



International Journal of Health care and Biological Sciences

An International Online Open access Referred Journal
Content available at www.saajournals.org Online ISSN: 2582-7499



LARVICIDAL ACTIVITY AND MIDGUT HISTOPATHOLOGY OF *BACILLUS THURINGIENSIS* WHOLE CELLS AND SPORES AGAINST *CULEX PIPIENS* (DIPTERA: CULICIDAE) LARVAE

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DOI: <https://doi.org/10.46795/ijhcbcs.v7i3.960>

ARTICLE HISTORY	ABSTRACT
Received on: 19-07-2026 Revised on: 11-08-2026 Accepted on: 03-09-2026 Keywords: <i>Culex pipiens</i>; <i>Bacillus thuringiensis</i>; bioinsecticide; larvicidal bioassay; midgut histopathology; Abbott's formula. *CORRESPONDING AUTHOR Jenan M. Ubaid	<i>Culex pipiens</i> is a vector of several human pathogens, and the chemical control of its larvae is losing ground to insecticide resistance. This study measured the larvicidal activity of whole cells and spores of a local <i>Bacillus thuringiensis</i> isolate against third-instar <i>Cx. pipiens</i> larvae and described the damage they cause to the larval midgut. Larvae were collected in Al-Hillah, central Iraq, between November 2024 and February 2025 and were reared in the laboratory for three generations. <i>Bacillus thuringiensis</i> was isolated from non-clinical food samples and identified by colony morphology on HiCrome™ <i>Bacillus</i> agar, Gram staining, biochemical tests and 16S rRNA gene sequencing. Three whole-cell concentrations (1.5×10^8, 7.5×10^7 and 3.7×10^7 CFU/mL) and three spore concentrations (1.5×10^7, 1.5×10^6 and 1.5×10^5 CFU/mL) were tested, each with three replicates of ten larvae. Beta-cypermethrin served as the positive control and dechlorinated water as the negative control. Dead larvae were counted at 24, 48 and 72 h, and mortality was corrected with Abbott's formula. Whole cells killed every larva at 72 h at all three concentrations (100 % corrected mortality) against 10 % in the negative control. Mortality was very low at 24 h and rose sharply between 48 and 72 h. Spores gave 100 %, 95.72 % and 89.30 % corrected mortality at 1.5×10^7, 1.5×10^6 and 1.5×10^5 CFU/mL, so their activity was close to that of whole cells. Histological sections stained with haematoxylin and eosin showed damage of the midgut that increased with the severity of the treatment: the epithelial lining was disrupted, muscle bundles were fragmented, and vacuoles, cellular debris and spore clusters filled the tissue. Control larvae kept a normal tissue architecture. The local <i>B. thuringiensis</i> isolate is therefore an effective, time-dependent larvicide against <i>Cx. pipiens</i>.

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1. INTRODUCTION

Culex pipiens is a blood-sucking arthropod and a major vector of Bancroftian filariasis, West Nile virus, Rift Valley fever, Japanese encephalitis, St Louis encephalitis, Murray Valley encephalitis and Ross River disease. It occurs in tropical and subtropical regions and passes through a complete metamorphosis from egg to adult (Coalson et al., 2021; Naik et al., 2025).

The adult of *Cx. pipiens* has a small slender body with dark brown scales, a globular head, a thick clypeus and black eyes. The life cycle begins with egg rafts that float on the water surface. The larva passes through four

instars and then reaches the pupal stage before the adult emerges (Yaba and Al-Muffti, 2024).

Adulticides and repellents reduce mosquito populations or suppress their olfactory activity, and they can lower the number of diseases spread by mosquitoes. These substances, however, may also affect soil, water and wildlife that were never their target, and overexposure to them may cause disease or death (Ren et al., 2023; Farag et al., 2024). Resistance to the insecticides used in vector control and in agriculture has now developed in the major disease vectors worldwide. More resistance data are needed to understand how this affects control

programmes and to improve resistance management (Van den Berg et al., 2021; Okeke et al., 2022).

The role of *B. thuringiensis* in mosquito control has grown over the past decades. Its crystal toxins, made of Cry and Cyt proteins, are among the most used ecological insecticides. Control relies mainly on the δ -endotoxins, which change the osmotic pressure of the midgut cells and cause cell lysis and insect death (Saibu et al., 2023; Naing et al., 2023).

This study investigates the bioactivity of *B. thuringiensis* against *Cx. pipiens* larvae and examines the histopathological changes that follow treatment. Whole bacterial cells and purified spores were compared side by side under the same conditions, which is the main point of difference from earlier reports on this species.

2. MATERIALS AND METHODS

2.1. Mosquito collection, identification and rearing

The study was carried out in Al-Hillah city, central Iraq. Mosquito larvae were collected between November 2024 and February 2025 from sites known for breeding activity: drainage ditches, small pools and stagnant water. Each sample was labelled with its location and date and was carried to the laboratory in sterile plastic containers filled with water from the collection site.

For identification, adults were placed in 70 % ethanol and larvae were transferred onto glass slides. Specimens were examined under a dissecting microscope for colour, size, antennae, proboscis, wings, legs and abdomen. Members of the *Cx. pipiens* group were identified with an accepted taxonomic key, and the identification was confirmed by a local source (Figure 1) (Harbach and Kitching, 1998; Polse et al., 2021).

After identification, larvae were fed a fish feed containing 35 % protein, 0.9–2 % calcium, 2.5 % fibre and 4 % fat. They were kept in cubic wooden cages with 500 mL of tap water for 72 h. Pupae were transferred to a plastic container placed inside the cages so that adults could emerge. The cages were held at 27 °C under a 12 h light : 12 h dark cycle. Females were fed on a chicken and males on a cotton pad soaked in sugar solution. Two to three days after oviposition the eggs were moved to sterile glass tanks containing water that had been left to stand for 24 h. The colony was reared for three generations before the experiments began, so that any trace of the chemical and biological pesticides used in the field was removed (Figure 2) (Wohl and McMeniman, 2023). The complete experimental sequence is summarised in Figure 3.

