Entomopathogenic potential of thermotolerant microorganisms isolated from infected *Dermestes maculatus* against *Culex pipiens* larvae

Potencial entomopatógeno de microorganismos termotolerantes aislados de *Dermestes maculatus* infectados contra larvas de *Culex pipiens*

Potencial entomopatogênico de microrganismos termotolerantes isolados de *Dermestes maculatus* infectados contra larvas de *Culex pipiens*

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Abstract

During summer, when temperatures reach extreme records, the inhabitants Sahara seek refuge in oases for shade and water. These ecosystems are characterized by a unique microclimate. Nevertheless, they face serious threats from two arthropod species: venomous scorpions and mosquitoes, which act as vectors of diseases. Chemicals impacting both biotic and abiotic components of the ecosystem, and more critically human health. Chitinolytic entomopathogenic microorganisms were isolated from dust samples collected from the cadavers of the *Dermestes maculatus*. Chitin extracted from shrimp shells (yield: 16.6 %) served as the sole carbon source in the selective culture media employed for their cultivation. Five strains were obtained: three fungi (*Aspergillus flavus*, *A. fumigatus*, *Mucor* sp.) and two bacteria (*Bacillus* sp. and Actinomycete). Bioassays against third-instar *Culex pipiens* larvae showed that Actinomycete (10^6 CFU.mL^{-1}) induced 90 % of mortality, followed by *A. fumigatus, Mucor* sp., and *Bacillus* sp. (80 %). Data were analyzed using one-way ANOVA and Duncan's test ($p<0.05$). Microscopic observations revealed severe larval deformities. These findings confirm the strong larvicidal potential of microorganisms as eco-friendly alternatives to chemical insecticides.

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Resumen

Durante el verano, cuando las temperaturas alcanzan valores extremos, los habitantes del Sahara buscan refugio en los oasis para obtener sombra y agua. Estos ecosistemas se caracterizan por un microclima único. Sin embargo, enfrentan graves amenazas de dos especies de artrópodos: los escorpiones venenosos y los mosquitos, que actúan como vectores de enfermedades. Los productos químicos utilizados afectan tanto los componentes bióticos como abióticos del ecosistema y, de manera más crítica, la salud humana. Se aislaron microorganismos entomopatógenos quitinolíticos a partir de muestras de polvo recolectadas de los cadáveres de *Dermestes maculatus*. La quitina extraída de caparazones de camarón (rendimiento: 16,6 %) se utilizó como única fuente de carbono en el medio selectivo para su cultivo. Se obtuvieron cinco cepas: tres hongos (*Aspergillus flavus*, *A. fumigatus*, *Mucor* sp.) y dos bacterias (*Bacillus* sp. y Actinomiceto). Los bioensayos contra larvas de tercer estadio de *Culex pipiens* mostraron que Actinomiceto (10^6 CFU.mL^{-1}) provocó un 90 % de mortalidad, seguido por *A. fumigatus*, *Mucor* sp. y *Bacillus* sp. (80 %). Los datos se analizaron mediante ANOVA de una vía y la prueba de Duncan ($p<0,05$). Las observaciones microscópicas revelaron deformidades larvales severas. Estos resultados confirman el fuerte potencial larvicida de los microorganismos como alternativas ecológicas a los insecticidas químicos.

Palabras clave: quitina, entomopatógenos, quitinolíticos, larvicida, ecológico.

Resumo

Durante o verão, quando as temperaturas atingem níveis extremos, os habitantes do Saara buscam refúgio nos oásis em busca de sombra e água. Esses ecossistemas são caracterizados por um microclima único. No entanto, enfrentam sérias ameaças de duas espécies de artrópodes: escorpiões venenosos e mosquitos, que atuam como vetores de doenças. Os produtos químicos utilizados afetam tanto os componentes bióticos quanto abióticos do ecossistema e, de forma mais crítica, a saúde humana. Microrganismos entomopatogênicos quitinolíticos foram isolados a partir de amostras de poeira coletadas de cadáveres de *Dermestes maculatus*. A quitina extraída de cascas de camarão (rendimento: 16,6 %) foi utilizada como única fonte de carbono no meio seletivo para seu cultivo. Cinco cepas foram obtidas: três fungos (*Aspergillus flavus, A. fumigatus*, *Mucor* sp.) e duas bactérias (*Bacillus* sp. e Actinomiceto). Os bioensaios realizados com larvas de terceiro instar de *Culex pipiens* mostraram que Actinomiceto (10^6 CFU.mL^{-1}) causou 90 % de mortalidade, seguido por *A. fumigatus*, *Mucor* sp. e *Bacillus* sp. (80 %). Os dados foram analisados por meio de ANOVA de uma via e teste de Duncan ($p<0,05$). As observações microscópicas revelaram deformidades larvais severas. Esses resultados confirmam o forte potencial larvicida dos microrganismos como alternativas ecológicas aos inseticidas químicos.

Palavras-chave: quitina, entomopatogênico, quitinolítico, larvicida, ecológico.

