Sumer University Journal for Pure Science (ISSN: 2790-7031)
SUJPS 2022 (November), Vol. 1, No. 1, p.p.: 17-25

Use of *Bacillus thuringiensis israelensis* in the manufacture of a nano bio-pesticide (Bti-AgNPs) to control on mosquitoes of *Culex quinquefasciatus* in laboratory.

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Key words :*Bacilus thuringiensis var:israelensis*, Nanotechnology, mosquitoes

Abstract

The *Bacillus thuringiensis israelensis* bacteria was obtained from the dead larvae that were collected during the survey and has been used as biocides within 24, 48 and 72 hours against the fourth instar larvae of *C.quinquefasciatus* mosquitoes that were put in a different water drainage water, a river and a swamp and it has gave mortality reached (97.1,96.8 and 82.2) % respectively. Bti-AgNPs was recorded within 24, 48 and 72 hours against the fourth instar larvae of *C.quinquefasciatus* mosquitoes that were put in a different water drainage water, a river and a swamp and it has gave mortality reached (99.4, 98.9 and 96.2) % respectively .

استخدام Bacillus thuringiensis israelensis في صناعة مبيدات الآفات الحيوية النانوية (Bti- AgNPs) للسيطرة على بعوض Culex quinquefasciatus في المختبر.

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الخلاصة

تم الحصول على بكتيريا *Bacillus thuringiensis israelensis* الإسرائيلية من اليرقات الميتة التي تم جمعها أثناء المسح وتم استخدامها كمبيدات حيوية في غضون 24 و 48 و 72 ساعة ضد يرقات الإصدار الرابع من بعوض C.quinquefasciatusالتي تم وضعها في مياه تصريف مياه مختلفة، نهر ومستنقع وقد أعطى معدل وفيات بلغ (97,1,96,8 و 82,2) في المائة على التوالي. تم تسجيل Bti-AgNPs في غضون 24 و 48 و 72 ساعة مقابل يرقات الإصدار الرابع من بعوض C.quinquefasciatus التي تم وضعها في مياه تصريف مياه مختلفة ونهر ومستنقع وأعطت معدل وفيات (99.4 و 98.9 و 96.2) على التوالي.

1.Introduction

Most of the inhabitants of the globe suffer from pests of medical and veterinary importance, especially those that transmit many dangerous diseases that affect both humans and animals [1] . These pests multiply in developing countries clearly as a result of the neglect of public health [2] . Mosquitoes are medically important insects, so special programs have been developed to combat them in different parts of the world in multiple, overlapping ways, such as filling or drying ponds and swamps and getting rid of mosquitoes [3]. Mosquitoes are living in water eggs, larva and pupa (Image,1) ,adult needs meal of blood to develop its eggs therefore it bited human or animal by it specialized mouth . Mosquitoes transmitted many pathogens example Zika virus, West Nile virus, dengue, and malaria (Image,2) . Bacteria of Bacillus is the most importance species of bacteria that use in biological control of insects . *Bactericidal thuringiensis* is one of the most specialized species in kill mosquitoes. Berliner in 1911 was a German researcher had been recorded *Bacillus thuringiensis* (Bt) When isolated from the Ephestia moth in Thuringia, Germany . Goldberg and Margalit in 1977 were discovered the Israelensis serotype var *Bacillus thuringiensis* in the Naqap Desert .[4] The World Health Organization in Palestine was able to sponsor a project presented by Goldberg and Margalit to study the development of control agents against mosquitoes that carry diseases and parasites, after this study demonstrated the ability of bacteria on mosquitoes and other insects. Conventional chemical pesticides caused problems in the environment but *Bacillus thuringiensis israelensis* is try to treatment this [5]. [6] indicated to Nanotechnology is a science studying the nanoparticles which use in the agriculture ,medical and biological etc, a nano materials give new chemical and biological proprieties. Nanoparticles are (molecular) atoms arranged up to one million spherical atoms to have a diameter of less than 200 nanometers [7]. [8] observed that nanoparticles prepared in different ways for control insect pests. [9] indicated to effect nanoparticles prepared by the water extract of Eclipta prostat leaves with size (35-60 nm) against the larvae of Anophelis and Culex, with killing rates reaching 100%.

