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Test of Pathogenicity of *Bacillus thuringensis* (Berliner) in Biological Control for the Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.)

ABSTRACT

The study was carried out to evaluate the pathogenicity of the bacteria, *Bacillus thuringensis* of the cotton leafworm, *Spodoptera littoralis* (Boisd) at the rate of 1×10^6 spores/ml. Laboratory results revealed that egg hatching percentage, at the age of 1-2 days were 0.0, 0.0 and 8.2 % 2 and 4 days after application respectively. While the hatching percentage of the 3-4 days old eggs was 6.8% at the age of 2-4 days after 2 days' treatment. The mortality rate of larval stages (2, 4, 6 instars) was 100% 14 days after application. When pupae were treated with the bacteria adult emergence percentage was 51.3 % 15 days after treatment compared to 100% emergence for the control treatment. The results in greenhouse show that the entomopathogenic bacteria also achieved mortality rate for 2, 4, 6 larval instars of the pest were 94.4, 91.8, 100, 88.4, 85.6 and 81.3 % for the soil and plant treatments respectively, 14 days after application.

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INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boisd) is one of the most serious insect pests that infecting Solanaceae and Malvaceae plants and caused significant economic damage, this insect spread throughout the year in the open and protected fields (Almallah, 2005). It is polyphagous that affect more than 27 plant families in Iraq (Rabii, 2002). It also causes damages to most parts of the plant as it effects on seedling, leaves, vegetable buds, flowerpots, flowers, and fruits especially in cases of severe injury on tomato, cotton, and clover. This type of bacteria has been used since 1870 and is still widely used and there are 90 breeds used in the fight against economic insect pests (Robert, 1981). Khoja et al. (2006) were found that these bacteria infect the insect after entering the midgut as it begins to fission and secreting endotoxin that works to tear the lining of midgut. These bacteria were used in Iraq to control a number of economic and storage pests and were found to be very effective in controlling olive leaf midge *Dusynura oleae* in the field by 98% (Jassim et al, 1988) and to control Khapra beetle *Trogoderma granarium* in the store (Ali et al., 2005) and (AL- Jamil et al., 2005).

The effect of bactospeine of *B. thuringiensis* was studied on the insect complying with chemical pesticides and produced a positive result in reducing insect damage (Zubaidi, 1988).

The population of cotton Leaf worm characterized by high resistance to pesticides and if this resistance develops it becomes difficult to insect Control chemical (Almallah, 2005) This is why the chemical control is not sufficient alone unless other methods are integrated with it, as well as the desire to avoid the use of chemical toxins because of the damage to the environment and human health and to complement previous studies in the field of integrated control cotton leaf Worm This research

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was conducted with the aim of testing the pathological effects of the bacteria *B. thuringiensis* on various stages of the cotton leafworm *S. littoralis* under laboratory and greenhouse conditions.

MATERIAL AND METHODS

The study conducted at the insect laboratory of the plant Protection Department at the Faculty of Agriculture and Forestry in 2012.

Sampling: Insect larvae at different ages are collected from infected plants in a field under eggplant plant cultivation as well as from the soil around the infected plants and under the leaves falling on an 50 cm area around the plant and a 2 cm depth below topsoil. The insect was laboratory-reared in 850 ml glass bottles covered with cloth and fed with eggplant leaves to get the different stages of the insect (Rabii, 2002). In this study, the Iraqi isolation of the bacteria *B. Thuringensis* obtained from Dr. Juhaina Idris Mohamed Ali in the Department of Plant Protection – Faculty of Agriculture and Forestry at the University of Mosul and this isolation has grown on the sterile food medium (nutrient agar). The bacteria were placed in an incubator at 25 m and relative humidity 80% for 7 days, after which several colonies of bacteria were obtained and the water commentator of the bacteria is equipped with a concentration of 1×10^6 spores/ml by the red blood cell count Haemocytometer, by which the concentration of spores bacteria in the water commentator was calculated Which is used in all trial transactions.