Introduction

Insects are the most diverse organisms on Earth, with about one million described and millions more yet undiscovered (Li and Wiens, 2023). While many play vital ecological roles, others seriously damage crops and affect human health (Bharadwaj *et al*., 2025). Mosquitoes (*Aedes*, *Culex*, *Anopheles*) remain major vectors of malaria, dengue, and other arboviral diseases. Although still widely used, chemical insecticides cause resistance, harm non-target species, and pollute fragile ecosystems like oases. In contrast, biological control using entomopathogenic microorganisms offers a safer and more sustainable alternative. Several biocontrol agents, notably *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus*, are currently applied worldwide against mosquito larvae. However, their efficacy decreases markedly under extreme desert conditions, where elevated temperatures and low humidity reduce spore viability and larvicidal performance. This limitation emphasizes the importance of identifying new thermotolerant entomopathogenic strains capable of maintaining activity in such environments. The main objective of the present study is to isolate and characterize new extremophilic entomopathogenic microorganisms from necrophagous insects, *Dermestes maculatus* beetles, inhabiting extreme desert environments of southwestern Algeria, using selective media prepared with extracted chitin. These poorly investigated regions of the Grand Sahara (some still unexplored by humans) offer a unique opportunity to discover microbial resources adapted to thermal and arid stress. The present study explores their potential for replacing synthetic insecticides, focusing on: (i) Eco-friendly approaches to preserve ecosystem balance. (ii) High specificity against target mosquito species. (iii) Human safety with no toxic residues or long-term risks. (iv) Cost-effective tools to overcome insecticide resistance (Bharadwaj *et al*., 2025). (v) Integration within sustainable Vector Management (IVM). (vi) Validation by scientific evidence for large-scale application. This study aims to isolate and identify chitinolytic microorganisms associated with necrophagous insects and to evaluate their pathogenic potential against *Culex pipiens*. The ultimate goal is to contribute to the development of sustainable biological control strategies for mosquito population management in vulnerable oasis ecosystems.

Materials and methods

This study focused on isolating entomopathogenic microorganisms with chitinolytic activity for sustainable mosquito control. Strains were recovered from necrophagous insect cadavers (*Dermestes maculatus* De Geer), cultured on chitin-based selective media, then purified and preserved. The most active isolates were characterized morphologically and tested for pathogenicity against *Culex pipiens* larvae (figure 1).

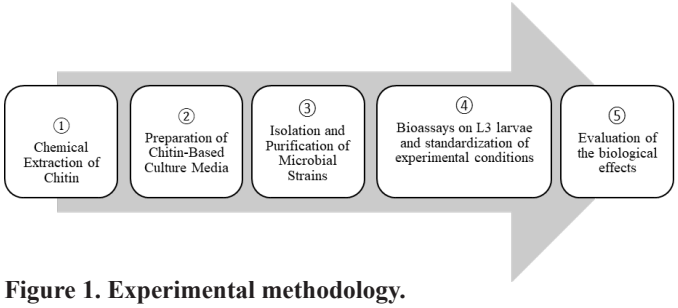

Figure 1. Experimental methodology.

Screening of potential entomopathogenic microorganisms

Chemical extraction of chitin

Chitin of marine origin (crustaceans), is commonly used to isolate chitinolytic microorganisms due to its structural similarity to natural chitin Izadi *et al*. (2025). Using chitin as the sole carbon source

allows specific selection of chitinase-producing bacteria and fungi. However, commercial chitin may contain impurities or mixed origins, potentially affecting microbial profiles. Locally purified chitin of known arthropod origin, properly treated to remove proteins, lipids, and minerals, can therefore provide greater specificity.

Shrimp shells were obtained from Mostaganem, an Algerian coastal city. The shells were peeled, thoroughly washed, dried at 45 °C, and then ground into a fine powder. Three grams of the powdered material were treated with 30 mL of 1 M HCl to remove mineral content (demineralization). The resulting residue was subsequently treated with 30 mL of 1.25 M NaOH at 90 °C for 2 hours to eliminate proteins (deproteinization). For optional depigmentation, hydrogen peroxide (H_2O_2) was applied, followed by rinsing with acetone to remove pigments and lipids, as described by Izadi *et al*. (2025). Finally, the purified chitin was dried at 80 °C for 10 hours.

Preparation of chitin-based culture media

We replaced the carbon source in CDA, GN culture media with a similar amount of purified chitin where it becomes the only carbon source available in the prepared culture media.

Isolation and purification of microbial strains

Dermestes maculatus cadavers were collected from IGLI oasis, which are likely serve as reservoirs of entomopathogenic microorganisms. Samples were collected under sterile conditions and transported to the laboratory within 24 h to preserve microbial viability.

Necrophagous insect carcasses were ground into powder, 5 g suspended in 45 mL sterile saline as stock, followed by serial dilutions (10^{-1}-10^{-4}). Aliquots were inoculated onto selective chitin-based media and incubated (bacteria 37 °C/24-48 h; fungi 3 days; Actinomycetes 28 °C/21 days). CDA_{chitin}, chloramphenicol for fungal isolation, GN_{chitin} solid medium for *Bacillus* and Chitin-based solid medium B vitamin for Actinomycetes isolation. Chitinolytic strains were isolated and identified via morphological, microscopic, and staining methods. All tests were performed in triplicate.