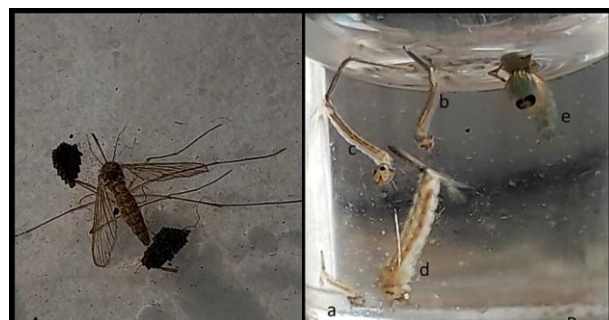


Figure 01: (A) Adult female *Culex pipiens* with two egg rafts laid on the water surface. (B) Developmental stages of *Culex pipiens* in water: (a) first-instar larva, (b) second-instar larva, (c) third-instar larva, (d) fourth-instar larva, (e) pupa.



Figure 02: (A) Mosquito breeding cage exposed to morning sunlight. (B) Adult mosquitoes feeding on sugar solution and on a severed chicken head.

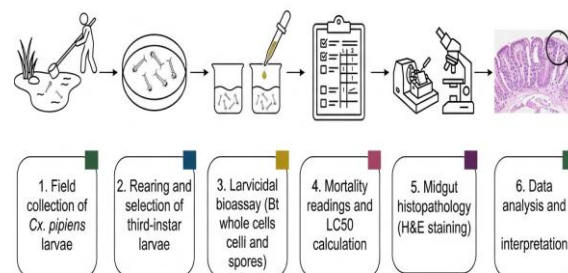


Figure 03: Workflow of the study, from field collection of *Culex pipiens* larvae to the larvicidal bioassay, mortality readings and histological analysis.

2.2. Isolation and identification of *Bacillus thuringiensis*

Bacillus spp. were isolated from non-clinical food samples collected in Babylon, and only non-pathogenic species were used in the experiment. The samples were kept until the isolation of *B. thuringiensis* began. They were suspended in brain–heart infusion broth, cultured on HiCrome™ *Bacillus* agar, and then examined by colony morphology, microscopy, biochemical tests and 16S rRNA gene sequencing. Pathogenic species were excluded because they did not meet the safety standards applied here (Jawetz et al.,

2019; Ganga et al., 2023). Sequencing details and accession numbers: [to be supplied by the authors].

2.3. Preparation of the bacterial and spore suspensions

Two kinds of suspension were prepared: whole bacterial cells and purified spores. Bacterial samples were cultured on nutrient media, and after 24 h *B. thuringiensis* colonies were taken from the plate and serially diluted. Three dilutions of whole cells were prepared, and each dilution was added to ten larvae held in a 100 mL cup with distilled water and fish feed. Each dilution was tested in three replicates under controlled environmental conditions (Fu et al., 2008). For the spore suspension, 5 mL of distilled water was added to the culture and the spores were harvested. The suspension contained 10 mL of distilled water and bacterial spores, and a heat-and-cold shock was applied to remove vegetative cells and retain the spores. The stock suspension was then placed in a test tube containing 9 mL of distilled water and adjusted against a 0.5 McFarland standard. Three decimal dilutions were prepared, and the results were recorded after 24, 48 and 72 h (Aube and Gagnon, 1969; Ishii and Ohba, 1993).

2.4. Reference chemical insecticide

The chemical pesticide beta-cypermethrin was tested against *Cx. pipiens* as a reference treatment. The product used was Icon Genta 10 % WP, a registered insecticide known to be effective against mosquitoes, especially in the immature stages. The pesticide was dissolved in distilled water to give a stock solution, and the working concentrations were prepared in steps of 0.3125 mg/L. This treatment served as the positive control (Yaseen and Sulaiman, 2021; Mahdi, 2022).

2.5. Bioassay design

Healthy, uninfected third-instar larvae of *Cx. pipiens* from the laboratory colony were used. Ten larvae of the same age were placed in a 100 mL plastic cup containing dechlorinated water and larval food, so that no larva died of starvation.

Three whole-cell concentrations (1.5×10^8 , 7.5×10^7 and 3.7×10^7 CFU/mL) and three spore concentrations (1.5×10^7 , 1.5×10^6 and 1.5×10^5 CFU/mL) were prepared. Ten millilitres of each concentration was mixed into 100 mL of water containing ten larvae and larval food. Each concentration was tested in three replicates. A negative control without any bacterial preparation and a positive control with the chemical pesticide were run alongside (Zhu et al., 1989).

2.6. Calculation of larval mortality

The number of dead larvae in each replicate was counted after exposure, including the control treatments. Data were collected at 24, 48 and 72 h by counting the larvae that showed signs of death such as immobility, discoloration or sinking to the bottom of the cup (Clements, 1999; Anwar, 2009).

Mortality was corrected with Abbott's formula (Abbott, 1925), also known as the Schneider–Orelli formula (Shabaan et al., 1991):

$$\text{Corrected mortality (\%)} = \frac{(T - C)}{100 - C} \times 100$$

where **T** is the percentage mortality in the treatment and **C** is the percentage mortality in the untreated control.

2.7. Histological analysis

Seventy-two hours after treatment the larvae were transferred to tubes containing formalin to fix the specimens. After one day they were passed through a graded alcohol series to remove the water and then through xylene to remove the alcohol.

The larvae were next placed in molten paraffin wax and embedded in solid wax blocks. Once the blocks had hardened, sections 5 μ m thick were cut with a microtome. The sections were mounted on glass slides and stained with haematoxylin and eosin (H&E), then covered with a cover slip and examined under a light microscope at 4X, 10X and 40X (Lucarotti et al., 2011).