Laboratory experiments

Eggs – Laboratory experience of three repeaters on two groups of eggs was conducted, the first group eggs with age 1-2 days and the second group eggs with age 3-4 days, put eggs in a petri dish diameter 9 cm equipped with paper filtration and 50 Eggs/petri dish (replicate) Sprinkle the dishes with 5 ml of the antibacterial water commentator with 1×10^6 concentration Spore/ml and sprayed dishes compared to sterilized water the dishes were placed in the incubator and the hatching of eggs was followed and the percentage of the seals was recorded after 2 and 4 days of treatment.

Larvae – The effect of bacteria in the second, fourth and sixth ages was tested by a cotton leafworm representing the all ages of caterpillar three independent experiments were conducted with 3 replicate of the treatment, 10 larvae were placed in each dish and the leaves of eggplant leaf were placed in petri dish plastic and the plant leaves were sprayed with 5 ml of the water commentator of the bacteria while spraying the control with sterile water only, the larvae were placed and the dish was covered from the top with a piece of cloth fastened with a rubber ring and then placed in the incubator at 25 °C and relative humidity 80% (Navon, 2000), the mortality percentage was recorded after 1, 3, 5, 7, 10 and 14 days of treatment and those mortality rates were corrected as Abbott's equation (Abbott, 1925).

% Corrected for mortality = % Mortality in treatment – % Mortality in Control / 100 – % Mortality in control.

Pupa – The larvae of cotton leafworm pupate inside clay rooms, the pupa collected from those rooms into breeding bottles and placed every 10 pupa in a petri dish with a 9 cm diameter equipped with filtration paper at three replicates of treatments each dish acts as one single, sprayed the dishes with 5 ml of the water suspension of the bacteria while spraying the control with water sterility only. Each dish was placed inside a bottle of a diameter of 13 cm and 17 cm high and its upper opened with a piece of cloth and attached to a rubber ring and hugged on 26 °C and relative humidity 80% The mortality percentages were recorded after 5, 10 days of treatment.

An Adult – Laboratory experience of three repeaters per treatment on male and female insect adults, a piece of cotton moistened with a 10% diabetic solution inside a small glass cup to feed the adult, sprayed the adults with 5 ml of the antibacterial water commentator and sprayed the treatment of the control with sterile water. The lantern bottle was blocked with a piece of cloth to prevent the exit of the adult and ventilation and was connected to a rubber ring and left in the incubator at 26 °C

and relative humidity 80% The mortality percentages were recorded after 1,3 and 5 days of treatment and those percentages were corrected by Abbott's equation. (Abbott, 1925).

Experiments of the greenhouse

Larvae – this experiment to see the effect of bacteria *B. thuringensis* on the second, fourth and sixth age of the cotton leafworm in the greenhouse. In the experiment, a local class eggplant was obtained from a vegetable nursery in the city of Mosul and planted in plastic pots diameter 20 cm and put one plant in each pot and left until the appearance of the fifth paper and then applied the following transactions:

- The first treatment – the water commentator of the bacteria with a concentration of 1×10^6 spraying on the plant.
- Second treatment – Control, plants spraying with sterile water.
- Third treatment – water suspension of bacteria with 1×10^6 concentration on soil.
- Fourth treatment – Control of soil spraying with sterile water.

These coefficients were applied with three repeaters each and independently for each age (II, IV and VI). After treating the plants, put in each pot five larvae, left to feed, and covered each pot with a cloth bag underneath it fastened with a rubber strap on the pot slot and hook from the top to prevent the exit of the larvae. Calculated mortality percentage after 4, 8 and 14 days of treatment and corrected according to the above-mentioned Abbott equation (Abbott, 1925).

Statistical analysis

A complete random design has been used of experience and analyzed the results using were compared with SAS and under test Duncan one-stop-center border at the level of risk 5%.

RESULT AND DISCUSSION

Laboratory experiments

The eggs – the results indicated in table (1) that bacteria *B. thuringensis* affected significantly the eggs with age 1-2-3-4 days, the hatching percentage was reduced to 2.8 and 4.6% after four days of treatment by water suspension of bacterial spores, respectively.

Table (1) Effect of *B. thuringensis* in egg of cotton leaf worm *S. littoralis*.

Treatment	% Egg hatching			
	1-2 Days age		3-4 Days age	
	Exposure time (day)			
	2	4	2	4
<i>B. thuringensis</i>	0	8.2 b	6.8 b	4.6 b
Control	0	98.4 a	98.2 a	98.2 a

Means that share similar characters in each column do not have significant difference depending on the Dunkin Test at the 5% probability level.