Bioassays on *Culex pipiens* larvae

Larval sampling

Sampling site: Larval sampling was carried out in the IGLI palm grove, Algeria (30.4440799 ° N, 2.2995554 ° W), at a stagnant drainage channel partially shaded by palm fronds and enriched with moderate organic matter.

Sampling period: Sampling was carried out on June 2020, corresponding to the peak seasonal abundance of *Culex pipiens* larvae in the study area, warm temperatures and the presence of stagnant water bodies ensure high population densities.

Sampling time: To minimize heat stress and reduce larval movement toward the bottom of the water column, which typically occurs at higher temperatures, collection was conducted during the early morning hours (06h00-08h00).

Sampling method: Larvae were collected using a 350 mL white plastic dipper at a 45 °C angle, with ten consecutive dips taken along a 15 m transect to account for spatial variability. Specimens were separated from debris in white enamel trays, then transferred to 500 mL containers (≤30 larvae/container) to avoid stress and cannibalism.

Criteria for choosing the larval stage for sampling: Sampling specifically targeted third-instar (L3) *Culex pipiens* larvae for the following reasons: (i) High susceptibility to entomopathogens with a sufficiently long larval period for post-treatment monitoring. (ii) Larger body size than L1-L2, allowing easier handling, sorting, dosing, and reliable morphological identification. (iii) Absence of metamorphosis changes seen in L4, reducing bias and misidentification. (iv) Lower mortality and pupation risk during handling and transport. (v) Optimal size and clear species-specific traits for laboratory experiments.

Selection and sorting of L3: Final sorting of L3 larvae was performed carefully in the laboratory under a stereomicroscope, following the morphological characteristics.

Standardization of experimental conditions: To replicate natural conditions inside laboratory, and ensure that mortality results from the tested microorganism, height controlled environmental parameters must be respected (table 1).

Table 1. Steps followed during the standardization of experimental conditions.

Parameter	Conditions	
Water Quality	Dechlorinated freshwater (pH 6.5-8, low conductivity, low-moderate hardness, low turbidity); replace periodically.	
Temperature	Optimal range for the species studied (25 ± 2 °C), sudden fluctuations were avoided.	
Photoperiod	Timeframe	12:12 h (light/dark).
	Intensity	Soft light intensity, were avoided direct strong light on containers.
RH (%)	15-25 % (close to the seasonal value)	
Dissolved Oxygen	Mechanical aeration was not required, but low-oxygen water was avoided and adequate surface area was ensured for larvae.	
Feeding Regime	Standard larval diet (fish food, yeast) in controlled amounts, 0.1-0.2 mg dry food per larva/day.	
Container Size and Density	Larval density was maintained within recommended limits to avoid competition and cannibalism.	
Handling Practices	To replicate natural abiotic conditions, mechanical disturbance was minimized, reducing stress and ensuring that mortality was due to the microbial treatment.	
Predators and Contaminants	No other organisms in the water (aquatic worms, predatory insect larvae, parasitic fungi, etc.). Handle with clean, disinfected tools.	

Microbial load measurement: Fragments from isolated colonies were aseptically transferred into 60 mL of sterile water. Microbial suspensions were homogenized in sterile distilled water with 0.05 % Tween 80, preheated to 40 °C for full dissolution (Goettel *et al*., 2010). Fungal and Actinomycete suspensions were adjusted to 10^6 CFU.mL^{-1} using a hemocytometer, and *Bacillus* suspensions to 10^6 cells.mL^{-1} via spectrophotometry. Two serial dilutions (10^5 and 10^4 CFU.mL^{-1}) were prepared for bioassays.

Data analysis: Twenty larvae were placed in each container and exposed to different concentrations of each entomopathogenic strain, with three replicates per treatment. Larval survival was monitored daily for seven days, and microscopic examinations were conducted to assess pathogenic effects. Control mortality (ranging from 5 to 20 %) was corrected using Abbott's formula (Abbott, 1925). Daily mortality data collected over seven consecutive days (N = 7 observation times) provided sufficient information to perform regression analyses and estimate LT_{50} values for each applied dose.

$$\% \text{ Corrected mortality} = \frac{\% \text{ Observed mortality} - \text{Control mortality}}{100 - \% \text{ Control mortality}}$$

Evaluation of biological effects

The entomopathogenic activity of each isolate was evaluated through three parameters: (i) larval mortality over seven days

to estimate cumulative death and LT_{50}; (ii) pupal inhibition and post-emergence mortality, reflecting developmental blockage or early adult death; and (iii) adult deformities and flight incapacity, indicating sublethal physiological effects. This approach allowed a comprehensive assessment of both lethal and sublethal impacts on *Culex pipiens* development.

Statistical analysis: All statistical analyses were performed using SPSS software. Data were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was conducted to evaluate the effect of microbial treatments on larval mortality. When significant differences were detected ($p<0.05$), means were separated using Duncan's multiple range test.