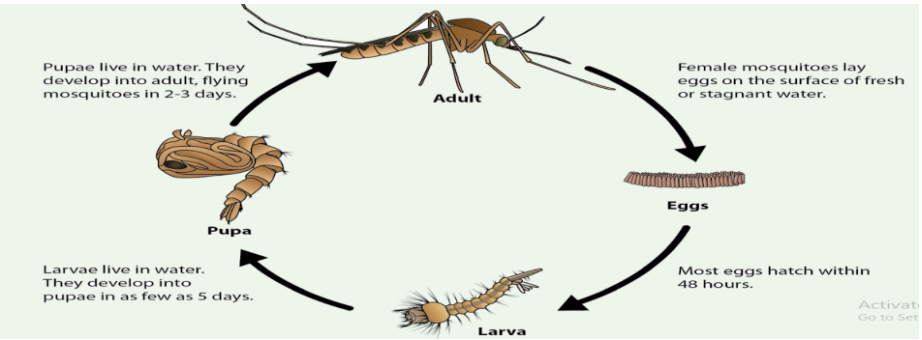

Image 1: life cycle of *Culex mosquitoe*s [10] .

A

A.Filariasis disease transmitted by mosquitoes (*Culex spp*)

B

B. Zika virus transmitted by mosquitoes (*Aedes aegypit*)

Image 2: Disease which transmits by *mosquitoe*s [10].

Metral and methods

(Bti) was collected from some dead *Culex quinquefasciatus* larvae in different aquatic environments . After applicated (Kokhs, hypothesis) (Bti) were isolated, purified, diagnosed. Bti was transformed into Bti-AgNPs through adding 5 ml of the 0.9% normal saline to the bacterial farm then separating the bacterial growth by L-shaped . Bacterial growth was pulled by syringe after removing the metal part and then filtering the suspension through two sheets of gauze stabilized on a glass funnel with repet 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.1 liter of Nutrient broth was prepared and sterilized then it added to bacterial suspension, incubated at 30 ± 2 degrees and then taken from the center 100 ml [11] . 100 ml was put in centrifuges for 15 minutes with range 5000 RPM, , added to glass flask containing 900 ml of $AgNo_3$ and deionized water after filtered by millipour (0.02 milligrams). The solution was became 1000 mL 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]. the solution of Bti-AgNPs (500,1000 and 1500) per part million were tested against 20 larvae of the fourth instar of mosquitoes *C. quinquefasciatus*. The larvae was put in a cup volume 500 ml is containing 350 ml of lake water. The experiment was repeated with the same previous procedures , but the larvae were

placed in swamp water, drains water and river water. Repeated the same previous experiment but added three concentrations of *Bacillus thuringiensis israelensis* (70×10^{-4}, 70×10^{-5} and 70×10^{-6}) CFU / ml .

3.statisticalanalysis

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

4-1. Optical and spectral properties with shape and size of silver nanoparticles prepared by bacteria (Bti)

the study observed that silver solution was changed to a light brown color within 48 hours at a temperature of 25 ° C when the bacteria (Bti) solution was added , where due to the biological reduction process [13]. The wavelength 442.9 nm recorded the highest absorption of silver nanoparticles prepared by Bti bacteria with other wavelengths such as 309.10 nm (Fig., 1)

