Original Article

Exploitation of Endemic Extremophilic Microbial Strains Isolated from Date Palm Fibrillium in the Saoura Region (Southwest Algeria) as Potential Biocontrol Agents Against Mosquitoes

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Abstract

Background: Algeria's extreme ecosystems, such as the Saoura region, represent unique reservoirs of rare microorganisms, inhabiting pristine and unexplored virgin territories with considerable biotechnological potential. Chitinolytic bacteria are particularly noteworthy for mosquito control due to their ability to degrade chitin, the major structural component of the insect cuticle. This study aims to: (i) identify new microbial strains adapted to extreme desert conditions, overcoming the limited efficacy of some bioinsecticides, (ii) address the spread of vector-borne diseases and mosquito resistance, (iii) reduce the environmental impacts of non-selective insecticides, and (iv) explore eco-friendly strategies and integrated biological control.

Methods: Shrimp chitin was extracted to prepare selective media for the isolation and purification of chitinolytic bacteria. The entomopathogenic activity of these chitinolytic strains at concentrations of 10^4, 10^5 and 10^6 CFU/mL was evaluated through bioassays on *Culex pipiens* larvae.

Results: We isolated three chitinolytic strains from *Phoenix dactylifera* bark, among which *Streptomyces* spp. 2 (STR2) was clearly the most virulent against *Cx. pipiens* larvae. This strain exhibited marked dose and time-dependent toxicity (LC_{50}-LC_{99}: 7.9×10^3-3.4×10^7 CFU/mL; DC: 6.8×10^7 CFU/mL; LT_{50}: 4.6 days). Beyond larval mortality, it also severely affected adults by reducing the proportion of flight-capable mosquitoes and increasing rates of flightlessness, post-emergence mortality, and deformity. However, an IGR-like mode of action remains speculative.

Conclusion: This study confirms the larvicidal activity of desert-adapted chitinolytic bacteria, but further research is needed to determine their selectivity toward non-target organisms before considering their application in mosquito management.

Keywords: Biological control; Chitinolytic microorganisms; *Culex pipiens*; *Phoenix dactylifera*

Introduction

Vector and pest control remains one of the greatest challenges at the intersection of public health, agriculture and environmental sustainability. For decades, chemical pesticides have formed the cornerstone of control programs; however, their widespread use has led to environmental pollution, adverse effects on human and ecosystem health and the rapid emergence of insecticide-resistant mosquito populations.

Mosquitoes (Culicidae), notably *Anopheles*, *Aedes* and *Culex*, are major vectors of malaria, dengue, West Nile virus and filariasis, causing over one billion cases and more than one million deaths each year (1, 2). *Culex pipiens* is widespread in temperate and arid regions, representing a significant public health and ecological concern (3, 4).

Between 2023 and 2025, Africa, the continent most severely impacted by vector-borne diseases, has seen the commercialization and operational deployment of several bacterial larvicides based on *Bacillus thuringiensis* variety *israelensis* (Bti), *Bacillus sphaericus* (Bs),

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or their combinations, notably within the urban malaria control programme (UMCP) and other large-scale larviciding (LSL) initiatives in Tanzania, as well as larval source management (LSM) programs in Burkina Faso and Cameroon (5). Microbial agents are particularly effective because they specifically target mosquito larval stages without adverse effects on non-target organisms. Bti produces Cry and Cyt toxins that bind selectively to mosquito gut receptors, causing larval death while sparing beneficial insects and mammals (6). Bs and *Streptomyces* produce binary toxins or chitinases that degrade larval exoskeletons with high specificity (7). The selectivity of these microbial agents makes them safe, efficient and environmentally sustainable alternatives to chemical insecticides (8).

In hot, arid environments of North Africa and the Middle East, intense solar radiation and extreme temperatures markedly reduce the residual activity of Bti- and Bs-based larvicides, leading to rapid loss of efficacy in small, transient breeding sites typical of desert regions (9). Harnessing locally adapted microorganisms offers a promising path toward more resilient and sustainable biocontrol. While *Bacillus*-based larvicides are well documented, endemic *Streptomyces* from extreme habitats remain largely unexplored, despite their remarkable metabolic versatility and inherent adaptation to arid ecosystems.

In this context, we investigated whether extremophilic chitinolytic microorganisms endemic to the Saoura region (Algeria) could offer an effective and climate-adapted alternative for mosquito control. Our study aimed to isolate entomopathogenic microorganisms from date palm fibrillium. This substrate is scientifically relevant and strategically valuable due to its biochemical richness; it is an excellent reservoir of extremophilic strains with strong potential against *Culex pipiens.* Key advantages include: (i) frequent accumulation of insect cadavers, pupal remains, exuviae and other organic debris, providing abundant chitin and lignocellulose while releasing metabolites that create selective pressures favoring the adaptation of chitinase-producing, entomopathogenic microorganisms, (ii) serving as a natural microhabitat for extremophiles adapted to the Saoura desert climate, (iii) acting as a reservoir of diverse microbial taxa, increasing the likelihood of isolating rare or novel entomopathogens, (iv) structural complexity of the fibrous matrix, favoring microbial colonization and long-term persistence and likely selecting for genetically adapted strains specialized for such arid environments, (v) represent an underexplored ecological niche, offering opportunities to discover strains with biotechnological value.

Specifically, our study aimed to isolate novel thermotolerant chitinolytic strains from palm date fibrillium and evaluate their entomopathogenic activity against *Cx. pipiens* larvae. We sought to identify the most effective strains based on larval mortality, as well as lethal concentration (LC_{50}) and lethal time (LT_{50}) values, assess their potential as sustainable, locally adapted biocontrol agents suitable for arid environments, and valorize underutilized local resources with socio-economic benefits. Additionally, the study also aimed to promote sustainability and biosafety by employing ecologically integrated strains adapted to the local climate.

Materials and Methods

Date palm fibrillium was used as a substrate for isolating entomopathogenic microorganisms. The collected material was first pretreated to suppress vegetative cells and promote sporulation, ensuring the selective recovery of spore-forming taxa. It was then cultured on chitin-based media designed to favor the growth of *Bacillus* sp. and *Streptomyces.* After purification, the recovered isolates were screened for their larvicidal activity against mosquito larvae to evaluate their

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potential for use in sustainable vector management programs.

Chemical extraction of chitin

Chitin is widely used as a substrate to isolate chitinolytic microorganisms; in this study, we employed locally purified chitin to improve specificity and experimental control. Compared with commercial products, locally prepared chitin offers higher purity (reduced proteins, pigments and minerals), better reproducibility and traceability (batch-level characterization) and greater cost-effectiveness and sustainability by valorizing shrimp shell waste. Its properties (particle size and degree of deacetylation) can also be tailored to experimental needs, providing a consistent substrate for entomopathogen isolation and pathogenicity assays. Shrimp shells were processed to chemically extract chitin (Fig.1) through the following steps:

- Demineralization: Shells were treated with 0.25 M HCl at room temperature (25 °C) for 2 hours to remove minerals (mainly $CaCO_3$) and then rinsed with distilled water until neutral.
- Deproteinization: The demineralized residue was treated with 1 M NaOH at 80 °C for 2 hours to solubilize proteins and washed thoroughly with distilled water.
- Depigmentation: Residual pigments were removed by treating the material with 30% H_2O_2 at 50 °C for 1 hour, yielding white, homogeneous chitin.
- Purification: The chitin was washed multiple times with distilled water, filtered and dried at 60 °C for 24 hours to remove chemical residues and stabilize the final product.
- Characterization: Purified chitin was analyzed using FT-IR spectroscopy, X-ray diffraction (XRD) to confirm its purity and structure (10, 11).

Preparation of chitin-based culture media

In this study, the carbon sources in Czapek Sucrose Agar medium (CDA), Glucose Nutrient medium (GN) and Vitamin B Chitin media were replaced by purified chitin, making it the sole carbon source available in the medium. The replacement was carried out by weighing the corresponding amount of chitin equivalent to the original carbon source (sucrose or yeast extract) and all other medium components were maintained as described. The pH was adjusted to 7.4±0.2 and the media were sterilized before inoculation.

This approach is consistent with previously reported methods for isolating and cultivating chitinolytic microorganisms using chitin as the sole carbon source (Table 1).

