# Evaluation of *Bacillus thuringiensis* (H14) in their natural and nano form on fourth-instar larvae of *Culex quinquefasciatus* put in lake water and knowledge of the effect of nanoparticles on non-target organisms

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# تقييم *Bacillus thuringiensis* (H14) بشكلها الطبيعي والنانوي على يرقات الطور الرابع لبعوض *Culex quinquefasciatus* وضعت في ماء بحيرة مع معرفة مدى تأثير الجسيمات النانوية على الاحياء غير المستهدفة

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**الخلاصة**

عزلت Bti من اليرقات الميتة لبعوض *C . quinquefasciatus* في مواقع الدراسة ، واستخدمت في تحول نترات الفضة $AgNO_3$ الى جسيمات نانوية بيولوجياً حيث حضرت منها ثلاثة تراكيز (1000، 2000 ، 3000) جزء بالمليون وقد اعطت نتائج عالية في قتل يرقات الطور الرابع لبعوض *C .quinquefasciatu*. تم اختبار (6 ml / L , 8 ml / L and 10 ml / L) من Bti-AgNPs وبتركيز 10,000 ppm لمعرفة تأثيرها علي اسماك الكوبي وقشريات الدافينا ككائنات حية غير مستهدفة ولم يكن هناك تأثير سالبي لها. وتم اختبار مدى تأثير *Bacillus thuringiensis* (H14) غير النانوية كمبيد حيوي طبيعي على نفس اليرقات المذكورة وقد اعطت نتائج قتل عالية جدا.

**الكلمات المفتاحية: بكتيريا *Bacillus thuringiensis* (H14), النانوتكنولوجي , البعوض , قشريات الدافينا ,اسماك الكوبي**

## Abstract

Bti-AgNPs tested againts Guppy fish and Daphina as non-target organisms. Bti was isolated from dead larvae of *Culex quinquefasciatus* mosquitoes in study places . Bti used to convert $AgNO_3$ into nanoparticles biologically (Bti-AgNPs ) , used three concentrations from it (1000, 2000, 3000) ppm .The results was high mortality in fourth instar larvae of mosquitoes *C.quinquefasciatus* . Tested (6 ml / L , 8 ml / L and 10 ml / L) of Bti-AgNPs on Guppy fish and Daphnia as non-target organisms,The concentration of them was 10,000 ppm .The effect was negative on them. Bti non-nanoparticles tested on the same larvae as natural biocide , too its gave high mortality results.


**Key words**:*Bacilus thuringiensis var:* (H14), Nanotechnology, mosquito , Daphnia,Guppy fish

# 1.Introduction

The species *Bacillus* is the most importance species of bacteria in the control of insects. *Thuringiensis*` type of bactericidal ( Gram positive and moved by a whip side) considered of the most specialized species in that space . These bacteria was recorded by German researcher Berliner (1911) When isolated from the Ephestia moth in Thuringia, Germany, where it was called *Bacillus thuringiensis* and its abbreviation (BT) [1]. In 1977, the study serotype H14 var *Bacillus thuringiensis* was discovered by [2] . The two studied project was sponsored by the World Health Organization in Palestine for a study to develop control factors against the mosquitoes which carry pathogenicity and parasites, after that study proved the ability of bacteria against mosquitoes and other insects. This study was started succeful in research and studies of bacteria. *Bacillus thuringiensis israelensis* used as a substitute for the use of conventional chemical pesticides in the environment after raising awareness of the problems caused by pesticides [3]. Nanotechnology is a science studying the use of (small and large) particles in the medical ,agriculture and biological etc, the a nano size gaves materials new chemical and biological proprieties[4] .Nanoparticles defined as an atomic or microcosmic cluster of a few atoms (molecular) to one million atoms are contact spherical together with a radius of less than 100 nanometers [5].Through several studies observed that nanoparticles prepared in different ways for control insect pests [6]. The nanoparticles prepared by the water extract of *Eclipta prostat* leaves with size (35-60 nm) effected against the larvae of mosquitoes Anophelis and Culex, with mortality rates reaching 100%. Leath concentration 50 was 27.49 mg