Results and discussion

The chitin yield was 16.6 %, which aligns with recent studies reporting that crustacean shells generally contain 15 to 40 % chitin, depending on species and extraction method used (Izadi *et al*., 2025).

Microbial cultures and purified strains: Following the inoculation of the stock solution of the necrophagous insects in the chitinous media CDA_{chitin}, GN_{chitin} and Vitamin B $_{Chitin}$, five distinct microbial isolates were purified. Three fungal (*Aspergillus flavus*, *Aspergillus fumigatus*, and *Mucor* sp.) and two bacterial (*Bacillus* sp. and Actinomycete).

Larval bioassay results

Control group: Control groups consistently showed 0 % mortality, full flight capability, and no post-emergence mortality, and are therefore presented as a common baseline for all treatments.

Effect of *Aspergillus flavus* on mosquito larvae and adult emergence

At 10^6 CFU.mL^{-1}, *A. flavus* caused the highest mortality, reaching 70 % by day 7, with only 10 % of adults retaining flight ability, indicating marked sublethal effects. At 10^5 CFU.mL^{-1}, mortality was moderate (50 %), and 20 % of adults remained flight-capable, suggesting reduced impairment. At 10^4 CFU.mL^{-1}, mortality declined to 40 %, but flightlessness (30 %) and post-emergence mortality (20 %) were more evident, reflecting severe physiological stress among survivors (figure 2). These findings confirm the dual action of entomopathogenic fungi, combining lethality with functional impairment. Similar results were reported by Lamy *et al*. (2025) and Valdez *et al*. (2025), highlighting their strong potential against mosquitoes and other insect pests.

Effect of *Aspergillus fumigatus* on mosquito larvae and adult emergence

For *A. fumigatus*, the concentration of 10^6 CFU.mL^{-1} induced rapid mortality, rising from 10 % on day 1 to 70 % by day 5, with no increase thereafter. Flight ability was strongly impaired, with only 10 % of adults able to fly, while 10 % exhibited flightlessness and 10 % died post-emergence. At 10^5 CFU.mL^{-1}, mortality plateaued at 40 % by day 5, but functional impairments were higher (30 % flightlessness, 20 % post-emergence mortality). At 10^4 CFU.mL^{-1}, mortality was lower (30 %), but 30 % of adults remained flight-capable, suggesting less damage among survivors (figure 3). These findings align with recent reports showing that entomopathogenic fungi both reduce adult survival and compromise adult fitness in mosquito hosts (Aguilar-Durán *et al*., 2023; Renuka *et al*., 2023).

Effect of *Mucor* sp. on mosquito larvae and adult emergence

At 10^6 CFU.mL^{-1}, *Mucor* sp. caused a sharp mortality increase from 10 % on day 1 to 60 % by day 7, with complete loss of flight ability among survivors (0 % flight-capable). At 10^5 CFU.mL^{-1}, mortality reached 80 % by day 7, with 10 % of adults retaining flight capacity but showing marked physiological impairment. At 10^4 CFU.mL^{-1}, mortality was also high (80 %) but with a slower progression, while 20 % of survivors were flight-capable and no post-emergence mortality was observed (figure 4). This suggests lower virulence at reduced doses but persistence of functional effects. Similar outcomes were reported by Zhu *et al*. (2023), who demonstrated strong pathogenic effects of *Mucor hiemalis* on insect hosts. Effect of ***Bacillus*** sp. on mosquito larvae and adult emergence

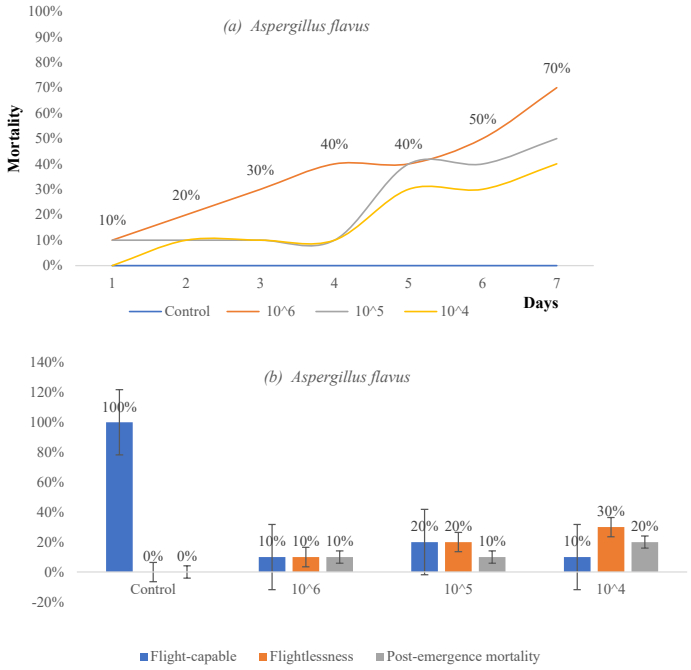

Figure 2. (a) Daily evolution of mortality rates following exposure to varying doses of *Aspergillus flavus*. (b) Post-exposure outcome of larvae surviving beyond 7 days.