While the percentage of eggs hatching in the treatment of compared 98.4% from the time of the insect eggs need 3.5 days to complete the embryonic development. The lower percentage of hatching of eggs is due to the intrusion of bacteria on the eggs of the cotton leafworm by penetrating the spores of the chorion and its effect on the embryo, resulting in a failure of growth and development.

Larvae – The results in Table (2) indicated that bacteria *B. thuringensis* has been greatly affected on the larvae of cotton leafworm, where the mortality percentage of larvae of the second, fourth and sixth age to 100% is 14 days of treatment and the increase in the effect of bacteria has been observed by increasing the time period of exposure, represented in The slow movement of the larvae and the lack of feeding and the larvae affected by the loss of the body weight before mortality and then vomiting of brown fluid from its bodies. The pathogenicity capacity of the bacteria may be due

to the fact that it has an active enzyme system for the analysis of the chitin and protein, which are the most important components of the insect's body (Jassim, 1984) and (Khoja et al., 2006).

The results of table (2) shows that the effect of bacteria in the larvae of the second, fourth and sixth ages was limited in the first five days of treatment then the effect of bacteria increased by increasing the duration of exposure, as the highest rates for the larvae of the sixth age reached 2%, 34%, 75% and 100% after 7, 10 and 14 days of treatment respectively. From here we conclude that the time factor is important in the biological control and the results converged with what he found (Al-Mansour et al., 2011) It also found an increase in the age of the treatment of bacteria and it was only possible to 0 2% after 10 days of treatment and the adults did not get out of these pupa compared to the comparison where I've excused all the larvae after 10 and 14 days of treatment.

Table (2) Effect of *B. thuringensis* in the larvae of the cotton leafworm *S. littoralis*.

Instars	Mortality percentage of larvae						Mean
	Exposure time (day)						
	1	3	5	7	10	14	
Second	4.2	4.8	7.6	28.8	75.4	100	36.2 b
Fourth	3.3	3.6	27	32	78.6	100	40.75 a
Sixth	3.3	4.8	10	34.2	75	100	37.9 a

Means that share similar characters do not have significant difference from each other according to the Dunkin Test at a probability level of 5%.

This may be due to the weak ability of the larvae infected with bacteria and the disruption of physiological and enzymatic processes that precede the process of intolerance. The results also indicate that the effect of bacteria increases with the increase in the age of the insect, where the mortality percentages of the larvae of the fourth and sixth age compared to the second age are observed, these results are consistent with what he found (Khaldooz et al., 2009) where he found that the highest mortality rate reached 94, 2% after 12 days of pain Agent for larvae of insect *Helicoverpa armigera* Hb with different doses *Bacillus thuringensis*.

Pupa – The results of a table (3) indicated that the effect of bacteria *B. thuringensis* in the Pupa phase was slow as the percentage of the emergence was 0.0, 12.3, and 51.3% after 5, 10 and 15 days of treatment respectively, compared to 0.0, 44.8 and 100% after 5 days of treatment in the control respectively.

Table (3) Effect of *B. thuringensis* in the pupa of cotton leaf worm *S. littoralis*.

Treatment	Adult emergence (%)		
	Exposure time (day)		
	5	10	15
<i>B. thuringensis</i>	0.0	12.3 b	51.3 b
Control	0.0	44.8 a	100 a

Means that share similar characters do not have significant difference from each other according to the Dunkin Test at a probability level of 5%.

The non-emergence of adults after 5 days of treatment in control as well as other transactions is explained by the incomplete period of the Pupa phase, which is 11-13 days in the cotton leafworm, also it noticed there is a deformation in a number of adults represented in the breakage of the wings and the lack of some scales covering the body of the insect. (AL- Zubaidi, 1988)

The Adults –(Table 4) shows the results that the effect of *B. thuringensis* in the cotton leafworm was limited at the beginning of the experiment and then began to increase after 5 days of treatment. The mortality percentage of adult treated with bacteria 2.4 , 2.26 and 6.28% after 1, 3 and 5 days of treatment respectively, the symptoms of infection with bacteria represented by weakness

of the adults and their lack of activity and their inability to fly and not to put eggs and the disappearance of some spots on the wings.

