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EFFECT OF BACTERIA *BACILLUS THURINGIENSIS* ON TWO SPOTS MITS *TETRANYCHUS URTICAE* UNDER LABORATORY CONDITION

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Abstract

This laboratory study was conducted at Al-Musayyib Technical College / Department of Biocontrol Technologies for the period from 1/9/2021 to 1/10/2021 in order to assess the efficiency of Bacillus thuringiensis in controlling the two-spotted mite Tetranychus urticae under laboratory conditions.The results showed that the bacterial suspension B. thuringiensis gave a significant effect on the roles of the two-spotted mite (larvae, nymphs and adults) at all concentrations, as it was observed that the mortality rate increased with increasing concentration and time period.While the bacterial sludge B. thuringiensis did not show any significant effect on egg mortality for all concentrations. It was observed that the concentration 33x 10^6CFU/ml increased the percentage of the destruction of motile stages of the two-spotted mite, which amounted to (63.33%, 53.33%, and 50.67%) for larvae, nymphs, and adults, respectively, for the time period (72) hours, which significantly differed from the control treatment, which amounted to (0.67%, 1.67%, and 1.33%) for larvae and nymphs.And adults, respectively, and for a period of time (72) hours. The lowest mortality rate for the moving roles of the concentration was 33x 10^2 CFU/ml (47.33%, 34.00%, and 32.00%) for larvae, nymphs, and adults, respectively, for the time period (72) hours, with a significant difference from the control treatment, which amounted to (0.67% and 1.67). % and 1.33% for larvae, nymphs and adults, respectively, for a period of time (72) hours.The study also showed an increase in the death rate with increasing the concentration of the bacterial sludge, where the highest concentration 33x 10^6 CFU/ml gave the highest mortality rate of (46.00, 41.56 and 41.44) for larvae, nymphs and adults, respectively, with a significant difference from the mortality rate for the lowest concentration 33x 10^2 CFU/ml, which reached (35.11, 23.44, and 26.22) for larvae, nymphs, and adults, respectively.It was observed that the mortality rate increased with the increase of the time period, where the period of 72 hours showed the highest mortality rate of (40.33, 34.17, and 31.08) for larvae, nymphs, and adults, respectively, with a significant difference from the lowest death rate for the 24-hour period, which amounted to (20.75, 16.92, and 19.58).) for larvae, nymphs and adults, respectively.

Keywords: Bacillus thuringiensis, bacterial suspension, two-spotted mite.

1 Introduction.

Tetranychus urticae is a widespread pest that infects various plants, including fruit trees, field crops, vegetables and ornamental plants, and causes great economic damage, especially when

infected in the initial stages of plant growth (Kumral and Çobanoğlu, 2016). Where this pest has a high potential to attack more than 1100 species of plants, including 300 species of economic importance and cause them many losses (Flore et al., 2019), It causes direct damage by absorbing plant juices, causing the appearance of yellow spots on the tissue of the leaf, which leads to a reduction of chlorophyll pigment by 60%, which leads to a decrease in the efficiency of the photosynthesis process in the leaf, as well as the accumulation of dust and dust on the spider web that the mites produces, which it uses to get rid of its enemies The vitality and disposal of pesticides and its ease of movement (Ebrahim et al., 2021). As for the indirect damage, it is the transfer of viral pathogens and their injection into the healthy plant, where it has piercing and absorbent parts of the mouth, such as the Wheat streak mosaic virus and the Tobacco mosaic virus (Hein et al., 2012). In view of the amount of losses caused by this pest on economic crops, as well as the speed of its reproduction and the multiplicity of its generations in a year (All Abas, 2021), Because of its high resistance to chemical pesticides (Zhang et al., 2021). This necessitated the search for other alternative means and solutions to chemical compounds harmful to humans and the environment, including biological control (Riba and Silvery, 1989), in which pathogens of pests, including Bacillus thuringiensis, are used (Entwistle et al., 1993). Where specialists resorted to introducing it within the principle of bio control and its development, as it is one of the important bio control elements due to its rapid growth and the ability to use various food sources in its growth (Al Zubaidi, 1992). The study aimed to evaluate the efficacy of B. thuringiensis in controlling T. urticae under laboratory conditions.

2-Materials and methods

1-2 Diagnosis and breeding of T.urticae in the laboratory.