Isolation and purification of microbial strains

Sampling procedure

Samples of *Phoenix dactylifera* (Faggous cultivar) fibrillium, weighing 20–25 g each, were aseptically collected from three randomly selected trees in Mazzer's palm grove, located in the Saoura region, southwestern Algeria (coordinates: 30°19′03.0″N, 2°15′42.2″W). Collection was performed at a height of 1.3 m using ethanol-sterilized tools. Samples were immediately placed in sterile Whirl-Pak bags, stored at 4–8 °C and transported to the laboratory within 6 hours. A field blank was included to monitor potential contamination.

Pretreatment of fibrillium samples

Chemical treatment: 5 g of crushed Fibrillium sample were mixed with 0.5 g of $CaCO_3$, placed on aluminum foil, then transferred into a sterile glass Petri dish containing moistened paper and incubated at 28 °C for one week to promote *Streptomyces* growth. This $CaCO_3$ enrichment step has been widely used to selectively favor *Streptomyces* over faster-growing bacteria (12).

Physical treatment: 5 g of crushed fibrillium were heated at 90 °C for 15 min to suppress vegetative cells and favor spore germination, especially for *Bacillus*. Sublethal heat shocks at this temperature and for this duration are consistent with studies on thermal activation of bacterial spores (13, 14).

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Preparation of stock solutions and serial dilutions

The pretreated fibrillium (5 g) was suspended in 45 mL of sterile saline (0.85% NaCl (w/v) in distilled water) and serial dilutions were prepared in triplicate. Serial dilution after chemical and physical pretreatments is a standard method to isolate slow-growing *Streptomyces* (12).

Sterile saline was used together with date palm fibrillium powder to isolate microbial strains and to prepare serial dilutions from 10^{-1} to 10^{-4}. This ensured cell integrity, prevented uncontrolled bacterial growth and allowed accurate and reproducible dilutions for subsequent bioassays (Fig. 2).

Inoculation and isolation of microorganisms

Sterile selective media were used to isolate and cultivate chitinolytic and general bacterial populations. Samples (0.1 mL from each stock solution and dilution) were inoculated onto the following media:

- CDA $_{chitin}$: a general medium (sucrose replaced by chitin) used to favor the growth of *Bacillus* species after heat shock.

- GN $_{chitin}$: supports the growth of general heterotrophic bacteria and *Bacillus* spores.

- VB $_{chitin}$: contains chitin as the sole carbon source and vitamins B, providing selectivity for chitinolytic microorganisms and supporting the growth of more fastidious strains.

- International *Streptomyces* Project medium 2 (ISP2): used for *Streptomyces* isolation, promoting growth of slow-growing species over 15–21 days.

For physically pretreated samples, after heat shock (90 °C, 15 min), CDA, GN and VBC plates were incubated at 37 °C for 24–48 h to favor *Bacillus* spore germination and outgrowth. For chemically pretreated samples, ISP2 plates were incubated at 28 °C for 15–21 days to allow *Streptomyces* colonies to develop, consistent with standard protocols (12).

Isolation, identification and purification of *Bacillus* and *Streptomyces*

Distinct colonies obtained from primary cultures were subcultured to obtain pure isolates. Preliminary identification was based on colony morphology (size, shape, color, margin, elevation), microscopic features (cell shape, arrangement, motility, presence of endospores), Gram and spore staining and basic biochemical tests (catalase activity, oxygen requirement) (15, 16).

Bacillus: Aerobic, spore-forming *Bacillus* isolates were streaked on GN-chitin agar and incubated at 37 °C for 24–48 h. Purification was performed by repeated streaking until colonies with uniform morphology and consistent microscopic and biochemical characteristics were obtained. Microscopic examination included assessment of rod shape, endospore location (central, terminal, subterminal) and motility (12, 17).

Streptomyces: Filamentous *Streptomyces* isolates were streaked on ISP2 agar and incubated at 28 °C for 7–15 days. Purification was achieved by repeated streaking until colonies with consistent macroscopic, microscopic and biochemical features were obtained. Identification was based on the presence of substrate and aerial mycelium, spore-chain formation, colony pigmentation, texture and catalase activity (18, 19).

Preparation of inocula

Three isolates (one *Bacillus* sp., two actinomycetes belonging to *Streptomyces* spp.) were purified. Standardized suspensions (10^6 CFU/mL) were prepared in sterile water, quantified by spectrophotometry for *Bacillus* and the Malassez chamber for *Streptomyces*. Serial dilutions (10^4–10^5 CFU/mL) served as inocula for bioassays.

The concentrations of 10^4, 10^5 and 10^6 CFU/mL were selected based on the following criteria: (i) Standard bioassay range: These doses cover the commonly used range for microbial larvicides against mosquitoes, allowing the assessment of dose-response rela-

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tionships, (ii) Dose-response evaluation: Using three orders of magnitude ensures that both sublethal effects (at lower doses) and maximal mortality (at higher doses) can be observed, facilitating LC_{50} estimation, (iii) Literature precedent: Similar concentrations have been reported in recent studies for *Bacillus* and *Streptomyces*, making the results comparable with previous work (20, 21), (iv) Safety and environmental consideration: Including lower concentrations helps identify the minimal effective dose, reducing potential non-target effects and environmental load (22–24).

Bioassays on *Culex pipiens* larvae

Larval collection

Study site

Larvae were collected from palm groves in Igli, located in the Saoura region of southwestern Algeria (coordinates: 30°26'43.3" N, 2°16'52.0" W). The collection site consisted of a shallow, stagnant water body within an agricultural drainage channel, partially shaded by palm fronds and characterized by moderate accumulation of organic matter.

Collection period

The sampling took place in June 2023, coinciding with the peak seasonal abundance of *Cx. pipiens* larvae in the study region, when elevated temperatures and stable stagnant aquatic habitats favor larval proliferation and ensure high population densities.

Collection time

To minimize thermal stress and reduce the tendency of larvae to move to the bottom of the water column, which is common at higher temperatures, sampling was conducted in the early morning between 06:00 and 08:00, when the water temperature was 30.6 °C and the relative humidity was 18%.

Collection technique

Larvae were collected using a 350 mL dipper, with ten successive scoops taken along a 15 m transect to account for spatial variability. Samples were transferred into enamel trays for separation, then placed in 500 mL containers (≤30 larvae each) filled with habitat water. Containers were covered with mesh, labeled with sampling metadata and transported to the laboratory within 2 h for species confirmation and experiments.

Criteria for selecting the larval stage

The collection specifically targeted third instar (L3) larvae of *Cx. pipiens*, as this stage is the most suitable for bioassays due to its high susceptibility to entomopathogenic agents while still allowing sufficient time before pupation. The choice of L3 was justified by the following characteristics:

(i) High vulnerability to entomopathogenic microorganisms, (ii) Adequate larval period remaining for post-exposure monitoring, (iii) Larger body size compared to L1 and L2, facilitating handling, manipulation and precise dosing, (iv) Absence of metamorphic physiological changes occurring at L4, reducing experimental variability, (v) Ease of morphological identification at this stage, (vi) Lower mortality risk during handling and transportation, (vii) Minimization of misidentification compared to earlier instars, (viii) Distinct species-specific traits visible at L3, (ix) Reduced probability of pupation during transport and handling and (x) Optimal body size for both laboratory and field bioassays.

Sorting of L3 larvae

The final selection of L3 larvae was performed in the laboratory of Tahri Mohamed University under a stereomicroscope. Larvae were identified using morphological diagnostic features, including body size, siphon length-to-width ratio, number of siphonal setae, presence of palmate hairs and pigmentation patterns. Only active and morphologically intact individuals were retained, handled carefully using a fine-tipped pipette.

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Key morphological traits for rapid identification of *Cx. pipiens* larvae include:

- Respiratory siphon: elongated, longer than the head; siphonal index approximately 3–4. This is a primary feature distinguishing *Culex* from *Anopheles*, which lacks a siphon.
- Subventral comb scales (peines): present at the base of the siphon; moderate number, unlike the dense combs observed in *Aedes* larvae.
- Setae (hairs): moderately dense on abdominal segments and siphon; lateral and dorsal setae clearly visible; less dense than in *Aedes*.
- Position in water: larvae suspended at the surface, head downward.
- Other characters: size and number of abdominal segments (8 segments) serve as confirmation, but are secondary.

Standardization of laboratory rearing conditions

Larvae were maintained under standardized abiotic and biotic conditions to reproduce their natural habitat and minimize external stress factors.