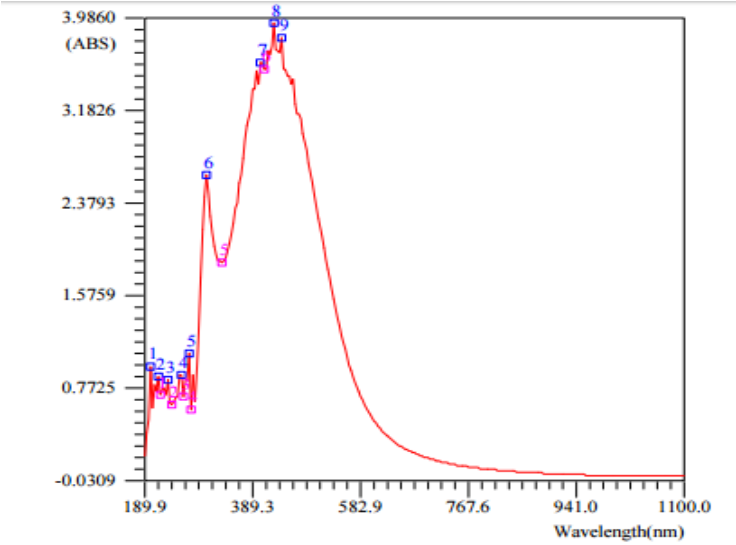

Figure (1): Absorption spectrum of silver nanoparticles prepared by Bti

The scanning electron microscope images showed that silver nanoparticles prepared by *B.t.israelensis* bacteria have oval or spherical shapes and sizes at a rate of 245.06 nm.(Image, 3).

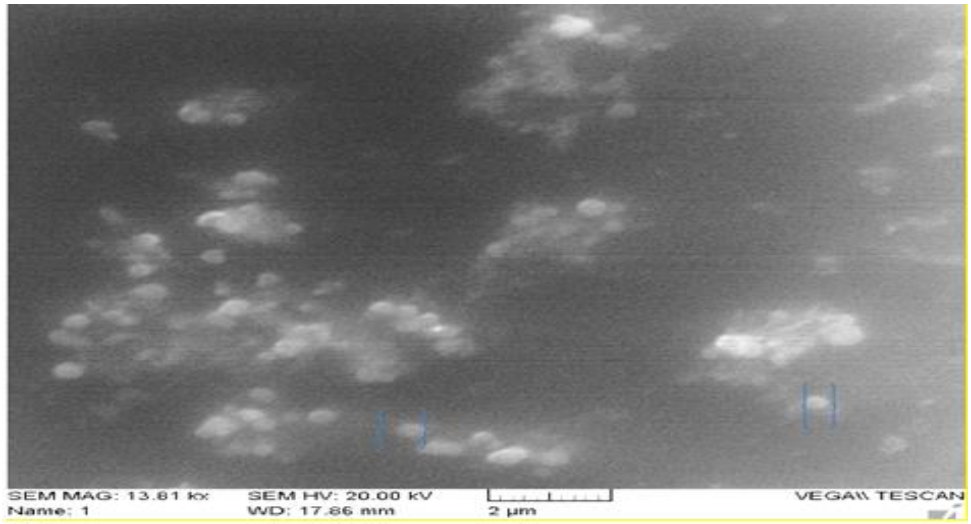

Image 3 : Scanning electron microscope images showing the shape and size of silver nanoparticles prepared by *Bacillus thuringienesis israelensis*.

4 -2 .Efficacy Bti-AgNPS to kill forth instar for larvae of *Culex quinquefasciatus* in different aquatic environments under laboratorial conditions.

The results indicate that silver Bti-AgNPs nanoparticles prepared by Bti bacterial isolate filter and placed in drains, river and swamp waters were highly effective towards the fourth instar larvae of *C.quinquefasciatus* mosquitoes, where the death rates were 99.4, 98.9 and 96.2%, respectively, with a significant difference between water drain and swamp water and between river water and swamp water, while no significant differences were observed between drainage water and river water in terms of the effect on the fourth instar larvae of *C.quinquefasciatus* mosquitoes at a probability 0.05 (Table ,1).Also the results showed that nanoparticle concentrations 1000, 2000 and 3000 ppm have achieved death rates of 92.2, 94.9 and 98.3%, respectively, with a significant difference at the probability level of 0.05 (Table ,1) . The table also shows the role of the time factor in increasing death rates during 24, 48 and 72 hours as it reached 95.1, 99.4 and 100%, respectively, with a significant difference between the time factor of 24 hours and the other times, while there was no significant difference between 48 and 72 hours. [14] showed that silver AgNPs nanoparticles that were prepared biologically by extract of Mimosa pudica leaves were used to control the larvae of C. quinquefasciatus , where during the first hours very high death rates were recorded in larvae.