T.urticae was obtained from infected eggplant plants and was diagnosed by A. Dr. Razak Shaalan Akl at the Natural History Research Center and Museum / University of Baghdad, The mites were bred in the laboratory according to the paper disc method of researchers (Kondo and Takafuji, 1985) by taking the leaves of the castor plant Ricinus communis containing all the stages of the two-spot mites and placing them upside down in a 9 cm diameter plastic petri dish containing a layer of medical cotton. Saturated with distilled water to ensure Wetting the leaves as long as possible.The dishes were placed in an insect-rearing incubator at a temperature of 25 ± 2 °C and relative humidity of 65 ± 5% (AL-Neami, 2007).

2-2 Diagnosis of Bacillus thuringiensis.

Isolation of Bacillus thuringiensis was obtained from the pathology laboratory for postgraduate studies in the Department of Biological control technical Department / Al-Musayyib Technical College and the bacteria were activated in the same laboratory where the isolate was placed in a water bath at a temperature of 70°C for 15 minutes (to kill all fungi and bacteria except Bacillus thuringiensis to get rid of any contamination) (Kitnamorti et al. 2011). They were cultured by sterile Loop on Petri dishes containing Nutrient Agar (NA) by the planning method. The dishes were kept in the incubator at a temperature of 30±2°C for 72 hours (Kersh (El- et al., 2012). After that, the growth of bacterial colonies in the dishes was observed, then some different diagnostic tests were performed to ensure that they were related to B. thuringiensis based on Bergey´s

classification method for the years (1974, 1984 and 1989), as well as (Logan and Vose, 2009), as it relied on Phenotypic characteristics and microscopic tests in diagnosis.

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3-2 Preparation of the bacterial suspension Bacillus thuringiensis.

The bacterial suspension was prepared by inoculating the liquid nutrient media (NB) directly from the growing bacterial colonies on NA and then incubated at 30±2°C for 48 hours (Stahly et al., 1991). In order to know the concentration of the main bacterial suspension of Stock Solution, a series of dilutions were prepared with three replications for each dilution (10-1 - 10-7) of the stock solution, then 1 ml of each dilution was transferred to the dishes containing a medium (NA). It was incubated at 30±2°C for 24 hours (Thiery and Frachon, 1997). After 24 hours, the bacterial colonies growing in the dishes were counted by direct counting method, where the 10-7 dilution was adopted. The rate of the developing colonies in three dishes was extracted and multiplied by the reciprocal of the dilution. According to the following equation mentioned (Clark, 1965).

Number of colonies in 1 ml of bacterial inoculum = average number of colonies growing in dishes x inverted dilution.Whereas, a bacterial suspension with a concentration of (Colony forming unit)

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CFU/ml 107 × 33 was obtained. For the purpose of obtaining the required concentrations, the equation (Lacey, 1997) was applied.

The volume (ml) taken from the original suspension=$\frac{required\ concentration}{concentration\ of\ the\ original\ suspension}$

Then the product was multiplied by the volume of the suspension and thus the required concentrations were prepared in the experiment CFU/ml (33 x 102, 33 x 104, 33 x 106).

4-2 Effect of the bacterial suspended B. thuringiensis on T.urticae two-spot mite eggs in laboratory.

It was transferred to castor leaves placed in petri dishes with a diameter of 9 cm and prepared in advance for 20 adults of T.urticae mites, 10 males and 10 females, and left for 48 hours for the purpose of mating. The adults and the excess eggs were removed with a soft brush (Al-Mallah, 2015) leaving 10 an egg , The dishes were sprayed with the bacterial suspension B. thuringiensis by a small hand sprayer with a capacity of 30 ml and at concentrations CFU/ml (33 x 106, 33 x 104, 33 x 102) at a rate of 1 ml per plate and 3 replicates for each concentration. As for the control treatment, it was sprayed with distilled water only. The dishes were incubated in the incubator at a temperature of 25±2°C and a relative humidity of 65%, and the percentage of egg mortality was calculated after 24, 48 and 72 hours of treatment.