- Water quality: Dechlorinated water (pH 6.5–8) with low conductivity, low to moderate hardness, and minimal turbidity was used. Water was renewed periodically to maintain hygiene without abrupt chemical or thermal changes.
- Temperature: A constant water temperature of 25±2 °C was maintained, avoiding sudden fluctuations.
- Photoperiod: A 12:12 h light-dark cycle was applied with soft illumination, preventing direct exposure to strong light.
- Relative humidity: Ambient humidity was kept at 15–25%, reflecting the arid climate of Southwestern Algeria (18%).
- Dissolved oxygen: No mechanical aeration was provided, but stagnation was avoided by ensuring sufficient water surface relative to larval density.
- Feeding regime: Larvae were fed finely ground fish food and yeast (0.1–0.2 mg/larva/day) in controlled amounts to avoid fouling.
- Container size and density: Each bioassay was conducted in 500 mL plastic cups, filled with 400 mL of dechlorinated water, containing 20 third-instar larvae per cup (density 0.05 larvae/mL). These conditions were selected to prevent overcrowding and cannibalism, ensuring uniform growth and exposure.
- Handling practices: Disturbance was minimized during routine observation and sampling.
- Absence of predators and contaminants: Rearing water was kept free from other organisms (worms, predatory larvae, fungi), and all manipulations were performed with disinfected tools.

Introduction of bacterial suspensions to larvae

Using a sterile syringe, 4 mL of the *Bacillus* sp. mother suspension (10^6 CFU/mL) was withdrawn after agitation and added to one of the experimental cups. The same procedure was used for the other dilutions (10^4 and 10^5 CFU/mL) and for the remaining microorganisms. Cups were labeled and covered with fine sterile gauze, both to prevent external contamination and to retain adults emerging from surviving larvae, which were later inspected under a stereomicroscope to document deformities and malformations. After dilution, the final concentrations in the cups were 10^6, 10^5 and 10^4 CFU/mL, with 10^6 CFU/mL considered the maximum bioassay dose.

Data processing

Larvicidal bioassays were conducted following WHO standard procedures, with minor adjustments for the environmental conditions of the Saharan desert, where the mosquito populations were collected. Twenty (20)

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third-instar (L3) *Cx. pipiens* larvae were placed in 500 mL containers covered with sterile gauze to prevent adult escape after pupation. A total of 720 larvae were used across the study. Larvae were exposed to three concentrations (10^4, 10^5, 10^6 CFU/mL) of each bacterial strain, *Bacillus* sp., *Streptomyces* spp. 1 and *Streptomyces* spp. 2, with one untreated control per strain. Each concentration and control were tested in triplicate, and all experiments were conducted separately for each strain to avoid cross-contamination.

Mortality was monitored daily for seven days to estimate LC_{50}, DC and LT_{50} values. Pathogenicity was confirmed microscopically, and control mortality ranging from 5% to 20% was corrected using Abbott's formula.

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

Evaluation of biological effects

The biological effects of each bacterial isolate on *Cx. pipiens* were assessed at multiple developmental stages. Larval mortality over seven days, including cumulative deaths, LC_{50}, diagnostic concentration (DC) and LT_{50}, quantified both lethality and speed of action. Pupal inhibition and post-emergence mortality revealed disruptions in development and early adult death, while adult deformities and flight incapacity reflected sublethal physiological consequences. This multi-parameter approach provided an integrated evaluation of both lethal and sublethal impacts (Table 2).

Statistical analysis

Larval mortality was calculated as the percentage of dead larvae at each concentration and exposure time. Dose-response data were analysed by log-probit regression, fitting probit-transformed mortality against $\log_{10}$ (concentration) for each strain and day to obtain the regression parameters (intercept a and slope b), LC_{50}, LC_{90} and LC_{99} (CFU/mL) and the coefficient of determination (R^2). In line with WHO recommendations, the DC for each strain was defined as twice the LC_{99} (DC= 2 × LC_{99}). Time mortality data at 10^6 CFU/mL were similarly analysed by log-probit regression of probit-transformed mortality on exposure time (days) to estimate LT_{50} for each strain. Post-treatment outcomes were classified as flight-capable, flightless, or post-emergence mortality and expressed as percentages. All bioassays were performed in triplicate, and results are presented as mean ± standard error (SE). All computations were carried out in Microsoft Excel using standard log-probit procedures.

Results

The chitin mass yield, calculated from dried and ground shrimp shells (68.26 g), resulted in a final recovery of 11.3 g of purified chitin, corresponding to 16.55% of the initial biomass.

Isolation and identification of microorganisms

Following the isolation process, one *Bacillus* sp. strain and two *Streptomyces* spp. strains were purified and characterized.

Bacillus

Microscopic examination revealed motile rod-shaped bacteria arranged in chains, with spores observed after 48 h. Gram staining confirmed Gram-positive bacilli, while spore staining highlighted free and intracellular spores. Biochemical assays showed facultative anaerobic growth and catalase activity.

Streptomyces

Morphological and cultural features identified both isolates as *Streptomyces*.

STR_1: colonies cottony and wrinkled with smooth edges; white vegetative mycelium and gray aerial mycelium.

STR2: dome-shaped, powdery colonies with rough margins; orange vegetative mycelium and beige aerial mycelium.

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Impact of *Bacillus* sp. on mosquito larval development and adult emergence

For *Bacillus* sp., the probit-$\log_{10}$ (concentration) plot at day 7 shows an almost perfectly linear dose-response, with a moderate positive slope (b= 0.26) and a very high goodness-of-fit (R^2= 0.999). Probit mortality increases steadily from the lowest to the highest tested dose (10^4–10^6 CFU/mL), in agreement with the progressive rise in larval mortality from 50% to 70% (Fig. 3). This dose-dependent pattern is consistent with the reduction in adult emergence and flight capacity observed at higher concentrations, where only a small proportion of larvae reaching pupation produced fully functional, flight-capable adults and post-emergence mortality increased (Fig. 3).

Impact of *Streptomyces* (STR 1) on mosquito larval development and adult emergence

For *Streptomyces* STR1, the probit-$\log_{10}$ (concentration) curve also displays a positive linear trend (b= 0.26), but with a lower coefficient of determination (R^2= 0.75), reflecting greater variability between the three doses. Probit mortality rises from 50% at 10^4 CFU/mL to around 70% at 10^5–10^6 CFU/mL, indicating a stronger larvicidal effect than *Bacillus* sp. at equivalent concentrations (Fig. 4). These probit-based patterns match the cumulative mortality recorded over time and the post-pupation outcomes, where STR1 reduced the proportion of flight-capable adults and produced consistent levels of flightlessness and post-emergence mortality among those that successfully reached pupation (Fig. 6).

Impact of *Streptomyces* (STR2) on mosquito larval development and adult emergence

The probit-$\log_{10}$ (concentration) plot for *Streptomyces* STR2 shows the steepest dose-response among the three strains, with a higher slope (b= 0.64) and a strong linear fit (R^2= 0.97). Probit mortality increases sharply between 10^4 and 10^6 CFU/mL, corresponding to the mortality observed rising from 50% to 90% at day 7. This steep relationship confirms the high sensitivity of larvae to STR2 and is consistent with the highest overall larval mortality in the time-course assays (Fig. 5). Moreover, at the pupal and adult stages, STR2 strongly reduced the proportion of flight-capable adults and increased the frequency of flightlessness and post-emergence defects or mortality, indicating that its larvicidal action extends beyond simple larval kill to markedly compromise post-pupation performance (Fig. 6).

Comparative analysis of LC_{50}, LC_{90} and LC_{99}

Log-probit analysis of the dose-response data (Probit mortality vs $\log_{10}$ concentration) showed that the linear model provided a good fit from day 4 onwards, with R^2 values in the range of 0.75–1.00 for most strain-day combinations. Early in the exposure period (days 1–3), mortality was too low or too similar across doses to yield reliable LC estimates, which explains the non-estimable LC values. By day 7, the estimated lethal concentrations (CFU/mL) were:

Bacillus sp. (day 7): LC_{50}: 1.03×10^4, LC_{90}: 7.82×10^8 and LC_{99}: 7.90×10^{12} CFU/mL, *Streptomyces* spp. 1 (day 7): LC_{50}: 4.64×10^3, LC_{90}: 3.54×10^8 and LC_{99}: 3.57×10^{12} CFU/mL, *Streptomyces* spp. 2 (day 7): LC_{50}: 7.86×10^3, LC_{90}: 7.82×10^5 and LC_{99}: 3.40×10^7 CFU/mL (Fig. 7).

These values confirm clear strain-to-strain differences in potency at the end of the 7-day exposure. In particular, *Streptomyces* spp. 2 required orders of magnitude lower concentration to reach LC_{99} compared with the other two strains, while *Bacillus* sp. exhibited the highest LC_{99}, indicating lower intrinsic activity under the tested conditions (Table 3).