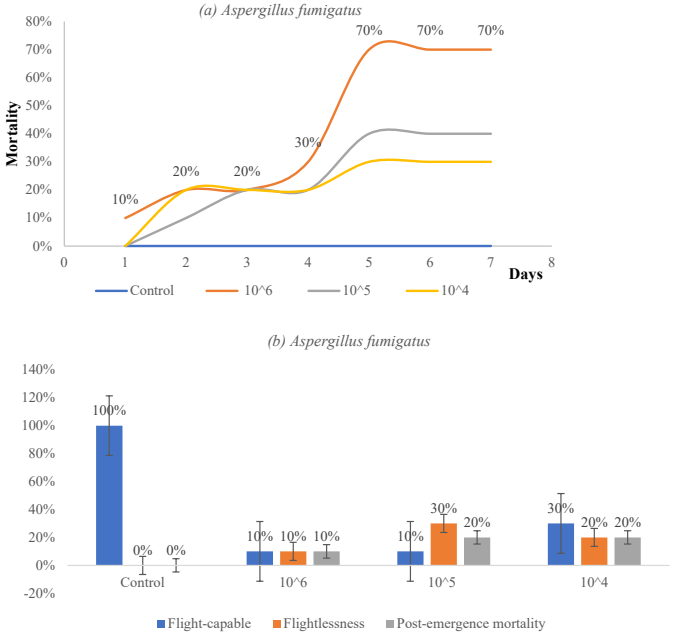

Figure 3. (a) Daily evolution of mortality rates following exposure to varying doses of *A. fumigatus*. (b) Post-exposure outcome of larvae surviving beyond 7 days.

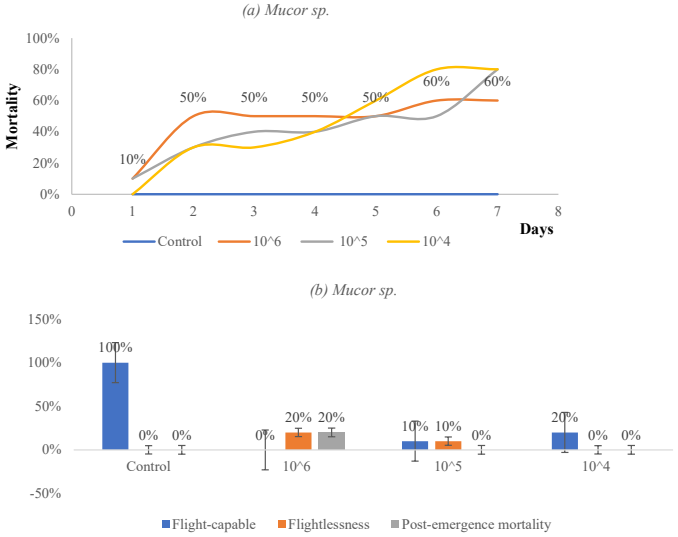

Figure 4. (a) Daily evolution of mortality rates following exposure to varying doses of *Mucor* sp. (b) Post-exposure outcome of larvae surviving beyond 7 days.

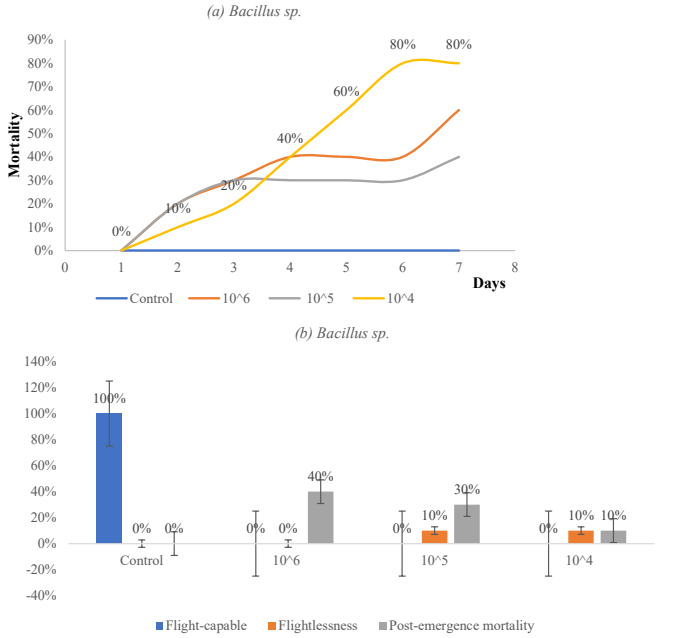

Figure 5. (a) Daily evolution of mortality rates following exposure to varying doses of *Bacillus* sp. (b) Post-exposure outcome of larvae surviving beyond 7 days.