2.8. Statistical analysis

All data were analysed with the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). A two-way analysis of variance (ANOVA) was used to test the effect of treatment and time. When the ANOVA showed significant differences among means ($p \leq 0.05$), the means were compared with Tukey's Honestly Significant Difference (HSD) test at the 5 % level. Results are given as mean $\pm$ standard deviation (SD), and means followed by different letters differ significantly.

3. RESULTS

3.1. Isolation and identification of *Bacillus thuringiensis*

Bacillus thuringiensis was identified as a rod-shaped, Gram-positive bacterium on the chromogenic medium HiCrome™ *Bacillus* agar. The isolate carried proteinaceous crystalline inclusions (Cry toxins) next to the spores, which separates it from closely related species. The morphological and biochemical characteristics are listed in Table 01, and the colonies and the Gram-stained smear are shown in Figure 04.

Table 01: Morphological and biochemical characteristics of the *Bacillus thuringiensis* isolate used in this study.

Test	Result
Growth on HiCrome™ <i>Bacillus</i> agar	Non-glossy appearance, dry texture, irregular shape, creamy white to pale ivory colour, no pigmentation
Gram stain	Gram-positive rods
Motility	+
Catalase	+
Haemolysis	+
Oxidase	+
Simmons' citrate	+
Voges-Proskauer	+

Test	Result
Indole	-
Methyl red	-
Urease	-

+ = positive reaction; - = negative reaction. Data taken from panel A of the original Figure 3 of the manuscript and presented here as a table.

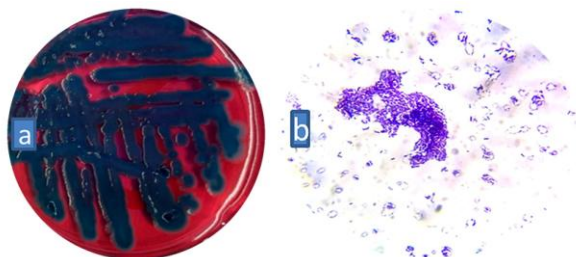


Figure 04: *Bacillus thuringiensis* isolate: (a) colonies on HiCrome™ Bacillus agar base; (b) Gram stain under the light microscope, oil immersion, 100X objective (total magnification 1000X).

3.2. Larvicidal activity of whole *B. thuringiensis* cells

Table 02 shows the effect of the three whole-cell concentrations (1.5×10^8 , 7.5×10^7 and 3.7×10^7 CFU/mL) on the mortality of third-instar *Cx. pipiens* larvae over 24, 48 and 72 h, together with the negative control, which reached 10 %, and the positive control with the chemical pesticide, which reached 100 %. Whole cells gave the highest larval mortality, and a longer exposure improved the effect. After correction with Abbott's formula, mortality reached 100 % at all three concentrations at 72 h against 10 % in the negative control (Figure 5A and Figure 06).

Table 02: Effect of whole *Bacillus thuringiensis* cells on third-instar larvae of *Culex pipiens*.

Concentration ⁿ (CFU/mL)	Mean $\pm$ SD of dead larvae at 24 h	Mean $\pm$ SD of dead larvae at 48 h	Mean $\pm$ SD of dead larvae at 72 h	Corrected mortality at 72 h (%)
1.5×10^8	0.0 ± 0.0 a	0.3 ± 0.01 b	10.0 ± 0.0 f	100
7.5×10^7	0.3 ± 0.01 b	1.7 ± 0.2 e	10.0 ± 0.0 f	100
3.7×10^7	0.3 ± 0.01 b	0.7 ± 0.1 c	10.0 ± 0.0 f	100
Negative control	0.0 ± 0.0 a	0.3 ± 0.01 b	1.0 ± 0.05 d	10
Positive control	9.0 ± 1.0 f	10.0 ± 0.0 f	10.0 ± 0.0 f	100

Values are the mean $\pm$ SD of the number of dead larvae out of ten per cup (three replicates). Different letters indicate statistically significant differences among the interaction treatments (concentration $\times$ time)

based on Tukey's HSD test at $P < 0.05$. Mortality was corrected with Abbott's formula.

Mortality was very low during the first 24 h. At that time the two lower concentrations (7.5×10^7 and 3.7×10^7 CFU/mL) already differed from the negative control, while 1.5×10^8 CFU/mL did not. At 48 h the three concentrations differed from one another, and 7.5×10^7 CFU/mL gave the highest count of dead larvae (1.7 ± 0.2). At 72 h the three concentrations reached the same level as the positive control and showed no difference among themselves, while all of them differed clearly from the negative control.

3.3. Larvicidal activity of *B. thuringiensis* spores

Table 3 shows the effect of the three spore concentrations (1.5×10^7 , 1.5×10^6 and 1.5×10^5 CFU/mL) on the mortality of third-instar *Cx. pipiens* larvae over the same three periods, compared with the negative control, which reached 6 %, and the positive control, which reached 100 %. The statistical analysis showed that the different bacterial preparations had a significant effect. The activity of the spores was close to that of the whole cells. No larva died during the first 48 h. At 72 h, corrected mortality was 100 % at 1.5×10^7 CFU/mL, 95.72 % at 1.5×10^6 CFU/mL and 89.30 % at 1.5×10^5 CFU/mL (Figure 5B and Figure 6).

Table 03: Effect of *Bacillus thuringiensis* spores on third-instar larvae of *Culex pipiens*.