Discriminating concentration (DC)

Following WHO recommendations, the discriminating concentration (DC) was defined as

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twice the LC_{99} estimated by log-probit analysis against the susceptible laboratory strain.

Using the day-7 LC_{99} values, DCs were *Bacillus* sp.: DC: 1.58×10^{13} CFU/mL, *Streptomyces* spp. 1: DC: 7.15×10^{12} CFU/mL and *Streptomyces* spp. 2: DC= 6.80×10^{7} CFU/mL

Thus, *Streptomyces* spp.2 required a much lower discriminating concentration than *Bacillus* sp. and *Streptomyces* spp. 1, again highlighting its superior larvicidal potency (Table 3).

Lethal time (LT_{50})

Time-mortality data at the highest test concentration (10^6 CFU/mL) were analyzed using log-probit regression of probit-transformed mortality against exposure time. The estimated LT_{50} values were: *Bacillus* sp.: LT_{50}: 5.56 days, *Streptomyces* spp. 1: LT_{50}: 5.43 days and *Streptomyces* spp. 2: LT_{50}: 4.58 days (Fig. 7).

These estimates confirm that *Streptomyces* spp.2 kills larvae more rapidly than *Bacillus* sp. and *Streptomyces* spp. 1 at the same high dose, while the difference between *Bacillus* sp. and *Streptomyces* spp. 1 is modest (Table 3).

Principal component analysis (PCA) projection illustrating the variability among *Bacillus* sp., STR1 and STR2

Principal component analysis (PCA) based on $\log_{10}$ (LC_{50}), LT_{50} and mortality at 10^6 CFU/mL (day 7) showed a clear separation among the three strains. PC1 (67.4 % of the variance) mainly contrasted high LC_{50} and long LT_{50} with high mortality, placing *Streptomyces* spp.2 on the side of higher mortality and shorter LT_{50}, while *Bacillus* sp. and *Streptomyces* spp.1 clustered together with similar LC_{50} and LT_{50} values. PC2 (32.6 % of the variance) contributed only marginally to strain separation, confirming that STR2 is the most potent and fastest-acting isolate in overall multivariate space (Fig. 8).

Figure 9 illustrates the spectrum of pathological symptoms induced by *Bacillus*, STR1 and STR2 at 10^6 CFU/mL in *Cx. pipiens*. Morphological alterations include somatic swelling, cuticular darkening (melanization) and tissue necrosis; deformation of pupal respiratory trumpets; and extensive cuticle degradation indicative of chitinolytic activity. Physiological impairments are evidenced by wing detachment due to flight muscle degradation, leg and antennal deformities and midgut epithelial damage. Behavioral and developmental disruptions are also visualized, such as premature adult emergence and arrested development in the pharate pupal stage. The composite figure summarizes the key pathologies leading to mortality and reproductive failure.

Table 1. Composition of selective chitin-based media (CDA, GN and Vitamin B) for isolating chitinolytic microorganisms. Major ingredients and concentrations (g/L) of the three media in which the carbon source was replaced by chitin. Media were adjusted to pH 7.4±0.2 and incubated at 37 °C

CDA_{chitin}		**GN_{chitin}**		**Vitamin B $_{Chitin}$**	
Ingredients	**Quantity g /L**	**Ingredients**	**Quantity g /L**	**Ingredients**	**Quantity g /L**
Sucrose replaced by chitin	30	Yeast extract	2	Chitin	2
	10	Replaced with chitin	2		
K_2HPO_4	1	meat extract	1	KH_2PO_4	0.35
KCl	0.5	Peptone	5	$MgSO_4$	0.2
$MgSO_4$	0.5	NaCl	5	$CaCO_3$	0.02
$FeSO_4$	0.01	Agar	15	$FeSO_4$	0.01
$NaNO_3$	3			$ZnSO_4$	0.001
Agar	15			$MnCl_2$	0.001
		pH 7.4±0.2 at 37 °C			

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Table 2. Parameters for assessing bacterial effects on *Culex pipiens*. Measured parameters include larval mortality (LC_{50}, LC_{90}, LC_{99} and LT_{50}), pupal inhibition/post-emergence mortality and adult deformities/flight incapacity. Combined, these provide a comprehensive assessment of lethal and sublethal effects

Parameter evaluated	Description	Biological significance
Larval mortality (7 days)	Measurement of cumulative larval deaths and calculation of LC_{50}, LC_{90}, LC_{99}, and LT_{50}	Indicates the lethality and speed of action of each bacterial isolate.
Pupal inhibition and post-emergence mortality	Observation of larvae failing to pupate, pupae not reaching adulthood, or adults dying shortly after emergence	Reflects developmental disruption and early adult mortality.
Adult deformities and flight incapacity	Detection of malformed adults showing defects (e.g., deformed wings/legs, flight inability)	Demonstrates sublethal physiological effects impacting adult fitness.
Overall evaluation	Combined interpretation of lethal and sublethal parameters	Provides a comprehensive assessment of the impact of isolates on *Cx. pipiens* development.

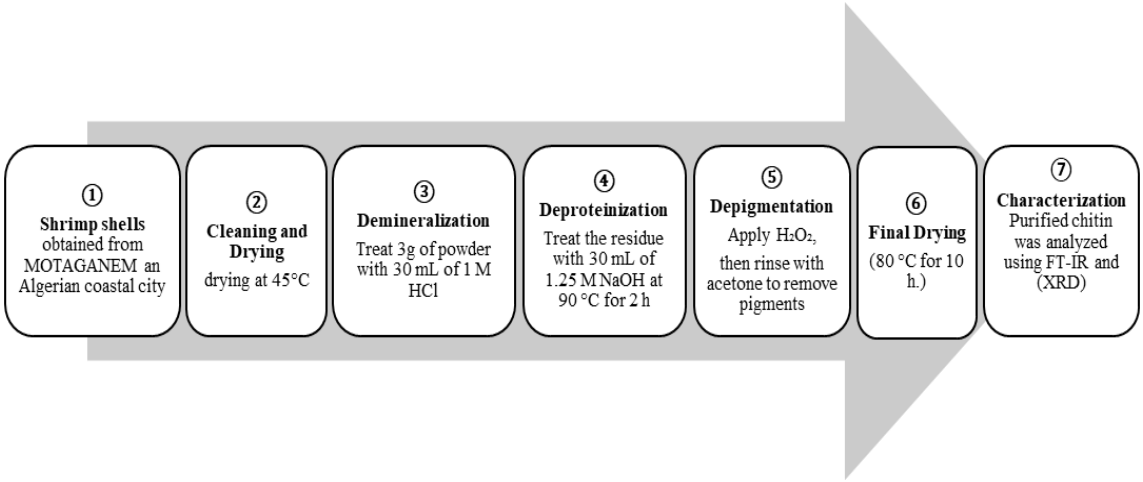

Fig. 1. Chemical purification of arthropod chitin. Continuous block process of chitin extraction: demineralization with 1 M HCl (2 h), deproteinization with 1 M NaOH (24 h, 65 °C), followed by washing and drying

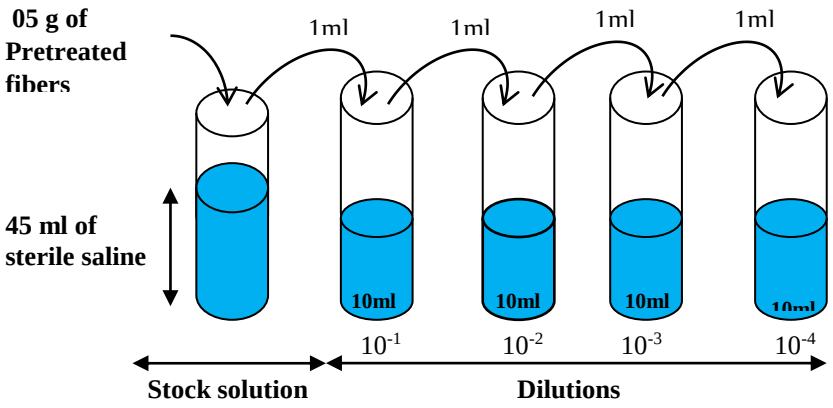

Fig. 2. Preparation of serial dilutions of the fibrillium stock solution was performed as previously described by Pochon and Tardieux (25). 0.5 g of pretreated fibrillium fibers were suspended in 45 mL of sterile saline (0.85 % NaCl, pH 7) at ambient temperature as the stock solution for serial dilutions