Corrected mortality rate=$\frac{\%\ of\ mortality\ in\ treatments - \%\ of\ mortality\ in\ control}{100 - \%\ of\ mortality\ in\ control}$×100%

5-2 Effect of B. thuringiensis on the motile stages of T.urticae (larva - nymph - adult) in laboratory

10 individuals from the motile stages for each stage separately (larva - nymph - adult) were placed on castor leaves placed upside down in petri dishes of 9 cm diameter prepared in advance and sprayed the dishes with the bacterial suspension B. thuringiensis by a small hand sprayer with a capacity of 30 ml and at concentrations CFU/ml (33 x 106, 33 x 104, 33 x 102), at a rate of 1 ml for each plate and 3 replicates for each concentration and for each mobile phase separately. As for the control treatment, it was sprayed with distilled water only. The dishes were also inspected and the percentage of damage was calculated in the same methods as before.

-6-2 Statistical analysis

The results of the study were statistically analyzed using the GenStat program (2011) according to a factorial experiment with a completely randomized design (C.R.D) completely randomized design, and the Least significant difference (L. S.D.) test was adopted under the 0.05 probability level to test the significance of the results (Al-Rawi and Khalafallah, 2000).).

3- Results and discussion.

1-3 Diagnosis of Bacillus thuringiensis.

The isolate was confirmed to be Bacillus thuringiensis according to the results of the diagnostic tests shown in Table (1).

Table (1) shows the results of the diagnostic tests for Bacillus thuringiensis.

result	test type	No.
+	Gram stain	1

+	Carbol Fuchsine	2
+	Voges – Proskauer	3
+	Enzyme test Catalase	4
+	Methyl red Test	5
+	Sugar Fermentation Test	6
+	Motility Test	7

2-3 Effect of the bacterial suspended B. thuringiensis on T.urticae two-spot mite eggs in the laboratory.

The results in Table (1) showed that mite eggs were not affected by the bacterial suspension B. thuringiensis in all periods and for all concentrations, where the percentage of eggs killing (0.00%, 1.33% and 2.00%) for the concentration (33 × 106) after (24, 48 and 72) hour of the treatment in a row, which did not show significant differences from the comparison treatment, which amounted to (0.00%, 1.00%, and 2.00%) for the same previous time period and there were no significant differences between all concentrations, as the mortality rate for eggs was (0.66, 0.89 and 1.11) for concentrations (33 x 102, 33 x 104, 33 x 106), respectively.Significant differences appeared between the different time periods, where the mortality rate was (0.00, 1.00 and 1.75) for the time periods (24, 48 and 72) hours, which is considered a normal mortality rate for the eggs of the two-spot mite (Golizadeh et al., 2017).It is also believed that the natural mortality rate of eggs is due to being affected by the brush as a result of the removal of excess eggs in the dish. The reason is due to the fact that the eggs are not affected by the bacterial suspension B. thuringiensis because these bacteria do not work on the eggs and the basis of their work is inside the stomach of the target pest (Hilbeck et al., 1998).This study agrees with other studies, where it was found (Aloysius, 2004) that the bacteria B. thuringiensis does not affect eggs, where the hatching rate was 98%, that is, a natural mortality rate of 2% This study also agrees with another study conducted by (Reza et al., 2011), where it was found that the bacterial suspension B. thuringiensis does not affect the rate of egg hatching, but it affects the length of the incubation period of eggs. The current study does not agree with a previous study, where it was found (Hall et al., 2004) that bacteria affect the rate of egg hatching and cause high fatalities.

Table (2) shows the mortality of T.urticae eggs treated with B. thuringiensis during 3 different time periods.

Concentration rate	**time periods/hour**			**concentration**
	72	**48**	**24**	**Cfu/ml**
0.66	**1.33**	**0.67**	**0.00**	$\mathbf{10^2}$**x33**
0.89	**1.67**	**1.00**	**0.00**	$\mathbf{10^4}$**x33**
1.11	**2.00**	**1.33**	**0.00**	$\mathbf{10^6}$**x33**
1.00	**2.00**	**1.00**	**0.00**	**control**
	1.75	**1.00**	**0.00**	**Periods rate**

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LSD concentration= 0.70 Periods= 0.61 interaction= 1.22

3-3 Effect of B. thuringiensis on the motile stages of T.urticae (larva - nymph - adult) in laboratory.