Concentration ⁿ (CFU/mL)	Mean $\pm$ SD of dead larvae at 24 h	Mean $\pm$ SD of dead larvae at 48 h	Mean $\pm$ SD of dead larvae at 72 h	Corrected mortality at 72 h (%)
1.5×10^7	0.0 ± 0.0 a	0.0 ± 0.0 a	10.0 ± 0.0 b	100
1.5×10^6	0.0 ± 0.0 a	0.0 ± 0.0 a	9.7 ± 1.3 b	95.72
1.5×10^5	0.0 ± 0.0 a	0.0 ± 0.0 a	9.0 ± 1.0 b	89.30
Negative control	0.0 ± 0.0 a	0.0 ± 0.0 a	1.0 ± 0.0 a	6
Positive control	9.0 ± 1.0 f	10.0 ± 0.0 f	10.0 ± 0.0 f	100

Values are the mean $\pm$ SD of the number of dead larvae out of ten per cup (three replicates). Different letters indicate statistically significant differences among the interaction treatments (concentration $\times$ time) based on Tukey's HSD test at $P < 0.05$. Mortality was corrected with Abbott's formula. The letters of Table 2 and Table 3 belong to two separate analyses and are not comparable between the tables.

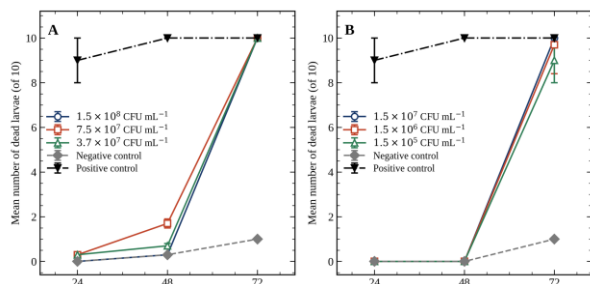


Figure 05: Mean number of dead *Culex pipiens* third-instar larvae (out of ten) over time. (A) Whole *Bacillus thuringiensis* cells. (B) *Bacillus thuringiensis* spores. Bars are standard deviations of three replicates and are truncated at the limit of ten larvae. Data from Tables 02 and 03.

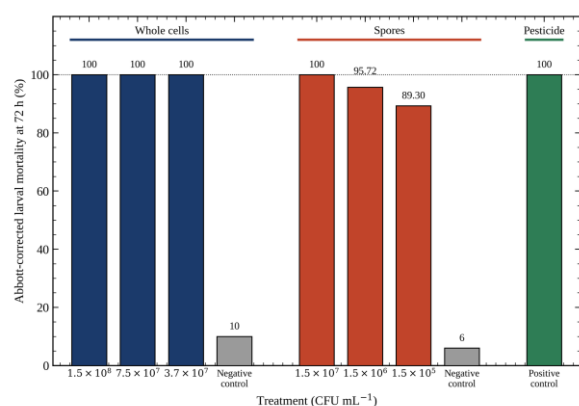


Figure 06: Abbott-corrected larval mortality at 72 h for every treatment. Values are those reported in Tables 2 and 3. The two negative controls belong to the whole-cell assay and the spore assay, respectively.

3.4. Histological changes in the larval midgut after treatment with *B. thuringiensis*

Larvae of the negative control kept healthy muscle tissue, well-defined and organised structures and a normal cellular density. No abnormal cell clusters, infiltrates or widespread damage were seen (Figure 07).

Larvae exposed to the chemical pesticide lost their cohesive structure. The sections showed dead and fragmented cells and cellular debris, and the tissue was left in a disorganised, non-functional state (Figure 08).

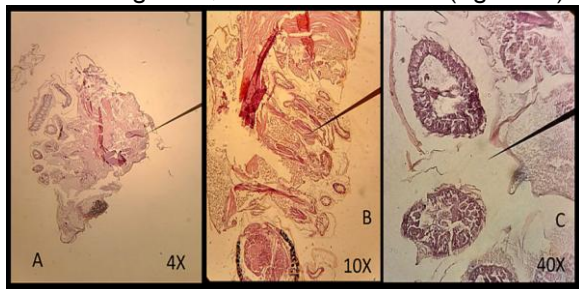


Figure 07: Cross and longitudinal sections of a third-instar *Culex pipiens* larva from the negative control treatment, stained with H&E and viewed at 4X (A), 10X (B) and 40X (C).

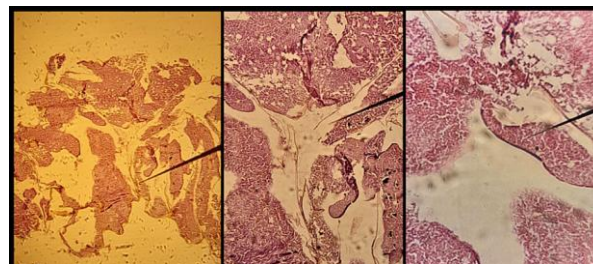


Figure 08: Cross section of a third-instar *Culex pipiens* larva treated with the chemical pesticide (positive control), showing severe histological change with widespread tissue disintegration and necrosis. Stained with H&E and viewed at 4X (A), 10X (B) and 40X (C). Larvae treated with 1.5×10^8 CFU/mL of whole cells appeared more disorganised than the control. The influx of immune cells and the tissue damage may explain this disarray. The muscle tissue looked degenerated, and the muscle bundles were less uniform and more fragmented in places than in the control. The gut wall showed signs of damage (Figure 09).

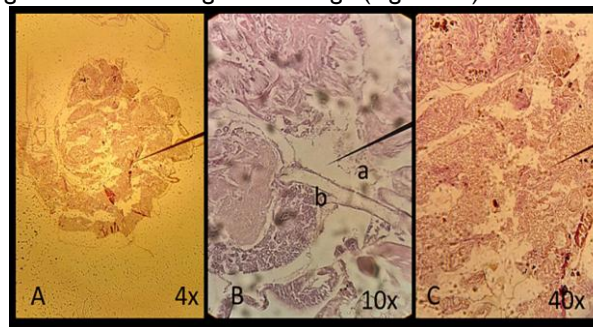


Figure 09: Longitudinal section of a third-instar *Culex pipiens* larva treated with 1.5×10^8 CFU/mL of whole *Bacillus thuringiensis* cells. (a) Muscle bundles appear fragmented. (b) The gut is damaged. Stained with H&E and viewed at 4X, 10X and 40X.