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Table 3. Linear regression parameters of probit-transformed mortality versus $\log_{10}$ (concentration) and derived LC_{50}, LC_{90}, LC_{99}, discriminating concentration (DC= 2× LC_{99}) and LT_{50} (at 10^6 CFU/mL) for *Bacillus* sp., *Streptomyces* spp. 1 (STR1) and *Streptomyces* spp. 2 (STR2) against third-instar *Culex pipiens* larvae after 7 days of exposure

Strain	Intercept (a)	Slope (b)	R^2	LC_{50} (CFU/mL)	LC_{90} (CFU/mL)	LC_{99} (CFU/mL)	DC (CFU/mL)	LT_{50} (Days)
BAC	3.948	0.262	1.000	1.03×10^4	7.82×10^8	7.90×10^{12}	1.58×10^{13}	5.562
STR 1	4.039	0.262	0.750	4.64×10^3	3.54×10^8	3.57×10^{12}	7.15×10^{12}	5.428
STR 2	2.504	0.641	0.968	7.86×10^3	7.82×10^5	3.40×10^7	6.80×10^7	4.577

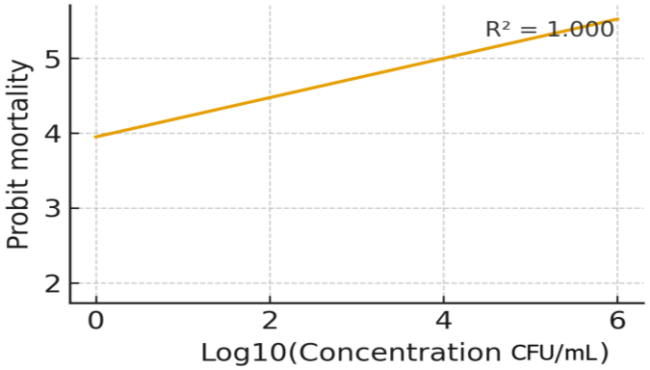

Fig. 3. Probit-$\log_{10}$ (concentration CFU/mL) dose-response of *Bacillus* sp. against third-instar *Culex pipiens* larvae after 7 days of exposure. Larvae (0.05 larvae/mL) were reared under standardized conditions (dechlorinated water pH 6.5–8, 25±2 °C, 12:12 h L:D, 15–25 % RH, controlled feeding 0.1–0.2 mg/larva/day). Points show probit-transformed mortality at 10^4, 10^5 and 10^6 CFU/mL and the line is the fitted log-probit regression; R^2 and derived LC_{50}, LC_{90}, LC_{99} and DC (2×LC_{99}) are reported on the plot and in Table 3

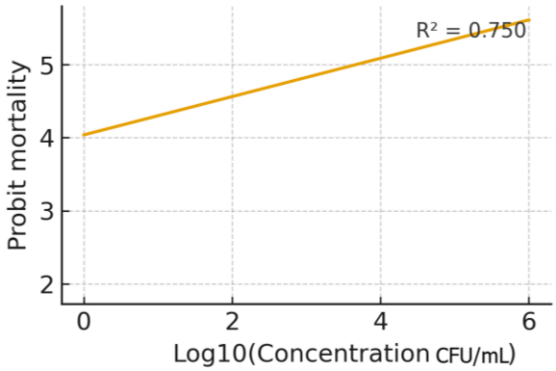

Fig. 4. Probit-$\log_{10}$ (concentration CFU/mL) dose-response of STR1 against *Culex pipiens* larvae after 7 days under standardized rearing (0.05 larvae/mL; dechlorinated water pH 6.5–8; 25±2 °C, 12:12 h L:D; 15–25 % RH, 0.1–0.2 mg/larva/day). Probit-transformed mortalities at 10^4, 10^5 and 10^6 CFU/mL are plotted versus $\log_{10}$(concentration) with the fitted log-probit line and R^2; resulting LC_{50}, LC_{90}, LC_{99} and DC are given in Table 3

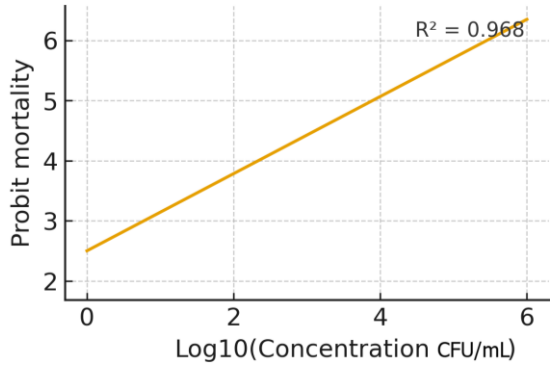

Fig. 5. Probit-$\log_{10}$ (concentration CFU/mL) dose-response of STR2 against *Culex pipiens* larvae reared for 7 days under the same standardized conditions (density 0.05 larvae/mL, dechlorinated water pH 6.5–8, 25±2 °C, 12:12 h L:D, 15–25 % RH, controlled feeding). Points represent probit mortalities at 10^4, 10^5, and 10^6 CFU/mL; the solid line represents the log-probit fit. R^2 and the corresponding LC_{50}, LC_{90}, LC_{99} and DC are summarized in Table 3

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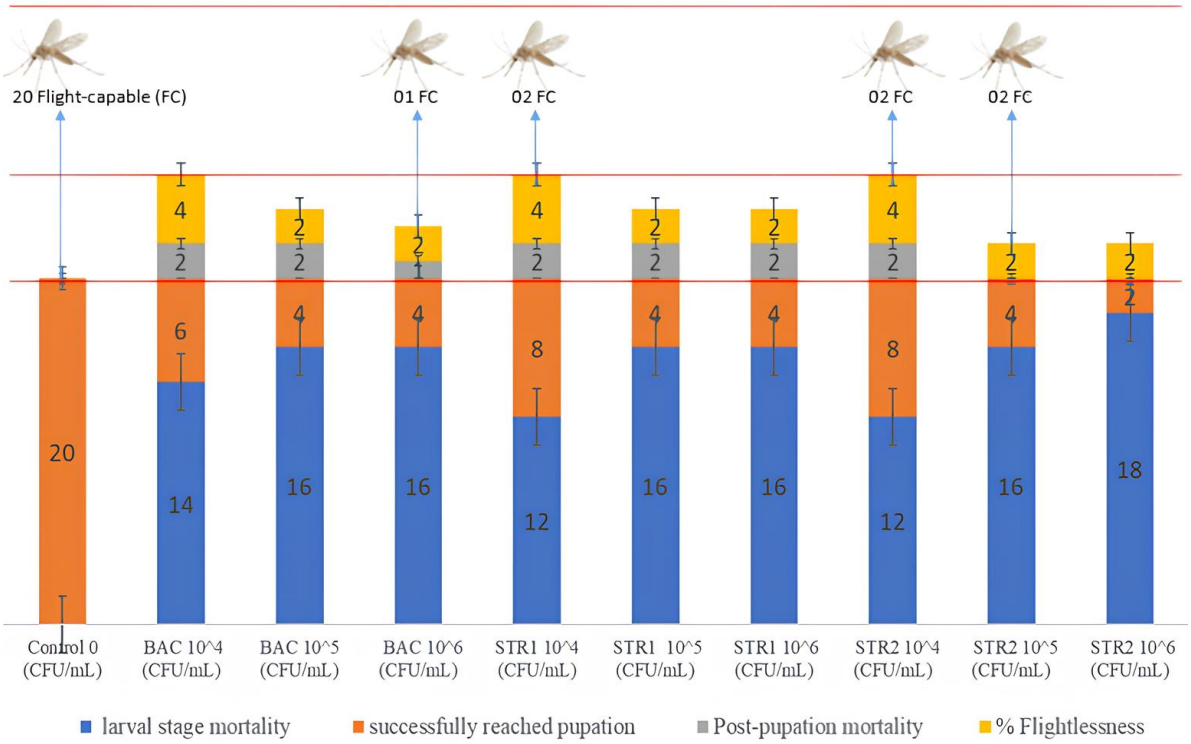

Fig. 6. Post-treatment fate of *Culex pipiens* larvae following exposure to *Bacillus* sp., STR1 and STR2. For each treatment, 20 third-instar larvae were exposed and subsequently classified as dead during the larval stage, pupated, emerged as flightless adults, or emerged as healthy flight-capable adults. Bars show the mean number of individuals in each category from three independent bioassay replicates (±SE)

