringiensis*, act as effective biocontrol agents by hydrolyzing lipids in insect cuticles, gut membranes, and energy reserves. A study by Pereira *et al*. [47] showed that the most relevant biocatalysts used to produce phytosterol ester compounds, mostly used in studies of effective green biocatalyst insecticides, are *Candida rugosa* lipases. These lipases are widely used enzymes in industry due to their high stability and ability to accelerate degradation when combined with other enzymes [47]. A study on aphid body wall degradation using extracellular enzymes showed that lipase activity was highest during the day when the aphid body stiffened with many tissues collapsing, suggesting the important role of lipase in the degradation process [48]. Lipase is also used to analyze Dichlorvos insecticide residues in vegetables by blending with glucose oxidase [49]. Pseudomonas-derived lipases facilitated the transesterification process for the production of insecticides, fungicides, and herbicides, such as (S)-indanofan, a compound used to control weeds and wild grass [50]. Lipase is proven effective in the production of wax-degrading bacteria as tested against the nymphs and adults of Solenopsis mealybug, *Phenacoccus*

solenopsis Tinsley, and the striped mealybug, *Ferrisia virgata* Cockerell, due to the hydrolytic lipase enzyme damaging the waxy cuticle [51]. A summary of enzyme efficacy and target species is shown in Table 2.

Table 2. Enzyme efficacy and target species.

Enzyme	Target Species	Mode of Action	Key Findings	Reference
Chitinase (*Beauveria bassiana*)	*Bactrocera dorsalis* larvae	Degrades cuticle by hydrolyzing β-1,4-N-acetylglucosamine	Purified chitinase caused larval mortality within 5 days	[35]
Chitinase (*B. bassiana* isolate AAUMB-29)	*Bemisia tabaci* nymphs	High extracellular chitinase activity correlates with virulence	Highest chitinase index; rapid whitefly mortality	[36]
Chitinase (*Metarhizium anisopliae*)	*Spodoptera frugiperda* larvae	Cuticle degradation	Contributed to 95% mortality at 1.5×10^7 conidia mL^{-1}	[37]
Chitinase (*Trichoderma* spp.)	*Spodoptera littoralis* larvae	High extracellular chitinase production	>80% larval mortality in oral bioassays	[38]
Co-produced Chitinase (*Metarhizium robertsii* + *B. bassiana*)	*Diatraea saccharalis* larvae	Enhances fungal biocontrol	Increased efficacy by 66.7%	[39]
Plant Chitinase (NkChit2b-1, *Nepenthes khasiana*)	*Spodoptera exigua*, *Nilaparvata lugens*	Antifeedant and feeding suppression	Broad-spectrum activity against multiple insect pests	[40]
Cold-adapted Chitinases (*Beauveria pseudobassiana*)	*Lobesia botrana* cocoons	Degrades the cocoon structure	Effective degradation under field conditions	[41]
Protease (Pr1, *Beauveria bassiana*)	*Aphis gossypii* nymphs	Hydrolyzes structural cuticular proteins	78% mortality within 48 hours	[42]
Protease (*Metarhizium anisopliae*)	*Spodoptera frugiperda* larvae	Rapid transcutaneous infection	≥1.2 U mL^{-1} Pr1 caused >90% mortality; LT_{50} reduced by 35%	[43]
Protease (*Trichoderma harzianum*)	*Helicoverpa armigera* larvae	Extracellular protease degradation	>85% mortality in leaf-dip assays	[44]
Protease (*Metarhizium robertsii* formulations)	*Lobesia botrana*	Protein degradation and synergy	Synergistic effect with *B. bassiana* products	[45]
Cold-adapted Proteases (Antarctic *B. pseudobassiana*)	Multiple cold-climate pests	Active at low temperatures	Effective suppression at 5°C	[46]
Lipase (*Candida rugosa*)	General insect pests (used for green biocatalysts)	Hydrolyzes lipids; accelerates degradation	Highly stable enzyme; useful in biocatalyst-based insecticides	[47]
Lipase (extracellular enzymes)	Aphids	Degradation of body wall lipids	Lipase activity is highest during the tissue collapse phase	[48]
Lipase + Glucose Oxidase	Pesticide residue detection	Hydrolyzes lipid components	Used for the analysis of dichlorvos residues in vegetables	[49]
Lipase (*Pseudomonas*-derived)	Agricultural pests (fungicides, herbicides)	Transesterification in pesticide synthesis	Used in the production of (S)-indanofan	[50]
Lipase (Wax-degrading bacteria)	*Phenacoccus solenopsis*, *Ferrisia virgata*	Destroys waxy cuticle	Effective against mealybug nymphs and adults	[51]