/ L and 4 mg / L respectively [7]. There are many fears of unleashing that technology in many society because nanoparticles are very small to penetrate the immune system of the human body [8]. They can also pass through the membranes of skin and lung cells, and what is even more disturbing is that It can cross the brain blood barrier. In 1997 a study at the University of Oxford showed that the nanoparticles of titanium dioxide in anti-sun burning ointments have damaged the DNA of the skin. These and other causes have led to an increase in studies and a research to assess the risks of this technology to humans and other non-target organism,[9] and [10]. The study aimed to evaluate larivicidal activity of Green synthesis Bti-AgNPs againts fourth instar of *Culex quinquefasciatus* larvae

## 2.Material and methods

*Bacillus thuringiensis* (H14) was obtained from dead *Culex quinquefasciatus* larvae collected from different aquatic environments .After applicated (Kokhs' hypothesis) they were isolated, purified, diagnosed, and tested for biological testing. *Bacillus thuringiensis* (H14) was transformed into Bti-AgNPs by adding 5 ml of the 0.9% normal saline to the bacterial farm, separating the bacterial growth by L-shaped . Bacterial growth pulled by a syringe after removing the metal part and then filtering the suspension through two sheets of gauze stabilized on a glass funnel with several additional 5 ml of sterile saline solution to ensure the descent of all bacteria and the removal of the biological environment of the food . The bacterial suspension collected in a 100 mL conical glass flask. Bacterial stock obtained from bacterial suspension .Supplied 1 liter from the Nutrient broth after sterilization its added to bacterial suspension, incubated at 30 ± 2 degrees and then taken from the center 100 ml [11] . It Put in centrifuges for 15 minutes 5000 RPM, filtered by millipour (0.02 milligrams) and added to glass flask containing 900 ml of Ag $No_3$ and deionized water. The volume completed to 1000 ml by adding then incubated for 72 hours with constant shaking .Bti-AgNPs were characterized by using UltraViolet tests and scanning electron microscopy (SEM) to make sure that it is transformed into a nanoparticle[12].Three concentrations of Bti-AgNPs (1000,2000 and 3000) per part million were tested againts 20 larvae of the fourth instar of mosquitoes *C. quinquefasciatus*. *C.quinquefasciatus* put in a cup volume 500 ml is containing 350 ml of lake water.

Added 1.5 g of rat food For feeding larvae. The experiment was three replicates per concentration with control treatment and the results were recorded after 24, 48 and 72 hours . Repeated the same previous experiment but added three concentrations of *Bacillus thuringiensis* (H14) (69 x $10^4$, 69 × $10^5$ and 69 × $10^6$ ) CFU / ml. supplied three different sizes of Green synthesis (Bti-AgNPs) (6 ml / L , 8 ml / L and 10 ml / L) the concentrations of them were 10,000 ppm ,They were tested on three glass basins (40 x 20 x 15 cm). Each basin full with 10 liters of tap water without chlorine . In each basin, 10 Guppy fish with control basin. The same concentrations were repeated on three glass basins containing 10 Daphnia with control tank [13]. The lighting period was 12 hours and the temperature was 2 ± 27 C°. The results were recorded during 24, 48, 96 hours

## 3.statistic analysis

Experiments were analyzed with complete randomized design (CRD).
Results were tested using the least significant difference (LSD). At the level Probability (0.05)

## 4.Results and discussion

### A.Effect Bti-AgNPS on forth instar for larvae of*Culex quinquefasciatus*

Table 1. showed to concentration of Green synhesis (Bti-AgNPS) 3000 ppm gave highest rate of mortality to the fourth instar larvae of *C.quinquefasciatus* reached 100% while the concentration 1000 ppm gave less mortality was 92.8% . The rate of mortality during 72 hours was 100% while the rate of mortality during 48 hours was 94.4 % .The concentration 3000 ppm gave highest rate of mortality was 100% during 24 hours while the concentration 1000 ppm gave rate of death reached 83.3% during 48 hours from time of the experiment . [12] suggested that the nanoparticles which transformed by Bti bacteria gave high killings in the *Aedes aegypti* . [14] also obtained LC50 and LC90 values of 0.69 and 1.10 ppm as well as 2.15 and 3.59 ppm of AgNPs synthesized by leaf extract of *N.nucifera* against *C.quinquefasciatus*