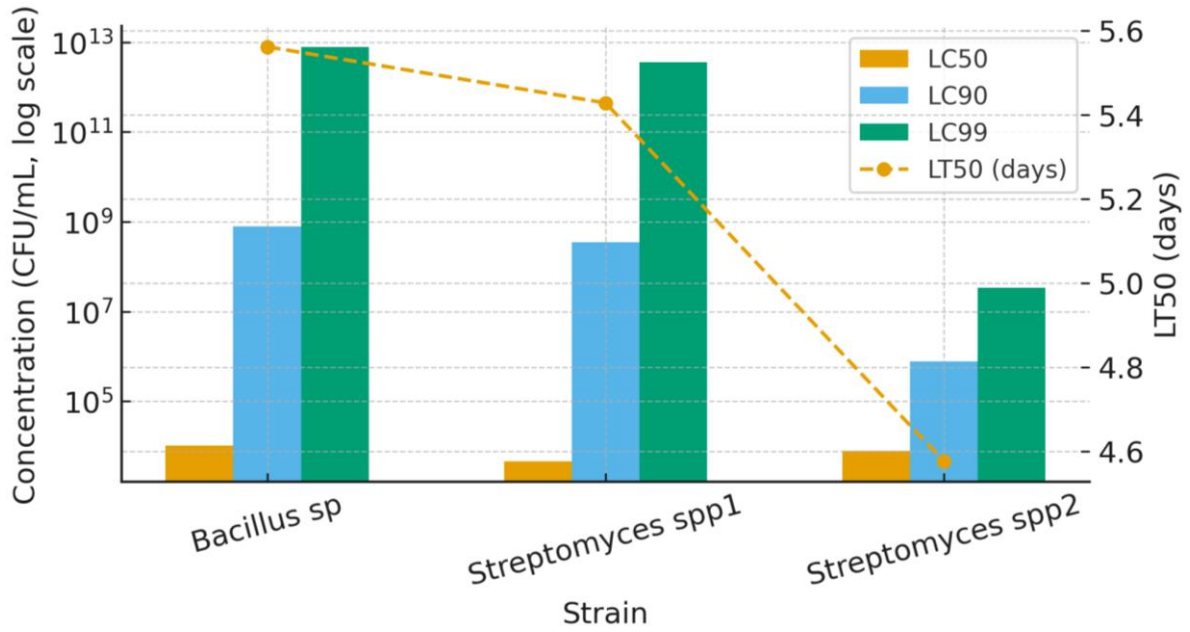

Fig. 7. Comparative LC_{50}, LC_{90}, and LC_{99} (bars, left y-axis) and LT_{50} (points, right y-axis) for *Bacillus* sp., STR1, and STR2 against third-instar *Culex pipiens* larvae. LC values and the corresponding LT_{50} were derived from log-probit dose-response and time-mortality regression models at day 7. Bars represent mean LC estimates (±95 % confidence intervals) on a logarithmic scale; LT_{50} points are shown with their 95 % confidence intervals

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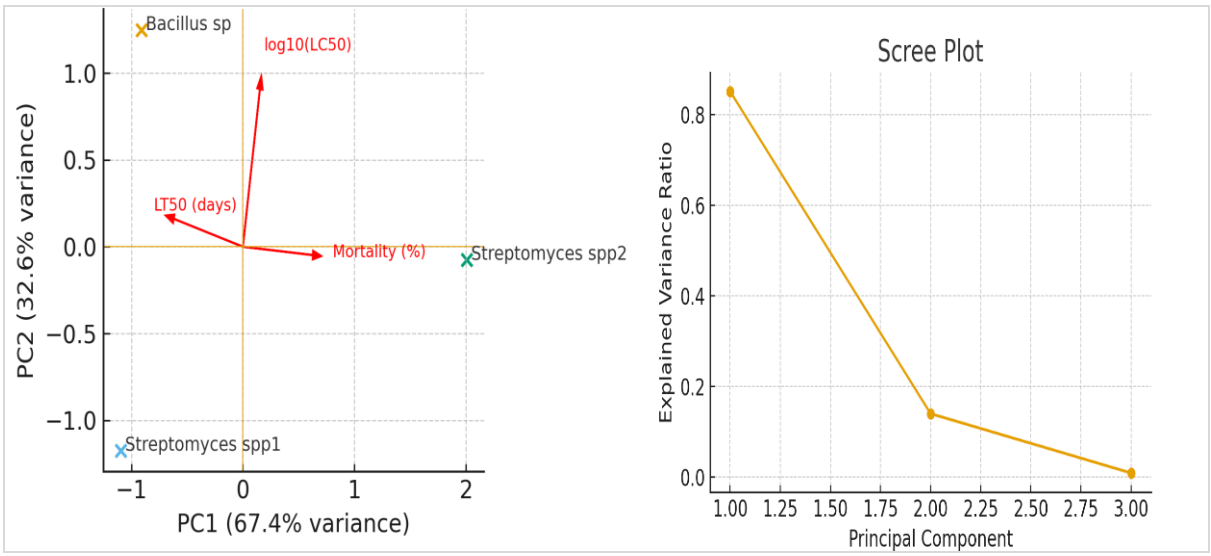

Fig. 8. Principal component analysis (PCA) of $\log_{10}$ (LC_{50}), LT_{50} and mortality of *Culex pipiens* larvae at 10^6 CFU/mL (day 7) for *Bacillus* sp., STR1 and STR2. (A) PCA biplot: PC1 and PC2 account for 67.4 % and 32.6 % of the total variance, respectively. Strains are represented by their scores (points) and variables by loading vectors (red arrows). (B) Scree plot showing the proportion of variance explained by each principal component, confirming that only the first two components are informative

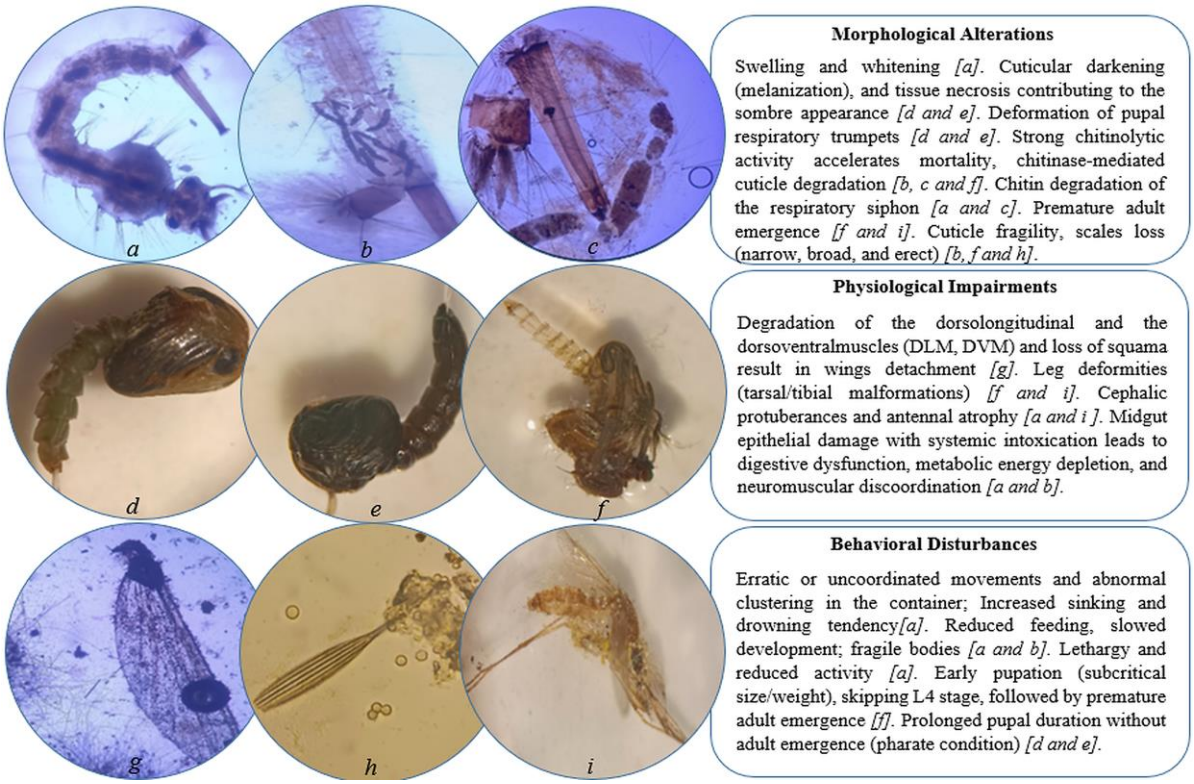

Fig. 9. Behavioral, morphological and physiological symptoms observed in *Culex pipiens* mosquitoes following treatment with *Bacillus*, STR1 and STR2 at 10^6 CFU/mL. Stereomicroscopic observations were performed at 10× and 40×. Panels (a–c) show larval-stage alterations; panels (d–f) show pupation and emergence-related abnormalities; panels (g–i) show adult-stage alterations

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Discussion

The chitin yield obtained from shrimp shells in this study was comparable to values reported for similar crustacean by-products, confirming this material as a suitable, low-cost substrate for enriching chitinolytic microorganisms (26). Using locally purified chitin rather than commercial material likely preserved native structural features important for colonization by chitin-degrading bacteria and actinomycetes and for mimicking natural cuticular substrates of mosquitoes (27). This strategy therefore supports both waste valorisation and the development of regionally adapted bioinsecticidal resources.