9. Application Strategies for Delivering Optimized Entomopathogenic Enzymes: Critical Feasibility and Scalability Assessment

Formulation enhancements represent the foundation of viable biocontrol deployment, yet feasibility varies substantially by context. Incorporation of enzymes into microbial products using oils, surfactants, and polymeric carriers effectively mitigates UV-induced degradation in controlled settings [52]. However, efficacy gains are highly dependent on climatic conditions--shelf-life extension benefits are greatest in arid and temperate regions but face critical limitations in tropical high-humidity environments where fungal contamination of formulations themselves becomes problematic [52]. Oil-based emulsions, while stabilizing enzymes, increase production costs by 30 to 40 percent and require cold-chain infrastructure often unavailable in resource-limited leishmaniasis-endemic regions [52]. This cost premium creates a significant barrier to adoption in endemic countries where health budgets are already severely constrained.

Nanoparticle encapsulation offers theoretically superior controlled release and prolonged shelf life [53], yet three major scalability barriers persist that substantially limit practical deployment. First, manufacturing costs remain two to three times higher than conventional formulations, effectively precluding adoption in low-income settings where cost-effectiveness is paramount. Second, production requires specialized equipment and quality control protocols not yet standardized across biocontrol manufacturers, meaning each facility would require independent validation and optimization. Third, regulatory pathways for nanoformulations remain undefined in most endemic countries, resulting in approval delays of 2 to 5 years that undermine the urgency of public health interventions [53]. Collectively, these barriers mean that nanoparticle approaches, despite their promise in laboratory settings, remain impractical for large-scale endemic-country implementation for at least the foreseeable future.

Genetic improvement via CRISPR-edited strains, such as Beauveria bassiana overexpressing chitinase or lipase, demonstrates impressive 2-fold increases in potency in laboratory trials [53]. However, this approach faces compounding feasibility challenges that severely limit its near-term applicability. Regulatory approval for environmental release of genetically modified entomopathogenic fungi remains prohibitive in most countries, reflecting genuine biosafety concerns about unintended ecological effects. Additionally, fitness costs associated with transgene expression may reduce competitiveness in field environments, potentially leading to the enhanced strains being outcompeted by wild-type fungi and losing their advantage. Most critically, the durability of enhanced traits across multiple generations under field conditions is poorly characterized, raising the genuine risk that any benefit would be lost within one to two seasons [53]. For endemic countries with limited biosafety infrastructure and regulatory capacity, genetic approaches may be impractical for ten years or longer, making this an unrealistic near-term strategy despite its theoretical appeal.

Delivery methods such as aerial or ground spraying of oil-emulsified fungal spores target breeding habitats efficiently in theory, but face significant scalability limitations in practice. Spray coverage variability ranges from 60 to 85 percent in optimal conditions, but this declines to 40 to 50 percent in dense urban settlements or highly vegetated peridomestic habitats typical of American leishmaniasis foci [54]. In resource-constrained settings, equipment maintenance becomes a persistent problem - spray nozzle clogging and uneven pressure distribution occur within 10 to 15 applications. Yet, replacement parts and technical expertise are often unavailable in endemic regions [54]. Beyond technical limitations, aerial

spraying is economically viable only for areas exceeding 500 hectares; smaller endemic zones, which are common in South American leishmaniasis contexts, simply cannot sustain cost-effective application using this method [54]. These constraints mean that aerial spraying, while theoretically efficient, remains practical only in exceptional circumstances with large, contiguous endemic areas and sustained technical support.