The results in Table (2) indicated the effect of all the concentrations used for the bacterial suspension B. thuringiensis in decreasing the percentage of mortality of the moving roles of T.urticae (larva - nymph - adult)

Where it differed significantly from the control treatment and the significantly of the concentration 10^6x33 CFU/ml in increasing the percentage of larval mortality of the two-spotted nipple, which amounted to (63.33%) for the time period (72) hours and the nymphs amounted to (53.33%).he percentage of adult mortality was (50.67%) for the same time period, which differed significantly from the control treatment, which amounted to (0.67%, 1.67% and 1.33%) for larvae, nymphs and adults, respectively, and for the time period (72) hours. While the mortality was (47.33%, 34.00%, and 32.00%) for larvae, nymphs and adults, respectively, treated with the bacterial suspension 〖10^2x33 CFU/ml for the time period (72) hours, with a significant difference from the control treatment, which amounted to (0.67%, 1.67%, and 1. 33%) for larvae, nymphs and adults, respectively, for a period of time (72) hours The results also showed an increase in the mortality rate with an increase in the concentration of the bacterial suspension, where the highest concentration 10^6x33 CFU/ml gave the highest mortality rate of (46.00, 41.56 and 41.44) for larvae, nymphs and adults, respectively, with a significant difference from the mortality rate for the lowest concentration 10^{2} x 33 CFU/ml which were (35.11, 23.44, and 26.22) for larvae, nymphs and adults, respectively

The results showed that the increase in the mortality rate is directly proportional to the time period, as the 72-hour period showed the highest mortality rate of (40.33, 34.17 and 31.08) for larvae, nymphs and adults, respectively, with a significant difference from the lowest mortality rate for the 24 hour period, which amounted to (20.75 and 16.92). and 19.58) for larvae, nymphs, and adults, respectively.The results indicate that the bacterial susceptibility B. thuringiensis significantly affected the larvae and nymphs more than the adults, as the mortality rate decreases with the increase in the development period. (Fillinger et al., 2003)The results also indicated that the death rate increases with the progression of time, which agreed with a previous study that showed that the infected pests stop feeding, as the poison begins to analyze the internal organs to die after several days, as the bacteria take time to multiply in the intestines of the pest and their crystalline toxins penetrate the internal organs (Martin and Wagih, 2005).Another study confirmed that B. thuringiensis reduces the development and feeding of motile roes more than adults and prevents the development and molting of nymphs from the first nymph to the second nymph, as well as reducing field populations of two-spot mites (Royalty et al., 1991). Another study indicated that the mortality rate of adult mites ing increased to 80% due to the exotoxin β-exotoxin secreted by bacteria (Hall et al., 2004). Another study indicated that B. thuringiensis was effective on mites, with a mortality rate of 59% (Isabel et al., 2015).

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Table (3) shows the percentage of mortality of the moving roles of T. urticae treated with B. thuringiensis during 3 different time periods.

average concentration	Percentage adult mortality after an hour			average concentration	Percentage nymphs mortality after an hour			Average concentration	Percentage larval mortality after an hour			concentration CFU/ml
	72	48	24		72	48	24		72	48	24	
26.22	32.00	25.33	21.33	23.44	34.00	20.67	15.67	35.11	47.33	34.67	23.33	10^2x33
33.89	40.33	36.67	24.67	34.89	47.67	36.00	21.00	38.89	50.00	39.00	27.67	10^4x33
41.44	50.67	41.67	32.00	41.56	53.33	40.33	31.00	46.00	63.33	42.67	32.00	10^6x33
0.78	1.33	0.67	0.33	0.78	1.67	0.67	0.00	0.33	0.67	0.33	0.00	control
	31.08	26.08	19.58		34.17	24.42	16.92		40.33	29.17	20.75	Periods average
LSD concentration= 3.89 Periods= 3.37 interaction= 6.74				LSD concentration =3.37 Periods= 2.92 interaction= 5.84				LSD concentration= 1.45 Periods= 1.25 interaction= 2.51				

4 Conclusions.

We conclude from the study that Bacillus thuringiensis was effective and had an effect on the destruction of T.urticae on larvae, nymphs and adults, and had no effect on the mortality of eggs under laboratory conditions.

The best concentration of the bacterial suspension in all stages of the mites was 〖10〗^6x33 CFU/ml, and the best time period with the highest mortality rate was 72 hours.

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