At 10^6 CFU.mL^{-1}, *Bacillus* sp. caused 60 % larval mortality by day 7 with high post-emergence death and complete flight loss, whereas at 10^5 CFU.mL^{-1} mortality was lower (40 %) but sublethal effects remained severe (figure 5). Interestingly, the highest mortality (80 %) occurred at 10^4 CFU.mL^{-1}, suggesting a non-linear dose-response. The lower virulence observed at 10^6 CFU.mL^{-1} compared to 10^5 CFU.mL^{-1} may result from bacterial aggregation and quorum-sensing inhibition at high densities, which reduce toxin production and trigger stronger host immune defenses (Lavine and Strand, 2002; Natrah *et al*., 2011; Hossain, *et al*., 2025). These results highlight both lethal and sublethal impacts of *Bacillus* sp. across concentrations, supporting its potential as an effective biocontrol agent.

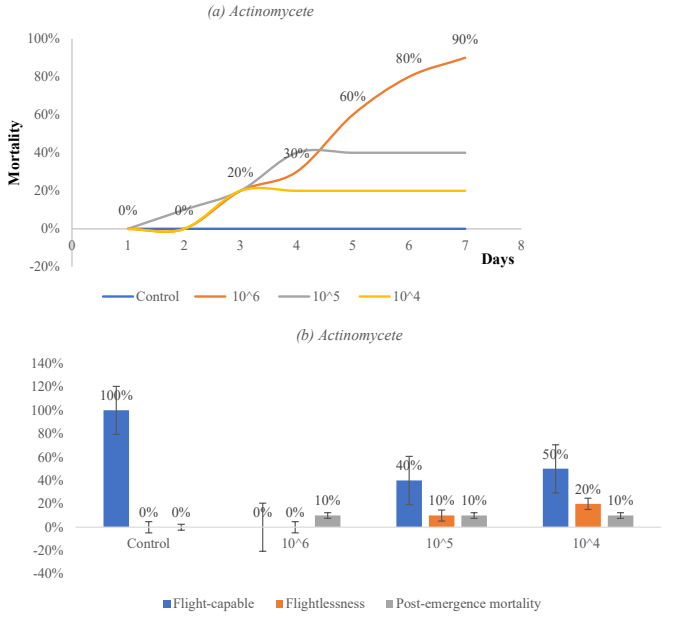

Figure 6. (a) Daily evolution of mortality rates following exposure to varying doses of Actinomycete. (b) Post-exposure outcome of larvae surviving beyond 7 days.

Effect of Actinomycete on mosquito larvae and adult emergence

At 10^6 CFU.mL^{-1} Actinomycete caused mortality up to 90 % by day 6, with no flight-capable adults and 10 % post-emergence mortality, confirming strong virulence (figure 6). At 10^5 CFU.mL^{-1}, mortality stabilized at 40 %, with 40 % of adults flight-capable, though 10 % exhibited flightlessness and 10 % died after emergence. At 10^4, mortality was low (20 %), but 50 % of adults retained flight, while 20 % showed flightlessness and 10 % post-emergence mortality. These results suggest dose-dependent effects, where higher concentrations strongly reduce survival, while lower ones primarily impair physiology. Comparable studies by Al-Nagar *et al*. (2024) and Singh and Dubey (2015) support the entomopathogenic potential of Actinomycetes through both lethal and functional impacts.

ANOVA revealed a significant effect of microbial treatment on larval mortality ($F = 5.87$, $p<0.001$). *Mucor* sp. and Actinomycete caused the highest mortality, while *Bacillus* sp. showed weaker effects; *A. flavus* and *A. fumigatus* produced moderate, similar responses. Fungal isolates, particularly *Mucor* sp., were the most virulent, likely due to rapid growth and chitinolytic activity. These results confirm the stronger biocontrol potential of fungi, with bacterial strains showing complementary, dose-dependent effects.

Comparative analysis of TL_{50} values

TL_{50} results (table 2) revealed clear differences in virulence among the tested microorganisms. Fungal isolates acted faster than bacterial ones, while bacterial effects were slower but cumulative. *Mucor* sp. was the most virulent (TL_{50} = 2 days at 10^6 CFU.mL^{-1}), consistent with reports of high pathogenicity of *Mucor* species against insect larvae (Zhu *et al*., 2023).

A. fumigatus showed moderate virulence (4.5 days), consistent with previous reports of its larvicidal activity against mosquito larvae (Balumahendhiran *et al.,* 2019), and *A. flavus* was slower (6 days), likely due to delayed toxin production (Arreguín-Pérez *et al*., 2023). Among bacteria, Actinomycete was most effective ($TL_{50} \approx 4.7$ days;

90 % mortality), in line with studies showing strong larvicidal activity of actinomycete metabolites against mosquito larvae (Seratnahaei *et al*., 2023). *Bacillus* sp. acted more slowly (6.5 days), consistent with the short residual activity and environmental sensitivity reported for *Bacillus*-based biolarvicides (Zogo *et al.,* 2019). Nonlinear dose-response trends suggested spore aggregation or host stress at high inoculum levels (Parco *et al*., 2023).

Table 2. Comparative TL_{50} values of tested entomopathogenic strains.