Bait stations packed with enzyme-secreting bacteria, such as Bti, offer targeted delivery with reduced environmental exposure, but operational feasibility is severely constrained by labor intensity. Placement, servicing, and monitoring of stations requires 1.5 to 2 hours per 100 habitats per week, translating to substantial recurrent personnel costs that most endemic-country health systems cannot sustain [54]. In endemic regions experiencing concurrent vector control efforts for dengue and malaria, competing resource demands often deprioritize leishmaniasis interventions, reducing adherence to placement schedules and undermining the consistency necessary for effectiveness. Moreover, efficacy data for bait stations in mixed-infection foci where Leishmania, Plasmodium, and dengue vectors coexist remain limited, leaving fundamental questions about effectiveness unanswered [54]. This combination of high labor demands, resource competition, and insufficient field validation means that bait station approaches, despite their theoretical appeal, face substantial implementation barriers in most endemic settings.

Integration of fungal sprays with bacterial larvicides simultaneously targets immature and adult sandflies, theoretically enhancing epidemiological impact [54]. However, this additive approach substantially increases formulation complexity and regulatory approval requirements. Compatibility testing between fungal and bacterial products is often inadequate or absent entirely; incompatible microbial combinations may reduce the efficacy of both agents by 20 to 35 percent relative to monotherapy, effectively negating the benefit of the combined approach [54]. Recommended application frequency of every 2 to 4 weeks during peak activity assumes sustained funding and personnel availability - parameters that are rarely maintained beyond 2 to 3 seasons in endemic countries experiencing the budget volatility and political instability that characterize many leishmaniasis-endemic regions [54]. The assumption that multiple coordinated interventions can be deployed simultaneously ignores the harsh reality of public health resource constraints in low-income settings.

The Colombian case study demonstrates proof-of-concept for integrated monitoring and delivery approaches [55,56]. Local health agents equipped sandfly traps coated with Beauveria bassiana conidia and supplemented with attractants were placed in domestic habitats and monitored every 10 days during peak season [56]. Captured insect counts declined significantly over three consecutive application cycles, confirming technical efficacy while maintaining low operational costs [56]. However, critical scalability constraints substantially limit the generalizability of these results to broader endemic contexts. The study operated in a geographically confined, politically stable region with established health infrastructure replication in fragmented leishmaniasis foci, such as Venezuela, parts of Sudan, or Syria, where health systems are actively compromised by conflict and instability, remains entirely unvalidated [56]. The effectiveness demonstrated relied upon consistent 10-day monitoring cycles requiring 4 to 5 person-hours per week, a level of personnel commitment that is unsustainable for routine public health budgets in low-income settings [56]. Furthermore, efficacy data span only three seasonal cycles; long-term surveillance extending five or more years and demonstrating sustained impact remains absent, leaving fundamental questions about durability, resistance development, and long-term ecological effects unanswered [56]. The

study did not measure heterogeneity across microhabitats or socioeconomic strata, potentially obscuring differential effectiveness in underserved populations and limiting the applicability of results to diverse epidemiological contexts [56].

Enzyme activity assays quantifying residual chitinase, protease, and lipase levels effectively indicate whether enzymes persist at biologically meaningful concentrations [57]. Yet laboratory capacity for these assays is severely limited in endemic countries; most require specialized equipment such as HPLC or spectrophotometry, trained personnel, and quality control protocols that are absent in 85 percent of primary-level healthcare facilities in sub-Saharan Africa and South Asia [57]. Outsourcing assays to reference laboratories introduces 3 to 6 week delays, rendering real-time adaptive management completely infeasible and preventing rapid response to efficacy failures [57]. This capacity gap means that countries most burdened by leishmaniasis are simultaneously the least able to conduct the surveillance needed to ensure that interventions remain effective.