Table 1: Efficacy Bti-AgNPS to kill forth instar for larvae of *Culex quinquefasciatus* in different aquatic environments under laboratorial conditions.

Aquatic environments	Concentrations Bti-AgNPs PPM	Percentages of larval death (*C.quinquefasciatus)*			Rate of death %
		After 24 hours	After 48 hours	After 72 hours	
drains water	500	96.6	99.7	100	98.8
	1000	98.3	100	100	99.4
	1500	100	100	100	100
	Rate	98.3	99.9	100	99.4
River water	500	95.0	99.1	100	98.0
	1000	96.6	100	100	98.8
	1500	100	100	100	100
	Rate	97.2	99.7	100	98.9
Swamp water	500	85.0	97.1	100	94.0
	1000	90.0	98.9	100	96.3
	1500	95.0	100	100	98.3
	Rate	**90.0**	**98.6**	**100**	**96.2**
	General rate	**95.16**	**99.4**	**100**	94.0

LSD < 0.05

Concen. =1.36
Enviro. = 1.36
Time = 1.36
Enviro.× Concen.= 2.35
Enviro.×Time =2.35
Concen.× Time = 2.45
Enviro.× Concen.× Time = 4.08

4-3. Efficacy Bti to kill forth instar for larvae of *Culex quinquefasciatus* in different aquatic environments under laboratorial conditions .

Table (2) showed the effect of Bti concentrations (70×10^{-4}, 70×10^{-5}, 70×10^{-6}) CFU/ml in fourth instar larvae of *C.quinquefasciatus* which live at drain , river and swamp water with death: (97.1, 96.8 and 82.2) % respectively, no significant different between drain and river water while it was observed significant differences between that water and swamp water . The high concentration (70×10^{-4}) CFU/ml was gave high rate mortality in all water (drain , river and swamp) while the concentration (70×10^{-6}) CFU/ml was the least. The time factor was impacted in increasing the death rates at (24, 48 and 72) hours, which amounted to (85.5, 93.4 and 97.2)%, respectively, with significant difference. [15] was indicated that mortliaty rates of Culex pipiens larvae were (91 - 100)% when use Iraqi isolates of Bti bacteria . [16] mentioned that concentration (Bti) 30 ppm was caused the death of *C.quinquefasciatus* larvae by 100%.

Table 2 : Efficacy Bti to kill forth instar for larvae of *Culex quinquefasciatus* in different aquatic environments under laboratorial conditions .

Aquatic environments	Concentrations Bti CFU/ml	Percentages of larval death (*C.quinquefasciatus)*			Rate of death %
		After 24 hours	After 48 hours	After 72 hours	
drains water	70×10^{-4}	98.3	100	100	99.4
	70×10^{-5}	93.3	99.6	100	97.6
	70×10^{-6}	88.3	94.9	100	94.4
	Rate	93.3	98.2	100	97.1
River water	70×10^{-4}	96.6	100	100	98.8
	70×10^{-5}	91.6	96.9	100	96.1
	70×10^{-6}	90.0	96.1	100	95.3
	Rate	92.7	97.6	100	96.8
Swamp water	70×10^{-4}	80.0	91.6	96.6	89.4
	70×10^{-5}	71.6	85.0	91.6	82.7
	70×10^{-6}	60.0	76.6	86.6	74.4
	Rate	70.5	84.4	91.6	82.2
	General rate	85.5	93.4	97.2	92.7

LSD < 0.05

Concen. =1.45
Enviro. = 1.45
Time = 1.45
Enviro.× Concen.= 2.52
Enviro.×Time =2.52
Concen.× Time = 2.52
Enviro.× Concen.× Time = 4.36

Conclusion

1. The possibility of converting bacteria(Bti) into a Nanopesticide by AgNo3
2. The Bti-AgNPS very active in all aquatic locations (Drains , River ,Swamp) against larvae of *C.quinquefasciatus* while Bti was less quality in Swamp water

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