	Entomopathogenics				
Doses	*A. flavus*	*A. fumigatus*	*Mucor* sp.	*Bacillus* sp.	Actinomycete
10^4 CFU.mL^{-1}	-	-	4.5	4.5	-
10^5 CFU.mL^{-1}	7.0	-	5.0		-
10^6 CFU.mL^{-1}	6.0	4.5	2.0	6.5	4.7

Treated *Culex pipiens* larvae showed strong pathological effects, including reduced vitality, impaired motility, and rapid decomposition, especially in the head, abdomen, and siphon. Microscopic analysis revealed severe cuticle degradation in chitin-rich areas (figure 7), confirming integument weakening as a main mortality mechanism. Pigmentation changes, such as whitening and darkening with reddish hues, indicated melanization and microbial metabolite activity (Vega and Kaya, 2012; Arreguín-Pérez *et al*., 2023). Development was also disrupted, with delayed or premature pupation, prolonged metamorphosis, and undersized non-viable pupae. Surviving adults exhibited malformations, including wing deformities and flight incapacity, consistent with entomopathogen exposure (Lacey, 2017). These results, in line with previous studies (Lacey, 2017), highlighted both lethal and sublethal impacts. Overall, the findings confirmed the strong pathogenic activity of tested microorganisms and their promise as eco-friendly agents in mosquito control.

Pathological symptoms in *Culex pipiens* larvae following treatments

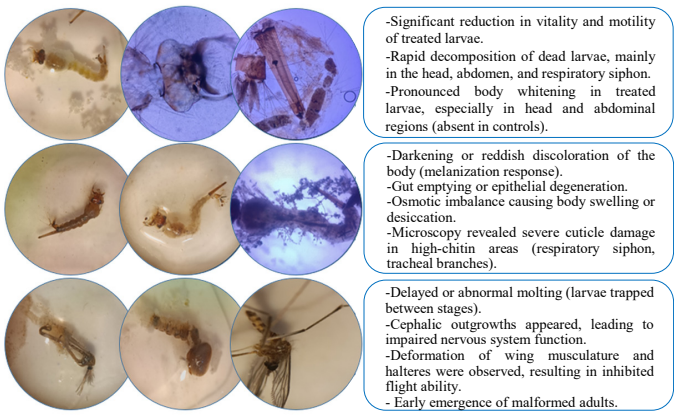

Figure 7. Different symptoms observed during treatments.

Conclusion

Protecting human health from mosquito-borne nuisances remains essential, while conventional chemical insecticides face growing limitations due to resistance and environmental concerns. In this study, chitin was successfully extracted from shrimp shells (yield: 16.6 %), highlighting their potential as a valuable resource.

Five chitinolytic microorganisms were isolated and identified: *Aspergillus flavus*, *Mucor* sp., *Bacillus* sp., *Aspergillus fumigatus*, and Actinomycete. Bioassays against *Culex pipiens* larvae confirmed their pathogenicity, with mortality increasing proportionally to concentration. Notably, strong larvicidal and sublethal effects were observed, indicating their potential use in mosquito biocontrol.

Although preliminary, these results support the use of chitinolytic microorganisms as eco-friendly alternatives to synthetic insecticides. Further molecular, biochemical, and ecological studies are needed to validate their efficiency under natural conditions.

Literature cited

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18*(2), 265-267. https://doi.org/10.1093/jee/18.2.265

Aguilar-Durán, J. A., Villarreal-Treviño, C., Fernández-Santos, N. A., Hamer, Gabriel L., & Rodríguez-Pérez, M. A. (2023). Virulence of entomopathogenic fungi isolated from wild mosquitoes against *Aedes aegypti*. *Entomological Research, 53*(2), 158-166. https://doi.org/10.1111/1748-5967.12640

Al-Nagar, N. M. A., Shawir, M. S., & Abdelgaleil, S. A. M. (2024). Mosquitocidal activity of extracts derived from soil Actinomycete isolates. *Alexandria Science Exchange Journal, 45*(4), 719-729. https://doi.org/10.21608/asejaiqjsae.2024.395086

Arreguín-Pérez, C. A., Miranda-Miranda, E., Folch-Mallol, J. L., & Cossío-Bayúgar, R. (2023). Identification of virulence factors in entomopathogenic *Aspergillus* isolates. *Microorganisms, 1*(8), 2107. Jhttps://www.ncbi.nlm.nih.gov/pmc/articles/PMC10457961/

Bharadwaj, N., Sharma, R., Subramanian, M., Ragini, G., Nagarajan, S. A., & Rahi, M. (2025). Omics approaches in understanding insecticide resistance in mosquito vectors. *International Journal of Molecular Sciences, 26*(5), 1854. https://doi.org/10.3390/ijms26051854

Goettel, M. S., Eilenberg, J., & Glare, T. R. (2010). Entomopathogenic fungi and their role in regulation of insect populations. In L. A. Lacey (Ed.), Manual of techniques in invertebrate pathology (2nd ed., pp. 387-432). Academic Press. https://doi.org/10.1016/C2010-0-66784-8

Izadi, H., Asadi, H., & Bemani, M. (2025). Chitin: A comparison between its main sources. *Frontiers in Materials, 12*: 1537067. https://doi.org/10.3389/fmats.2025.1537067