Table 1. Larvicidal effect of Bti-AgNPs against the Forth instar of *C.quinquefasciatus* larvae put in water of a lake

| Cumulative percentage of mortality (%) | Percentage of mortality (%) | Mortality of *C.quinquefasciatus* (%) | | | Bti-AgNPs PPM | N0. |
|---|---|---|---|---|---|---|
| | | After 72 h | After 48 h | After 24 h | | |
| 100 | 92.8 | 100 | 83.3 | 95.0 | 1000 | 1 |
| 100 | 98.9 | 100 | 100 | 96.7 | 2000 | 2 |
| 100 | 100 | 100 | 100 | 100 | 3000 | 3 |
| | | 100 | 94.4 | 97.2 | Rate | |
| Interfere | | Concentrations | | Times | L.S.D | |
| 16.56 | | 9.57 | | 9.57 | | |

## 2.Effect of *Bti* on forth instar larvae of *Culex quinquefasciatus*

Table 2. Showed to bacterial dilution ($69 \times 10^4$ )ml / CFU recorded the highest rate of mortality 98.9 % while the bacterial dilution ( $69 \times 10^6$ ) CFU / ml gave less rate of mortality 83.7% without a significant difference between concentrations. The rate of mortality during 72 hours was 100% While the lowest rate was 75.9% during 48 hours from the time of treatment with a significant difference between the time , the highest kill rate of *C. quinquefasciatus* was got by first dilution ($69 \times 10^4$ ) ml / CFU where reached 100% during 48 hours of treatment, while the mortality rate was 61.1% For the third concentration during 48 hours of the treatment period . [15] reported that the concentration of ($30 \times 10^6$) ml / CFU *Bti* gave high mortality in larvae of *C. quinquefasciatus*

Table 2. Larvicidal effect of Bti against the Forth instar larvae of *C.quinquefasciatus* put in water of a lake

| **Cumulative percentage (%) of mortality** | **Percentage of mortality (%)** | **Mortality of *C.quinquefasciatus* (%)** | | | **Bti CFU/ml** | **NO** |
|---|---|---|---|---|---|---|
| | | **After 72 h** | **After 48 h** | **After 24 h** | | |
| **100** | **98.9** | **100** | **100** | **96.7** | **$10^4$× 69** | **1** |
| **100** | **86.1** | **100** | **66.7** | **91.7** | **$10^5$ × 69** | **2** |
| **100** | **83.7** | **100** | **61.1** | **90.0** | **$10^6$ × 69** | **3** |
| | | **100** | **75.9** | **92.8** | **Rate** | |
| **Interfere** | | **Concentrations** | | **Times** | | **L.S.D** |
| **26.60** | | **15.36** | | **15.36** | | |

## C. Larvicidal effect of Bti-AgNPs on Guppy fish and Daphnia

There was no injurious effect of the Bti-AgNPs on Guppy fish and Daphnia during the four-day trial period. These organisms are considered to be the most affected by toxicity, especially bio toxins in their nanoparticles . the experiment evidence that there is no show effect of nanoparticles on thes organisms as non-target compared with other target organisms such as mosquitoes. [16] were showed in a study of silver nanoparticles are one of the contaminants that affect other microbial and aquatic organisms but, recent studies have shown that some algae can be used to prepare nanoparticles to reduce their toxicity to certain aquatic organisms. [17] showed that silver particles prepared by algae helped to reduce the toxicity of those particles towards the direction of fleas of water *Daphnia* spp. [18] showed that there is no toxicity of AgNPs on *Poecillia reticulata* fish. In another study on this subject was observed that there is no toxicity of *Bacillus thuringiensis israelensis* bacteria in the embryos Zebrafish [19]

Table 3.Larvicidal effect of Bti-AgNPs on Guppy fish and Daphnia

| **Mortality After 96 hour** | | **Mortality After 48 hour** | | **Mortality After 12 hour** | | **Mortality After 6 hour** | | **Volume of** Bti-(AgNPs) |
|---|---|---|---|---|---|---|---|---|
| **Daphnia** | **Guppy** | **Daphnia** | **Guppy** | **Daphnia** | **Guppy** | **Daphnia** | **Guppy** | |
| **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **6 ml/L** |
| **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **8 ml/L** |
| **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **10 ml/L** |
| **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **control** |

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