Bio-efficacy bioassays exposing laboratory-reared sandflies to treated surfaces provide field-condition verification [58], but introduce substantial standardization problems that undermine their utility. Results vary significantly with sandfly colony origin, rearing conditions, and environmental humidity during testing. Inter-laboratory reproducibility is often below 70%, complicating cross-site comparisons and making it difficult to assess whether observed differences reflect true biological variation or methodological inconsistency [58]. Conducting quarterly assays as recommended requires maintained sandfly colonies, specialized containment facilities, and trained entomologists, a capacity present in fewer than 20 endemic countries [58]. The requirement for sustained colony maintenance adds substantial recurrent costs and technical demands that exceed the capacity of most endemic-country institutions.

Leishmania detection via parallel PCR on field-captured sandflies enables transmission-risk assessment [59], yet implementation barriers are substantial and often underappreciated. PCR infrastructure is available in only 30 to 40 percent of national reference laboratories in endemic countries, which are typically concentrated in capital cities far from leishmaniasis foci [59]. Chain-of-custody and sample-preservation protocols are inconsistently followed in resource-limited settings, resulting in false-negative rates of 15 to 25 percent that undermine the reliability of surveillance [59]. Most fundamentally, detecting Leishmania DNA alone does not confirm vector competence, whereby the presence of parasites does not necessarily indicate transmission risk if parasite density is sub-infectious or strains are attenuated [59]. Cost per sample runs 20 to 50 USD in endemic countries where household incomes average 2 to 5 USD per day, making population-level screening economically completely unrealistic without sustained external funding [59]. These multiple barriers mean that PCR-based surveillance, while theoretically valuable, remains impractical for endemic countries seeking to deploy and monitor enzyme-based biocontrols independently.

Resistance monitoring combines target-site sequencing with serial bioassays to detect early susceptibility shifts [59]. However, this approach assumes that resistance mechanisms are well-characterized for fungal pathogens, an assumption that is dangerously false. Unlike in bacteria or insects, the genetic determinants of fungal resistance to chitinase-derived biocontrols remain poorly mapped and poorly understood [59]. Serial bioassays require parallel maintenance of susceptible and field-collected sandfly colonies over multiple generations, demanding sustained investment and expertise that are rarely available in resource-limited settings [59]. Detecting early resistance shifts typically requires two to three years of baseline

data; intervention programs may collapse from resistance development before resistance becomes technically detectable, creating a fundamental mismatch between the timescale of surveillance and the timescale of program sustainability [59]. This gap between detection capacity and intervention durability represents a critical vulnerability in the proposed surveillance strategy.

Non-target assessments, including quarterly monitoring of pollinators, soil microbes, and aquatic invertebrates, are critical for ensuring environmental stewardship and long-term sustainability [60] yet face substantial methodological and resource constraints that are often underestimated. Baseline characterization of non-target communities in pre-intervention phases is often incomplete or absent, complicating the attribution of post-intervention changes to enzyme applications rather than natural temporal variation [60]. Quarterly monitoring frequency may be insufficient for detecting rapid shifts in soil microbiota or aquatic communities that can occur within weeks. Yet, increasing monitoring frequency multiplies costs exponentially while adding marginal information value [60]. Protocol standardization across sites is poor, reducing comparability and preventing meta-analyses that are essential for identifying systemic environmental risks across multiple endemic settings [60]. In practice, non-target monitoring is frequently deferred or abandoned when budgets tighten, leaving long-term ecological impacts undetected and creating a false impression of safety based on incomplete surveillance [60].

To advance the feasibility and scalability of enzyme-based biocontrol strategies, five urgent research and implementation priorities must be addressed. First, cost reduction and formulation optimization specifically designed for resource-limited settings must be prioritized. Current oil-based formulations at 20-40 USD per hectare preclude adoption in endemic countries without sustained external funding [52]. Targeting the development of tropical-stable formulations that do not require cold chains, with a realistic cost target of less than 15 USD per-hectare application, would substantially increase the feasibility of adoption in endemic countries. Second, surveillance methods must be fundamentally reimagined to match endemic-country capacity. Laboratory-dependent assays such as HPLC-based enzyme activity quantification are impractical in resource-limited settings. Replacing them with rapid, field-deployable methods, such as colorimetric or lateral-flow assays that require no electricity or specialized equipment, would transform surveillance feasibility [57]. However, regulatory approval pathways for such rapid tests are currently lacking and must be developed in parallel with test development.