Larvae treated with 7.5×10^7 CFU/mL of whole cells showed moderate pathological change compared with the control. The epithelial lining of the gut was disrupted, the tissue around the digestive tract was disorganised, and the muscle tissue was mildly degenerated. Some regions also showed vacuolation or cellular lysis (Figure 10).

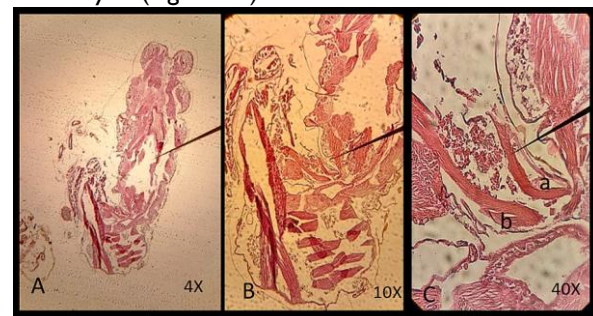


Figure 10: Cross section of a third-instar *Culex pipiens* larva treated with 7.5×10^7 CFU/mL of whole *Bacillus thuringiensis* cells. (a) Muscle bundles appear fragmented. (b) The gut is damaged. Stained with H&E and viewed at 4X, 10X and 40X.

Larvae treated with 3.7×10^7 CFU/mL of whole cells showed severe tissue damage compared with the control. The tissue architecture was lost and the midgut epithelium was extensively damaged. The surrounding structures and the muscle tissue had deteriorated noticeably. Cellular debris and large vacuoles indicated advanced lysis, and some structural elements had disintegrated completely (Figure 11).

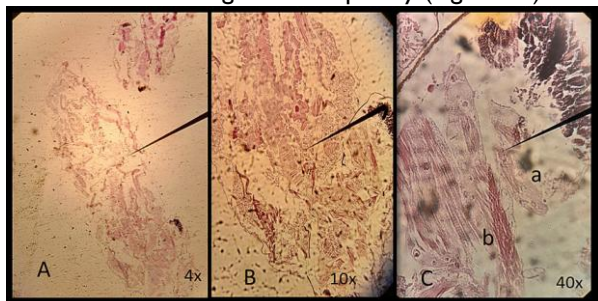


Figure 11: Cross section of a third-instar *Culex pipiens* larva treated with 3.7×10^7 CFU/mL of whole *Bacillus thuringiensis* cells. (a) Muscle bundles appear fragmented. (b) The gut is damaged. Stained with H&E and viewed at 4X, 10X and 40X.

Larvae treated with 1.5×10^7 CFU/mL of spores showed severe tissue degradation compared with the control. Normal tissue had been replaced by dense spore clusters, the midgut was damaged, and signs of inflammation were present (Figure 12).

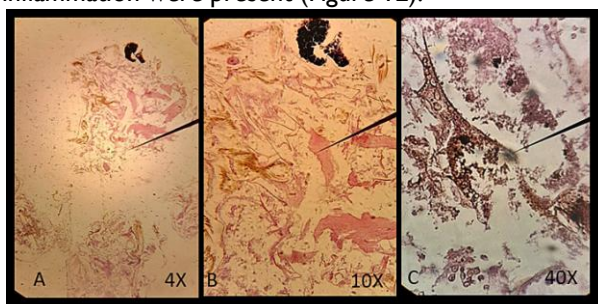


Figure 12: Cross section of a third-instar *Culex pipiens* larva treated with 1.5×10^7 CFU/mL of *Bacillus thuringiensis* spores, showing acute damage and tissue necrosis. Stained with H&E and viewed at 4X, 10X and 40X.

Larvae exposed to 1.5×10^6 CFU/mL of spores showed moderate structural disruption compared with the control. The epithelial lining was partly degenerated in the middle region. Spores were present but more sparsely dispersed than at 1.5×10^7 CFU/mL, and the adjacent musculature showed some damage (Figure 13).

Larvae treated with 1.5×10^5 CFU/mL of spores showed only mild histological change compared with the control. Spores were dispersed through the tissue and the gut lumen, tissue integrity was largely preserved, and damage was focal. The muscles were mostly undamaged (Figure 14).

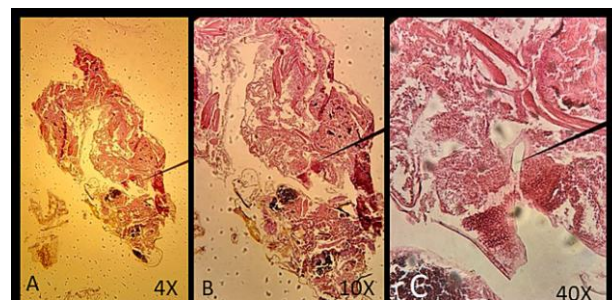


Figure 13: Cross section of a third-instar *Culex pipiens* larva treated with 1.5×10^6 CFU/mL of *Bacillus thuringiensis* spores, showing less severe pathology. Stained with H&E and viewed at 4X, 10X and 40X.

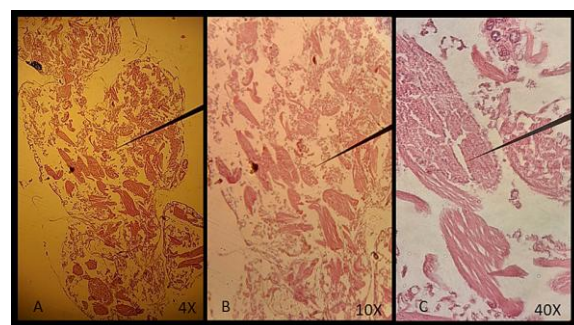


Figure 14: Cross section of a third-instar *Culex pipiens* larva treated with 1.5×10^5 CFU/mL of *Bacillus thuringiensis* spores, showing early signs of infection. Stained with H&E and viewed at 4X, 10X and 40X.