From this chitin-based system, we isolated one *Bacillus* sp. and two morphologically distinct *Streptomyces* strains (STR1 and STR2). The clear differences in virulence between STR1 and STR2 are consistent with the high metabolic and genetic diversity of *Streptomyces*, which is a major source of bioactive natural products, including insecticidal metabolites and potent chitinases (28, 29). Probit analysis of dose-response and time-mortality data revealed a robust potency gradient STR2 > STR1 > *Bacillus* sp., reflected by lower LC_{50}, LC_{90} and LC_{99} values, shorter LT_{50} and lower discriminating concentrations (DC) for STR2 compared with the other isolates.

The LC values obtained for STR1 and STR2 fall within the range reported for promising actinobacterial larvicides. *Streptomyces* spp. KSF103 and related isolates have shown strong activity against *Aedes*, *Anopheles* and *Culex* larvae with relatively low LC_{50} values and limited non-target effects (30, 31). Our STR2 strain thus fits into this emerging group of mosquitocidal actinomycetes. However, its potency remains lower than optimized formulations of *Bacillus thuringiensis israelensis* (Bti) and *Lysinibacillus sphaericus* which achieve very low LC_{50} and near-complete kill at ppm levels in WHO larval bioassays (32, 33). This indicates that optimization of culture conditions, concentration of active metabolites and formulation will be necessary before STR2 can realistically compete with established microbial larvicides.

The DC values derived from our probit models provide additional insight. According to WHO recommendations, diagnostic doses are used to distinguish susceptible from resistant mosquito populations and to monitor resistance to microbial larvicides (33). Although our bioassays were performed on extremophile strains, the particularly low DC of STR2 compared with STR1 and *Bacillus* sp. suggests that STR2 could be more economical and practical for future resistance monitoring or operational use. In contrast, the higher DCs of *Bacillus* sp. and STR1 imply that, under the tested conditions, these isolates would require larger quantities to achieve full control, making them more suitable as components of integrated or combined strategies rather than as stand-alone larvicides (34).

Time-mortality analysis at 10^6 CFU/mL yielded LT_{50} values of roughly 4–6 days, with STR2 consistently killing larvae faster than STR1 and *Bacillus* sp. Such LT_{50} indicates a relatively slow but progressive larvicidal effect, typical of biological agents acting through chronic intoxication, tissue degradation, or metabolic disruption (35). Plant-derived and microbial larvicides often show comparable or longer LT_{50}s yet still provide satisfactory control when persistent and environmentally safe (34). Microbial larvicides such as Bti and *L. sphaericus* also display variable LT_{50}s depending on formulation and mosquito species (32). In this context, the shorter LT_{50} of STR2 is advantageous, as it reduces the window during which larvae can feed and develop into adults.

Beyond acute mortality, all three isolates induced marked developmental and post-emergence effects. In larvae, reduced feeding, growth retardation, swelling, whitening,

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and fragile bodies are consistent with midgut damage, systemic stress and osmotic imbalance, as reported for other bacterial and actinomycete entomopathogens (27, 31). Pronounced chitinolytic activity and visible degradation of the cuticle and siphon suggest that extracellular chitinases and proteases contribute to lethality by weakening the integument and interfering with respiration (29, 30). At the pupal and adult stages, premature pupation at sub-optimal size, prolonged pupal duration with pharate adults, melanization, necrosis, deformities of respiratory trumpets, incomplete or abnormal adult emergence, poorly sclerotized cuticles, twisted or shortened legs, wing detachment and antennal atrophy were frequently recorded (Fig. 9). Similar malformations have been described in mosquitoes exposed to fungal pathogens, bacterial toxins and certain plant extracts, where disruption of cuticle sclerotization, neuromuscular function and energy metabolism leads to severely reduced survival and flight capacity (34, 35). These developmental and post-emergence defects raise the question of whether our isolates exert true insect growth regulator (IGR) activity or mimic IGR-like syndromes through chronic toxicity and tissue degradation. Classical IGRs, such as juvenile hormone analogues or chitin synthesis inhibitors, typically reduce pupation and adult emergence and induce malformed adults by disrupting endocrine control of molting and cuticle formation (36). The premature pupation extended pupal duration and malformed, non-flying adults observed here resemble this pattern. However, our current data do not demonstrate the production of genuine IGR molecules. It remains plausible that intense chitinolytic and proteolytic activity, combined with midgut and systemic damage, secondarily disrupts development in a way that phenotypically mimics IGR exposure (27, 31). Clarifying this issue will require targeted endocrine and molecular assays and chemical identification of the active metabolites.

Finally, the origin of STR1 and STR2 from an arid environment is consistent with growing evidence that desert actinomycetes are rich sources of structurally novel and often highly potent bioactive metabolites (28, 37). Desert-derived *Streptomyces* have been repeatedly highlighted for their high chitinase production and strong insecticidal profiles compatible with eco-friendly pest management (29, 31). Our findings support this view and show that such strains can affect *Cx. pipiens* not only by killing larvae but also by profoundly impairing the development and functionality of survivors. Overall, STR2 and to a lesser extent STR1 emerge as a promising candidate for further development as a biological control agent, warranting optimization of production and formulation and evaluation under semi-field and field conditions alongside established microbial larvicides such as Bti and *L. sphaericus* (32, 33).

Conclusion

Chemical pest control negatively impacts ecosystems, human health, and fosters pest resistance, highlighting the need for eco-friendly alternatives such as biological control. In this study, three chitinolytic bacterial strains (one *Bacillus* and two *Streptomyces*, isolated from date palm bark in IGLI's palm groves, (Saoura region, Southwestern Algeria)) were tested against *Cx. pipiens*. All strains caused significant larval mortality and developmental defects, with *Streptomyces* STR2 showing the highest virulence (70% mortality, LT_{50}= 4 days). The main mechanisms involved are cuticle degradation by chitinases and for *Bacillus*, gut damage induced by Cry proteins. Treated mosquitoes exhibited delayed development and malformations that ultimately led to death.

These findings confirm the strong potential of chitinolytic microorganisms as promising alternatives to chemical insecticides. Future work should focus on: (i) molecular

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identification of the strains and their chitinases, (ii) testing on other insect pests under both laboratory and field conditions, (iii) isolating and comparing additional chitinolytic microorganisms for their biocontrol efficacy, (iv) Evaluation of the adaptive potential of the studied strains to survive and function in harsh and extreme desert ecosystems, (v) Comprehensive assessment of the potential impacts and safety of the studied strains on human health.

The isolation of microorganisms from extreme desert environments offers unique opportunities, as their metabolic adaptations often translate into rare bioactive compounds with strong entomopathogenic potential. Valorizing this unexplored microbial diversity is therefore essential for developing innovative and sustainable biocontrol strategies.

Acknowledgments

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Ethical Considerations

This experimental study involved mosquito specimens and entomopathogenic bacterial isolates only. No human participants or vertebrate animals were involved. Therefore, ethical approval and informed consent were not required.

Conflict of interest statement

The authors declare there is no conflict of interest.