Third, capacity building and technology transfer must move beyond rhetoric to concrete institutional development. Rather than relying on northern-hemisphere suppliers with six-to-twelve-month lead times and prohibitive costs, endemic regions should establish regional biocontrol production and quality-control hubs, perhaps one facility per five to ten countries capable of producing formulations locally and training personnel in production and quality control [52]. The current supply-chain model is inherently vulnerable to disruptions and economically unsustainable for countries with severely constrained public health budgets. Fourth, long-term effectiveness and durability studies extending beyond the current three-to-five-year timeframe are essential for informed decision-making. Current data are insufficient to assess sustainability, resistance development, and ecological side effects. Extending field monitoring to at least seven to ten years would provide the evidence base necessary for responsible large-scale deployment [56]. Fifth, regulatory pathways for entomopathogenic fungi in endemic countries must be clarified and streamlined, reducing the current two to five-

year approval delays that undermine the public health responsiveness necessary for disease control [53].

While enzyme-based biocontrol strategies demonstrate genuine promise in controlled settings and selected field studies, widespread feasibility and scalability remain substantially constrained by multiple interconnected barriers. Formulation costs, surveillance capacity limitations, infrastructure requirements, and insufficient long-term field evidence collectively impose realistic limits on what can be achieved with current approaches. Current application strategies are viable for geographically confined, well-resourced pilot programs operating in politically stable regions with established health infrastructure, contexts that describe only a small fraction of actual leishmaniasis-endemic areas. These strategies are not yet ready for large-scale implementation in endemic countries without substantial, sustained technical and financial support, and many of the proposed surveillance methods remain impractical without dramatic improvements in laboratory capacity and field-deployable technologies. Without such targeted interventions, enzyme-based biocontrol remains a high-income-country solution to a low-income-country problem. Application strategies for delivering optimized entomopathogenic enzymes are shown in Table 3.

Table 3. Application strategies for delivering optimized entomopathogenic enzymes.

Strategy category	Key approach	Main advantages	Feasibility and scalability	Major limitations and cost considerations	Ref.
Formulation enhancement	Incorporation of enzymes into conidial powders or liquid suspensions using oils, surfactants, or polymeric carriers	Enhances enzyme stability, UV protection, and environmental persistence	High feasibility in arid and temperate regions, with scalability exceeding 10,000 hectares under optimal conditions	Requires cold-chain storage, higher formulation cost, and is susceptible to fungal contamination in humid environments; estimated cost USD 20–40/hectare	[52]
Nanoparticle encapsulation	Encapsulation of enzymes within nanoparticles for controlled and targeted release	Improves shelf life, controlled release, and enzyme stability	Laboratory-proven with moderate scalability (1,000–5,000 hectares)	High manufacturing cost, lack of standardized production protocols, and uncertain regulatory approval; estimated cost USD 40–80+/hectare	[53]
Genetic improvement (CRISPR-edited strains)	Development of genetically modified *Beauveria bassiana* strains overexpressing insecticidal enzymes	Increases insecticidal potency and overall biocontrol efficacy	Limited feasibility due to regulatory restrictions and low deployment scalability	Biosafety concerns, uncertain long-term trait stability, and extensive approval processes requiring advanced laboratory infrastructure	[53]
Aerial or ground spraying	Application of oil-emulsified fungal spores using aerial or ground spraying systems	Enhances dispersion and contact with larval and adult sandflies	Suitable for medium- to large-scale vector control operations (500–5,000 hectares)	Coverage inconsistency, equipment maintenance requirements, and operational limitations in endemic regions; estimated cost USD 20–40/hectare	[54]
Bait stations and combined biological larvicides	Use of bait stations containing enzyme-secreting bacteria and integration with *Bacillus thuringiensis israelensis* (Bti)	Enables targeted adult control while simultaneously affecting immature stages	Moderate feasibility with operational scalability depending on workforce and infrastructure	Labor-intensive implementation, compatibility issues between biological agents, and increased regulatory complexity; estimated	[54]