4. DISCUSSION

The results of this study agree with those of Benhissen et al. (2017), who showed that *B. thuringiensis* is toxic to *Cx. pipiens* larvae and that both the exposure time and the bacterial concentration were positively related to mortality. Their toxicological analysis reported good larvicidal activity, with mortality reaching 90 % at the highest concentrations. Ma et al. (2023) isolated new *B. thuringiensis* strains that were highly effective against mosquitoes. Eight strains carried genes coding for endotoxins, and preparations containing crystals and spores were particularly effective against larvae and adults. These findings point to a sustainable and environmentally responsible approach to mosquito control.

Khodyrev et al. (2024) obtained three *B. thuringiensis* isolates from the gut of a natural population of *Aedes* larvae. The strains differed in their protein endotoxin profiles and showed strong insecticidal activity; half-maximal lethal concentrations against *Cx. pipiens* were reached after 24 h of treatment. Mataba et al. (2023) examined how trophic level changes the impact of the biolarvicide *B. thuringiensis* on the oviposition and survival of *Culex* larvae. The application reduced larval survival and density but did not affect oviposition. Bouaziz et al. (2023) tested *B. thuringiensis* on *Culex* larvae and found a strong concentration–response relationship; mortality of *Cx. pipiens* reached 89.33 % at the lower concentration tested.

Whole bacterial cells produced a slightly higher mortality than spores alone. Live cells secrete enzymes and other metabolites that degrade larval tissue and increase the potency of the toxins, and they also produce toxic crystal proteins during the vegetative phase. Spores, in contrast, have no metabolic activity after application and rely only on the pre-formed toxins (Empey et al., 2025).

The sequence that leads from ingestion to death is summarised in Figure 15. The crystal is solubilised in the alkaline midgut, midgut proteases release the active δ -endotoxin, the toxin binds receptors on the brush border, and the resulting pores cause osmotic imbalance and lysis of the epithelial cells. The histological sections obtained here are consistent with each of these stages.

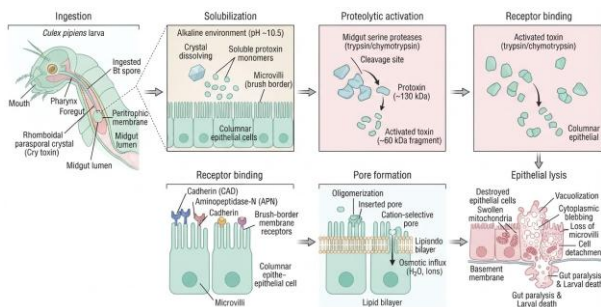


Figure 15: Mode of action of *Bacillus thuringiensis* in the mosquito larval midgut: ingestion of spores and parasporal crystals, solubilisation of the crystal in the alkaline midgut, proteolytic activation of the protoxin, binding of the toxin to brush-border receptors, pore formation and epithelial lysis. Drawn from the mechanism described in the cited literature.

Histological work on other systems supports these observations. The midgut of third-instar larvae was heavily damaged 24 h after oral administration of *B. thuringiensis* toxin complexes, with cytoplasmic extensions, nuclear and cellular degeneration and disruption of the peritrophic membrane and the microvilli. Nuclear disintegration and degradation of chromatin and nucleoli were also reported, while the midgut epithelium of untreated larvae remained intact (Ahmed et al., 2017). Similar findings were obtained with another *Bacillus* species: fourth-instar *Cx. pipiens* larvae treated with *Bacillus sphaericus* showed progressive damage of the midgut epithelium over time. Early vacuolation and cell expansion appeared first, with toxin crystals between the cells and the microvilli. After 10 h the vacuolation had increased, part of the microvilli was lost and spores had invaded the epithelial layer. By 14 h the degradation was extensive, the microvilli were disrupted and the nuclei had deteriorated, which indicates a time-dependent cytopathological effect that ends in larval death (Mohamed et al., 2023).

When *B. thuringiensis* was tested on *Aedes aegypti*, micro-computed tomography of fourth-instar larvae showed gradual damage of the midgut epithelium with oedema, hypertrophy and cellular lysis. The larvae also

changed the way they moved, which made it difficult for them to rise and breathe. Osmotic shock deformed the cross-sectional shape of the body and collapsed the tracheal trunk, so death followed the disruption of both digestion and respiration (Alba-Tercedor and Vilchez, 2023). Another study found that *A. aegypti* larvae treated with *B. thuringiensis* had disintegrated epithelial cells, no identifiable structures and bacterial spores inside the tissue. This destruction is attributed to the endotoxins produced during sporulation, which bind to specific receptors in the brush border membrane of the midgut cells and cause extensive lysis and death (Begum et al., 2015).

Comparable damage has been reported with other biological agents. Fourth-instar *Cx. pipiens* larvae treated with *Beauveria bassiana* showed marked structural damage and degeneration of the epidermal cells. The fungus penetrated the larval barrier, the intestinal epithelial cells disintegrated, the muscle layers deteriorated and the energy reserves fell. Fat cells of the adipose tissue also disintegrated, and the haemocytes in the haemolymph enlarged and changed shape, which suggests a systemic immune response (Benzina et al., 2018). Liu et al. (2020) reported that ar-turmerone, a cytotoxic compound from *Curcuma longa*, changed the histology and ultrastructure of fourth-instar *Cx. pipiens* larvae: the Malpighian tubules enlarged, the cytoplasmic density fell and the brush border became thinner. Twelve hours later the midgut epithelial cells and the muscle fibres were disorganised, the fibres were asymmetric and sparse, and the myofibrils were disrupted. These results support the use of such compounds as bioinsecticides and agree with our own findings.

Several reasons may explain the different degrees of tissue degradation observed here. First, the extent of damage depends on the type and the concentration of the bacterial preparation, since spores or higher bacterial loads can intensify the pathogenic effect. Second, the innate immune response, the age and the nutritional status of individual larvae affect how susceptible they are. Third, different tissues react differently to the toxins, so the muscles, the gut and the cuticle are not affected to the same degree. Temperature and water quality during the experiment may also influence both bacterial activity and the tissue response.