Balumahendhiran, K., Vivekanandhan, P., & Shivakumar, M. S. (2019). Mosquito control potential of secondary metabolites isolated from *Aspergillus flavus* and *Aspergillus fumigatus. Biocatalysis and Agricultural Biotechnology, 21*, 101334. https://doi.org/10.1016/j.bcab.2019.101334

Lacey, L. A. (2017). Entomopathogens used as microbial control agents. In Microbial control of insect and mite pests (pp. 3-12). Academic Press. https://doi.org/10.1016/B978-0-12-803527-6.00001-9

Lamy, D. L., Gnambani, E. J., Sare, I., Millogo, S. A., Sodré, F. A., Namountougou, M., Viana, M., Baldini, F., Diabaté, A., & Bilgo, E. (2025). *Metarhizium pingshaense* increases susceptibility to insecticides in highly resistant malaria mosquitoes *Anopheles coluzzii*. *Wellcome Open Research, 9*, 290. https://doi.org/10.12688/wellcomeopenres.21238.2

Lavine, M. D., & Strand, M. R. (2002). Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology, 32*(10), 1295-1309. https://doi.org/10.1016/S0965-1748(02)00092-9.

Li, X., & Wiens, J. J. (2023). Estimating global biodiversity: The role of cryptic insect species. *Systematic Biology, 72*(2), 391-403. https://doi.org/10.1093/sysbio/syac069

Natrah, F. M. I., Ruwandeepika, H. A. D., Pawar, S., Karunasagar, I., Sorgeloos, P., Bossier, P., & Defoirdt, T. (2011). Regulation of virulence factors by quorum sensing in *Vibrio harveyi*. *Veterinary Microbiology, 154*(1-2), 124-129. https://doi.org/10.1016/j.vetmic.2011.06.024

Parco, J., Valverde-Rodríguez, A., Cornejo, A., Briceño, H., Barrionuevo, L., & Romero, J. (2023). Efficiency of entomopathogenic bacteria and fungi on *Oligonychus yothersi in vitro* and on *Persea americana* Mill. plants. *Revista de la Facultad de Agronomía (LUZ), 40*(4), e234033. https://produccioncientificaluz.org/index.php/agronomia/article/view/40991/47103

Hossain, M. S., Anjum, M. F., Rabbani, M. A., Hasan, M. R., Sohag, M. M. H., Tasnim, N., Karmakar, D., Akter, S., Rahman, M. M., & Karim, M. R. (2025). *Bacillus altitudinis*: a sustainable mosquito controlling biopesticide isolated from the rhizospheric soil of *Nypa fruticans* in mangrove forest. *BMC Microbiology, 25*, 733. https://doi.org/10.1186/s12866-025-04168-0

Seratnahaei, M., Eshraghi, S. S., Pakzad, P., Zahraei-Ramazani, A., & Yaseri, M. (2023). Larvicidal effects of metabolites extracted from *Nocardia* and

Streptomyces species against the forth larval stage of *Anopheles stephensi* (Diptera: Culicidae). *Journal of Arthropod-Borne Diseases, 17*(2), 187-196. https://doi.org/10.18502/jad.v17i2.13623

Renuka, S., Vani, H. C., & Alex, E. (2023). Entomopathogenic fungi as a potential management tool for the control of urban malaria vector *Anopheles stephensi* (Diptera: Culicidae). *Journal of Fungi, 9*(2), 223. https://doi.org/10.3390/jof9020223

Singh, R., & Dubey, A. K. (2015). Endophytic Actinomycetes as emerging source for therapeutic compounds. *Indo Global Journal of Pharmaceutical Sciences, 5*(2), 106-116. https://doi.org/10.35652/igjps.2015.11

Valdez, D., Farah, S., Espinoza, W., Veliz, F., Villon, H., & Herrera, L. (2025). Biocontrol of *Cosmopolites sordidus* using entomopathogenic fungi under laboratory conditions, Ecuador. *Revista de la Facultad de Agronomía (LUZ), 42*(2), e254217. https://doi.org/10.47280/RevFacAgron(LUZ).v42.2

Vega, F. E., & Kaya, H. K. (Eds.). (2012). Insect pathology (2nd ed.). Academic Press. https://doi.org/10.1016/B978-0-12-384984-7.00001-4

Zogo, B., Tchiekoi, B. N., Koffi, A. A., Dahounto, A., Ahoua Alou, L. P., Dabiré, R. K., Baba-Moussa, L., Moiroux, N., & Pennetier, C. (2019). Impact of sunlight exposure on the residual efficacy of biolarvicides *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* against the main malaria vector *Anopheles gambiae*. *Malaria Journal, 18*, 55. https://doi.org/10.1186/s12936-019-2687-0

Zhu, G., Ding, W., Zhao, H., Xue, M., Chu, P., & Jiang, L. (2023). Effects of the entomopathogenic fungus *Mucor hiemalis* BO^{-1} on the physical functions and transcriptional signatures of *Bradysia odoriphaga* larvae. *Insects, 14*(2), 162. https://doi.org/10.3390/insects14020162

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