Strategy category	Key approach	Main advantages	Feasibility and scalability	Major limitations and cost considerations	Ref.
				cost USD 30–70/hectare	
Timed application during peak activity	Repeated enzyme application synchronized with seasonal sandfly activity and habitat management	Maximizes treatment efficiency and vector contact rates	Effective during seasonal outbreaks but dependent on sustained operational coordination	Requires repeated applications, continuous funding, and long-term personnel commitment; seasonal cost may reach USD 100–240/hectare	[54]
Continuous field monitoring and trap-based deployment	Deployment of *Beauveria bassiana*-coated traps with periodic monitoring	Enables real-time efficacy assessment and population reduction tracking	Technically feasible in stable endemic regions with moderate scalability (100–1,000 hectares)	High labor demand, dependence on stable infrastructure, and limited long-term validation across endemic regions	[55,56]
Post-application enzyme activity assays and bio-efficacy bioassays	Evaluation of residual enzyme activity and laboratory bioassays using treated sandflies	Confirms enzyme persistence, potency retention, and treatment effectiveness	Limited by laboratory infrastructure and insectary availability in endemic regions	Requires specialized equipment, trained personnel, and maintenance of sandfly colonies; analytical costs remain high	[57,58]
PCR-based surveillance and resistance monitoring	Molecular detection of *Leishmania* DNA and monitoring of resistance-associated genetic changes	Assesses disease transmission risk and detects emerging resistance patterns	Limited implementation in endemic regions due to insufficient molecular facilities	High operational cost, long monitoring periods, and a lack of baseline resistance data require molecular laboratories and technical expertise	[59]
Non-target safety assessment	Monitoring impacts on pollinators, soil microorganisms, and aquatic organisms in treatment areas	Supports environmental risk assessment and ecological sustainability evaluation	Moderately feasible but resource-intensive for long-term monitoring	Requires trained ecologists, ecological baseline data, and continuous funding; annual monitoring cost estimated at USD 10,000–20,000	[60]

10. Conclusion

An alternative to chemical insecticides, which often raise concerns about human health and ecosystems, has been introduced: the use of entomopathogenic enzymes to control sandfly populations. It is a promising, sustainable, and environmentally friendly approach that needs further research to improve its efficacy. The use of enzymes such as chitinase, protease, and lipase has been proven effective in disrupting the structural barriers of sandflies, reducing their survival rates and populations. Their versatility and potential for vector control are also proven by their use in various industrial and biological applications. Strategic formulations, such as nanoparticle encapsulations, can be used to protect enzyme activity, enhance stability, and control release under harsh environmental conditions, thereby maximizing their pathogenic potential in the field. Effective implementation also requires well-developed application methods, such as bait stations, spraying techniques, and strategic trapping systems, to reduce sandfly populations. Post-application evaluation, with consistent field monitoring, is also important to ensure the consistency and efficiency of the enzymes throughout the year. Suggested further research should focus on large-scale field studies, long-term ecological assessments, and the development of a clear regulatory framework to support rapid and safe implementation. Vector management programs in regions with a high rate of sandfly-borne

diseases can be improved by integrating scientific innovation with an environmentally friendly, enzyme-based biocontrol system.

Author Contributions

Conceptualization, A.M.M.M.; methodology, M.N.H.B.J.; investigation, A.M.M.M.; writing—original draft preparation, A.M.M.M.; writing—review and editing, M.N.H.B.J., I.S.T., and Y.H.T.; supervision, T.H.; formal analysis, T.H. and M.N.H.B.J.; funding acquisition, T.H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

Authors declare no competing interests.

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