The way each larva reacts to bacterial exposure therefore varies with its physiological state, its immune system, its nutritional status and its capacity to tolerate the toxins. These differences can affect the extent of tissue damage and the overall susceptibility to infection. Finally, complete mortality caused by *B. thuringiensis* spores increased significantly only at 72 h, which shows that the effect of the preparation was gradual in this experiment. The toxic crystals may need time to accumulate in the gut to a lethal level, or the larvae may ingest only a small part of the dose during the first days. This slow onset is the main practical limitation of the spore preparation compared with the chemical

pesticide, which killed 9.0 ± 1.0 larvae out of ten within 24 h.

5. CONCLUSION

Whole cells and spores of a locally isolated *Bacillus thuringiensis* strain are effective larvicides against third-instar *Culex pipiens* larvae. Whole cells killed all larvae at 72 h at every concentration tested, and spores reached 89.30–100 % corrected mortality over the same period, so the two preparations performed almost equally well. The effect was time-dependent: it was small at 24 h and became complete between 48 and 72 h. Histological sections showed that the midgut is the main target, with disruption of the epithelial lining, vacuolation, fragmentation of the muscle bundles and invasion of the tissue by spores. The local isolate is therefore a promising and environmentally acceptable alternative to chemical larvicides in Iraqi breeding sites. Field trials, determination of LC_{50} and LC_{90} values by probit analysis, and tests of persistence under field conditions are the next steps.

REFERENCES

- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18(2), 265–267.
- Ahmed, A. M., Hussein, H. I., El-Kersh, T. A., Al-Sheikh, Y. A., Ayaad, T. H., El-Sadawy, H. A., Al-Mekhlafi, F. A., Ibrahim, M. S., Al-Tamimi, J., & Nasr, F. A. (2017). Larvicidal activities of indigenous *Bacillus thuringiensis* isolates and nematode symbiotic bacterial toxins against the mosquito vector, *Culex pipiens* (Diptera: Culicidae). *Journal of Arthropod-Borne Diseases*, 11(2), 260–277.
- Alba-Tercedor, J., & Vilchez, S. (2023). Anatomical damage caused by *Bacillus thuringiensis* variety israelensis in yellow fever mosquito *Aedes aegypti* (L.) larvae revealed by micro-computed tomography. *Scientific Reports*, 13, 8759.
- Anwar, M. (2009). Antixenotic and antibiotic impact of botanicals for organic management of stored wheat pest insects (Ph.D. thesis). University of Agriculture, Pakistan.
- Aube, C., and Gagnon, C. (1969). Effect of carbon and nitrogen nutrition on growth and sporulation of *Trichoderma viride* Pers. ex Fries. *Canadian Journal of Microbiology*, 15(7), 703–706.
- Begum, M., Akbar, H., Saha, M. L., & Khan, H. R. (2015). Efficacy of *Bacillus thuringiensis* var. israelensis against the larvae of *Aedes aegypti*. *Journal of the Asiatic Society of Bangladesh, Science*, 41(1), 33–44.
- Benhissen, S., Habbachi, W., & Ouakid, M. L. (2017). Effect of *Bacillus thuringiensis* var. kurstaki on the mortality and development of *Culex pipiens* (Diptera: Culicidae). *International Journal of Mosquito Research*, 4(2), 20–23.
- Benzina, F., Hamid, S., Mohand-Kaci, H., Bissaad, F., & Sahir-Halouane, F. (2018). Histological changes in the larvae of the domestic mosquito *Culex pipiens* treated with the entomopathogenic fungus *Beauveria bassiana*. *Scientific Research and Essays*, 13(1), 1–10.
- Bouaziz, A., Aissaoui, L., & Boudjelida, H. (2023). The activity of *Bacillus thuringiensis* Vectobac G on the biochemical compositions of *Culex pipiens* and *Culiseta longiareolata* (Diptera: Culicidae) mosquito larvae. *Ecology, Environment and Conservation*, 29(1), 1–9.
- Clements, A. N. (1999). *The Biology of Mosquitoes: Sensory Reception and Behaviour* (Vol. 2). Wallingford, UK: CABI Publishing. ISBN 0-85199-313-3.
- Coalson, J. E., Anderson, E. J., Santos, E. M., Madera Garcia, V., Romine, J. K., Luzingu, J. K., ... & Ernst, K. C. (2021). The complex epidemiological relationship between flooding events and human outbreaks of mosquito-borne diseases: a scoping review. *Environmental health perspectives*, 129(9), 096002.
- Empey, M. A., Reyes, Y. M., Ethier, J. P., Rosa, C. G. T., & Trudeau, V. L. (2025). Toxicity of *Bacillus thuringiensis* israelensis and deltamethrin in three anuran species. *Environmental Pollution*, 382, 126702.
- Farag, S. M., Moustafa, M. A. M., Fónagy, A., Kamel, O. M. H. M., & AbdelHaleem, D. R. (2024). Chemical composition of four essential oils and their adulticidal, repellence, and field oviposition deterrence activities against *Culex pipiens* L. (Diptera: Culicidae). *Parasitology Research*, 123, 110.
- Fu, Z., Wang, L., Hou, Y., Chen, Y., Mo, X., Xia, L. (2008). Optimization of fermentation conditions of *Bacillus thuringiensis* strain bt 0601. *Acta Laser Biology Sinica*. 17(6):819–23.
- Ganga, G. C., Banjara, M. R., Gautam, I., Ghimire, P., and Rijal, K. R. (2023). Phylogeny of a Mosquitocidal *Bacillus thuringiensis* var. israelensis. *Tribhuvan University Journal of Microbiology*, 76–81.
- Harbach, R.E. and Kitching, I.J. (1998) Phylogeny and classification of the Culicidae (Diptera). *Systematic Entomology*. 23(4):327-70.
- Ishii, T., and Ohba, M. (1993). Diversity of *Bacillus thuringiensis* environmental isolates showing larvicidal activity specific for mosquitoes. *Journal of General Microbiology*, 139(11), 2849–2854.
- Jawetz, C., Bushlaibi, M., Alrefaei, R., Ndegwa, E., Kaseloo, P., and Wynn, C. (2019). Influence of prior pH and thermal stresses on thermal tolerance of foodborne pathogens. *Food science and nutrition*, 7(6), 2033–2042.
- Khodyrev, V. P., Polenogova, O. V., Artemchenko, A. S., Krivopalov, A. V., & Glupov, V. V. (2024). New strains of *Bacillus thuringiensis* subsp. israelensis highly toxic for *Aedes aegypti* and *Culex pipiens pipiens*. *Microbiology*, 93(6), 921–928.