References

1. Rodhain F, Perez M (1985) Mosquito-borne diseases: epidemiology and control. Bull World Health Organ. 63(5): 827–839.
2. World Health Organization (WHO) (2022) World malaria report 2022. World Health Organization, Geneva. Available at: https://www.who.int/publications/i/item/978924006489.
3. Sharma A, Singh P, Gupta R (2022) *Actinomycetes* as potential biocontrol agents: chitinase-mediated insecticidal activity. J Appl Microbiol. 132(2): 789–802.
4. Ghosh A, Chandra G, Sahu SK (2023) Sustainable strategies for mosquito control: biological approaches. Acta Trop. 246: 106–115.
5. Munyakanage D, Niyituma E, Mutabazi A, Misago X, Musanabaganwa C, Remera E, Rutayisire E, Ingabire MM, Majambere S, Mbituyumuremyi A, Ngugi MP, Kokwaro E, Hakizimana E, Muvunyi CM (2024) The impact of *Bacillus thuringiensis* var. *israelensis* (Vectobac® WDG) larvicide sprayed with drones on the bio-control of malaria vectors in rice fields of sub-urban Kigali, Rwanda. Malar J. 23(1): 281.
6. Van Nieuwpoort JC, Schrama M, Spitzen J, Boerlijst SP (2025) Beyond the target insects: impacts of Bti on aquatic macrofauna communities. Parasit Vectors. 18(271): 1–8.
7. Surya W, Chooduang S, Choong YK, Torres J, Boonserm P (2016) Binary toxin subunits of *Lysinibacillus sphaericus* are monomeric and form heterodimers after in vitro activation. PLoS One. 11 (6): e0158356.
8. Hegazy MI, Hegazy AM, Saad AM, Salem HM, El-Tahan AM, El-Saadony MT, Soliman SM, Taha AE, Alshehri MA, Ahmed AE, Swelum AA (2022) Some biologically active microorganisms have the potential to suppress mos-

quito larvae (*Culex pipiens*, Diptera: Culicidae). Saudi J Biol Sci. 29(4): 1998–2006.
9. Zogo B, N'Cho Tchiekoi B, Koffi AA, Dahounto A, Ahoua Alou LP, Dabiré RK, Baba-Moussa L, Moiroux N (2019) Impact of sunlight exposure on the residual efficacy of biolarvicides Bacillus thuringiensis israelensis and *Bacillus sphaericus* against the main malaria vector, *Anopheles gambiae*. Malar J. 18: 55.
10. Hasan S, Boddu VM, Viswanath DS, Ghosh TK (2022) Preparation of chitin and chitosan. In: Chitin and Chitosan: Science and Engineering. Springer International Publishing, Cham, pp. 17–50.
11. El-araby A, El Ghadraoui L, Errachidi F (2022) Physicochemical properties and functional characteristics of ecologically extracted shrimp chitosans with different organic acids during demineralization step. Molecules. 27(23): 8285.
12. Tiwari D, Shouche S, Bhati P, Das P (2021) A consolidated method for selective isolation of actinomycetes based on choice of substrate. Int Ann Sci. 11 (1): 10–21.
13. Alvarenga VO, Campagnollo FB, Silva RA, Hubinger MD, Lang É, Amorim-Neto DP, Sant'Ana AS (2025) Impact of sublethal treatments on dormant and non-dormant populations of *Bacillus cereus* spores during spray drying and on their recovery during whole milk powder storage. Int Dairy J. 165: 106208.
14. Wen J, Smelt JPPM, Vischer NOE, de Vos AL, Setlow P, Brul S (2022) Heat activation and inactivation of bacterial spores: is there an overlap? Appl Environ Microbiol. 88(5): e02324.
15. Reva ON, Sorokulova IB, Smirnov VV (2001) Simplified technique for identification of the aerobic spore-forming bacteria by phenotype. Int J Syst Evol Microbiol. 51(4): 1361–1371.
16. Wildermuth H (1972) Morphological surface characteristics of *Streptomyces glaucescens* and *S. acrimycini*, two streptomycetes with 'hairy' spores. Arch Microbiol. 81(4): 321–332.
17. Hellany H, Assaf JC, Matta J, Khalil MI (2024) Fungal isolation, detection, and quantification of aflatoxins in nuts sold in the Lebanese market. Processes. 12 (5): 1018.
18. Komaki H (2023) Recent progress of reclassification of the genus Streptomyces. Microorganisms. 11(4): 831.
19. Xu X, Kovács ÁT (2024) How to identify and quantify the members of the *Bacillus* genus? Environ Microbiol. 26(2): e16593.
20. Alnahari AA, Kusa AM, Al Ghamdi AK, Algashgari B, Alqurashi SI, Mahyoub JA, Baeshen MN (2025) Evaluating bioassay efficacy of extremophile *Bacillus* species for environmentally safe control of *Aedes aegypti* larvae. Sci Rep. 15: 24469.
21. Seratnahaei M, Zahraei-Ramazani A, Eshraghi SS, Yaseri M, Pakzad P (2023) Larvicidal effects of metabolites extracted from Nocardia and *Streptomyces* species against the fourth larval stage of *Anopheles stephensi* (Diptera: Culicidae). J Arthropod Borne Dis. 17(2): 187–196.
22. Rajchanuwong P, Peaboon S, Ngoen-Klan R, Rattanawannee A, Noosidum A, Promdonkoy B, Chanpaisaeng J, Chareonviriyaphap T (2025) Larvicidal activity of *Bacillus thuringiensis* strains against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. Curr Res Parasitol Vector Borne Dis. 7: 100245.
23. Ahmed M, Hassan R, El-Sayed A (2022) Biological control of *Aedes aegypti* mosquitoes using *Bacillus thuringiensis*. Egypt J Biol Sci For. 10(3): 45–55.
24. Li Q, Zhang X, Zhao Y (2023) New native *Bacillus thuringiensis* strains induce

high insecticidal action against *Culex pipiens* pallens larvae and adults. BMC Microbiol. 23: 100, 1–14.
25. Pochon J, Tardieux P (1962) Techniques d'analyse en microbiologie du sol [Techniques of analysis in soil microbiology]. Éditions de la Tourelle, Saint-Mandé, France.
26. Younes I, Rinaudo M (2015) Chitin and chitosan preparation from marine sources: structure, properties and applications. Mar Drugs. 13(3): 1133–1174.
27. Paul MK, Mathew J (2023) Mosquito larvicidal activity of chitinase of *Pseudomonas putida* Mb 12 against the human vector *Aedes aegypti*. J Pure Appl Microbiol. 17(1): 403–410.
28. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk HP, Clément C, Ouhdouch Y, Van Wezel GP (2016) Taxonomy, physiology, and natural products of actinobacteria. Microbiol Mol Biol Rev. 80(1): 1–43.
29. Ganesan P, Anand S, Sivanandhan S, David RHA, Paulraj MG, Abdullah N, Ignacimuthu S (2018) Larvicidal, ovicidal and repellent activities of *Streptomyces enissocaesilis* (S12–17) isolated from the Western Ghats of Tamil Nadu, India. J Entomol Zool Stud. 6 (2): 1828–1835.
30. Amelia-Yap ZH, Low VL, Saeung A, Ng FL, Chen CD, Hassandarvish P, Tan GYA, AbuBakar S, Azman AS (2023) Insecticidal activities of *Streptomyces* sp. KSF103 ethyl acetate extract against medically important mosquitoes and non-target organisms. Sci Rep. 13(1): 4.
31. Sharif MA, Raza FA, Sajid I (2024) Evaluation of larvicidal potential of *Streptomyces* extracts against *Aedes aegypti*. J Microbiol Mol Genet. 5(3): 189–203.
32. Silva-Filha MHNL, Romão TP, Rezende TMT, Carvalho KdS, Gouveia de Menezes HS, Alexandre do Nascimento N, Soberón M, Bravo A (2021) Bacterial toxins active against mosquitoes: mode of action and resistance. Toxins. 13(8): 523.
33. World Health Organization (2005) Guidelines for laboratory and field testing of mosquito larvicides (WHO/CDS/WHOPES/GCDPP/2005.13). World Health Organization, Geneva.
34. Lima EP, Goulart MOF, Rolim Neto ML (2015) Meta-analysis of studies on chemical, physical and biological agents in the control of *Aedes aegypti*. BMC Public Health. 15: 858.
35. De Souza Wuillda ACJ, Campos Martins RC, Costa FDN (2019) Larvicidal activity of secondary plant metabolites in *Aedes aegypti* control: an overview of the previous 6 years. Nat Prod Commun. 14(7): 1934578X19862893.
36. Suman DS, Wang Y, Bilgrami AL, Gaugler R (2013) Ovicidal activity of three insect growth regulators against *Aedes* and *Culex* mosquitoes. Acta Trop. 128(1): 103–109.
37. Bull AT, Asenjo JA (2013) Microbiology of hyper-arid environments: recent insights from the Atacama Desert, Chile. Antonie Van Leeuwenhoek. 103(6): 1173–1179.