Table (4) the effect of *B. thuringensis* in adult of cotton leaf worm *S. littoralis*.

Treatment	Mortality of adult (%)		
	Exposure time (day)		
	1	3	5
<i>B. thuringensis</i>	2.4	2.26 b	6.28 a
Control	0.0	0.0	0.0

Means that share similar characters do not significant difference from each other according to the Dunkin Test at a probability level of 5%.

Greenhouse experiences

Larvae - The table (5) results showed that the Mortality percentage of larvae has risen after 14 days of treatment of the breeding age (second, fourth and sixth) with bacteria *B. thuringensis* in soil or Rasha on the plant, which reached 69, 6% after 14 days of treatment of soil in the plants planted with eggplant plant with bacteria *B. thuringensis* and reached 49, 4% after 14 days of treatment for the total vegetative Plant with bacteria. The mortality percentage of the larvae of the fourth age was 100% after 14 days of treatment of soil with bacteria and 88, 4% after 14 days of treatment, vegetative total of eggplant plant with bacteria and the larvae of the sixth age the mortality percentage after 14 days of treatment with bacteria 85, 6 and 81, 3% for my treatment Bacteria to the soil and the plant's total vegetation respectively. Thus, the differences between the treatment of soil and the vegetative total of bacteria in the fourth and sixth years were not the same in the second age.

Table (5) Effect of *B. thuringensis* in the larvae of the cotton leafworm *S. littoralis* in the greenhouse.

Instars	Treatments	Larval mortality (%)			Mean
		Exposure time (day)			
		4	8	14	
Second	Soil spray	22.3	52.2	94.4	56.3 b
	Plant spray	7.8	48.6	91.8	49.4 c
Forth	Soil spray	20.2	88.6	100	69.6 a
	Plant spray	6.8	43.3	88.4	46.16 c
Sixth	Soil Spray	14.3	32.2	85.6	44.03 c
	Plant spray	6.7	41.2	81.3	43.06 c
Control	Soil spray	0.0	3.3	6.6	3.3 d
	Plant spray	0.0	3.3	6.6	3.3 d

Means that share similar characters do not have significant difference from each other according to the Dunkin Test at a probability level of 5%.

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أختبار كفاءة البكتريا *Bacillus thuringensis* (Berliner) في مكافحة الاحيائية لدودة ورق القطن *Spodoptera littoralis* (Boisd.)

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المستخلص

درست الكفاءة المرضية للبكتريا *Bacillus thuringensis* (Berliner) على الاطوار المختلفة لدودة ورق القطن *Spodoptera littoralis* (Bboisd.) بتركيز 10×10^6 بوغ /مل تحت ظروف المختبر والبيت الزجاجي. أظهرت نتائج الدراسة المختبرية ان النسبة المئوية لفقس البيض بعمر 1-2 يوم المعاملة بالبكتريا بلغت 0.0 و 8.2% بعد 2 و 4 ايام من المعاملة على التوالي. بينما بلغت 6.8 % عند العمر 2-4 يوم وبعد يومين من المعاملة. وكانت النسبة المئوية لموت اليرقات في اعمارها الثاني والرابع والسادس 100% بعد 14 يوم من المعاملة. وعند معاملة العذارى بالبكتريا فقد بلغت نسبة بزوغ البالغات 51.3 % بعد 15 يوم من المعاملة مقارنة بمعاملة المقارنة والتي بلغت عندها النسبة 100% عند ذات الفترة الزمنية. كما كان للبكتريا تأثيرا ملموسا في البالغات فكانت النسبة المئوية لموت البالغات 4.2 و 26.2 و 28.6% بعد 1 و 3 و 5 ايام من المعاملة على التوالي. وظهرت نتائج الدراسة في البيت الزجاجي ان النسبة المئوية لموت يرقات العمر الثاني والرابع والسادس 94.4 و 91.8 و 100 و 88.4 و 85.6 و 81.3 % في معاملتي التربة والمجموع الخضري على التوالي وبعد 14 يوم من المعاملة.

الكلمات المفتاحية: دودة ورق القطن، كفاءة مرضية، *Bacillus thuringensis* ، *Spodoptera littoralis* .