20. Liu, J., Fernandez, D., Gao, Y., Pierre, S., Gao, Y., & Dai, G. (2020). Enzymology, histological and ultrastructural effects of ar-turmerone on *Culex pipiens pallens* larvae. *Insects*, 11(6), 336
21. Lucarotti, C. J., Whittome-Waygood, B. H., & Levin, D. B. (2011). Histology of the larval *Neodiprion abietis* (Hymenoptera: Diprionidae) digestive tract. *Psyche: A Journal of Entomology*, 2011(1), 910286.
22. Ma, X., Hu, J., Ding, C., Portieles, R., Xu, H., Gao, J., ... & Borrás-Hidalgo, O. (2023). New native *Bacillus thuringiensis* strains induce high insecticidal action against *Culex pipiens pallens* larvae and adults. *BMC microbiology*, 23(1), 100.
23. Mahdi, M. N. (2022). Toxicological and biochemical effects of imidacloprid. *Iraqi Journal of Agricultural Research*, 26(1), 175–185.
24. Mataba, G. R., Clark, N. W., Kweka, E. J., Munishi, L., Brendonck, L., & Vanschoenwinkel, B. (2023). Interactive effects of dragonfly larvae and *Bacillus thuringiensis* var. *israelensis* on mosquito oviposition and survival. *Ecosphere*, 14(9), e4653.
25. Minard, G., Mavingui, P., and Moro, C. V. (2013). Diversity and function of bacterial microbiota in the mosquito holobiont. *Parasites and Vectors*, 6, 146.
26. Mohamed, D. M., Wassim, N. M., Khaled, H. E., & Youssef, T. N. (2023). Study the role of intestine stem cells in the midgut epithelium homeostasis of *Culex pipiens* post *Bacillus sphaericus* infection. *Egyptian Academic Journal of Biological Sciences. A, Entomology*, 16(2), 39–49.
27. Naik, B. R., Dinesh, D. S., Siddaiah, M., Daravath, S., & Tyagi, B. K. (2025). Biology of Mosquitoes. In *Mosquitoes of India* (pp. 219-236). CRC Press.
28. Naing, Z. L., Soe, E. T., Zhang, C., Niu, L., Tang, J., Ding, Z., ... Liang, G. (2023). Cadherin is a binding protein but not a functional receptor of *Bacillus thuringiensis* Cry2Ab in *Helicoverpa armigera*. *Applied and Environmental Microbiology*, 89(7), e00625-23.
29. Okeke, P., Luka, J., & Yina, G. I. (2022). An overview of insecticide usage and resistance of insect vector to insecticide: an implication to public health. *Journal of Public Health and Diseases*, 5(2), 16-20.
30. Polse, N. N., Faraj, A. M., & Mawlood, N. A. (2021). Description of common house mosquito, *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) from Erbil Governorate, Kurdistan Region—Iraq. *International Journal of Aquatic Science*, 12(2), 5147–5153.
31. Ren, Z., Cai, T., Wan, Y., Zeng, Q., Li, C., Zhang, J., Ma, K., He, S., Li, J., & Wan, H. (2023a). Unintended consequences: Disrupting microbial communities of *Nilaparvata lugens* with non-target pesticides. *Pesticide Biochemistry and Physiology*, 194, 105522.
32. Saibu, S., Nsa, I. Y., Odunsi, A. A., Akinyemi, N. M., Oyetibo, G. O., & Adebuseye, S. A. (2023, May). Draft genome sequence of *Bacillus thuringiensis* SS2, from dibenzofuran contaminated soil, reveals putative aromatic compound degradation genes. *Microbiology Resource Announcements*, 12(5), e0019823.
33. Shabaan, D., Awad, M., and Al-Mallah, N. M. (1991). A study on the effect of some volatile and fixed oils extracted from certain plants against the southern cowpea beetle. *Journal of Rasidin Agriculture*, 23(2), 179–187.
34. Van den Berg, H., da Silva Bezerra, H. S., Chanda, E., Al-Eryani, S., Nagpal, B. N., Gasimov, E., & Yadav, R. S. (2021). Management of insecticides for use in disease vector control: a global survey. *BMC Infectious Diseases*, 21(1), 468.
35. Wohl, M. P., and McMeniman, C. J. (2023). Batch rearing *Aedes aegypti*. *Cold Spring Harbor Protocols*, 2023(3), pdb-prot108017.
36. Yaba, D. B., & Al-Mufti, S. A. (2024). Study of life history traits of *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) in Laboratory in Erbil province-Iraq. *Polytechnic Journal*, 14(2), 1.
37. Yaseen, A. T., and Sulaiman, K. A. (2021). Insecticidal activity of some chemicals of mosquitoes *Culex pipiens molestus* Forskal. *Baghdad Science Journal*, 18(1)716–722.
38. Zhu, Y. S., Brookes, A., Carlson, K., and Filner, P. (1989). Separation of protein crystals from spores of *Bacillus thuringiensis* by Ludox gradient centrifugation. *Applied and Environmental Microbiology*, 55(5), 1279–1281.