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EVALUATION OF THE EFFICIENCY OF DIFFERENT CONCENTRATIONS OF BACTERIAL STRAINS *BACILLUS THURINGIENSIS* IN CAUSING DEATH TO MICROCEROTERMES DIVERSUS SILVWORKERS IN LABORATORY CONDITIONS

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Abstract

The present study was carried out to evaluate the efficacy of different concentrations of *Bacillus thuringiensis* strains in the death of microcarotermes diversus Silvestri (MDS) (Isoptera : Termitidae) workers by treating the dietary medium cellulose and agar in natural laboratory conditions. The results of the study showed that the efficacy of *Bacillus thuringiensis* bacteria in the death of the MDS workers depends on the strain, the concentration used and the duration of exposure, showing a gradual increase in the death rates of the workers by the time of the treatment and the high concentrations were the most efficient in causing death and the fastest in achieving death 100%. The obtained results of the present study showed that the speed of achieving mortality rates of 100% increases with increasing concentrations. The mortality rate in the first infection was 100% after 42, 39 and 36 days for commercial strain B.t. Kurstaki and 39, 36 and 33 days for the local strain B.t. Kurstaki and 36, 33 and 30 days for the local strain B.t. israelensis at concentrations of 5, 10 and 20 (1×10^{-6} spore/ml), respectively. Additionally, strains of B.t. remained effective for 180 days after treatment. The fourth infection after 180 days of treatment achieved 100% mortality after 39, 33 and 30 days of treatment with commercial bacterium and 33, 30 and 27 days of treatment with local bacterial strain Kurstaki B.t. and 27, 24 and 21 days of treatment with local bacterial strain B.t. israelensis for concentrations 5, 10, 20 (1×10^{-6} spore/mL), respectively. The third infection was the fastest in achieving 100% mortality compared to other infections due to the appropriate temperature for the growth and activity of bacteria in this infection. The high temperatures have a positive role in the efficiency of bacteria and the speed of achieving death, as low temperatures in the first infection has caused a significant reduction in the efficiency of bacteria and the speed of causing death. The results of the study also proved that different concentrations of B.t. israelensis were the fastest in achieving the death of 100% of MDS workers.

Key words : MDS workers, *Bacillus thuringiensis*, temperature, private colonies.

Introduction

Termites (white ants) is known to be one of the most important social insects. It lives in private colonies of thousands of individuals (Arab *et al.*, 2005), spends most of its life hidden from light and plays an important environmental role through its major contribution to the improvement of most ecosystems in the world as well as helps in soil ventilation, increase soil fertility and play an important role in the recycling of the high carbon element (Raychoudhury *et al*., 2013). It also considered one of the most successful insects on the earth that has colonized most of the land except Antarctica and their queens live longer than any other insect in the world where they live for 50 years (Bignell *et al*., 2010). The *M. diversus* type is the most important species in the Iraqi governorates, causing a major economic losses and problems (Al-Jassani, 1996).

In the United States, the residential and commercial buildings damage was estimated at more than 1 billion US $ per year. The earth dwelling termite is one of the most destructive species, with 95% damage (Marcus, 2008) and the cost of fighting the earth dwelling termite using chemical pesticides has been estimated at approximately 1.5 billion $ a year in the United States (Miller *et al*., 2010). Furthermore, the damage caused by the termite of the world has reached 40 billion US dollars annually (Evans *et al.*, 2013). The chemical pesticides such as organochlorine pesticides for termite

control (Mahapatro *et al*., 2014) have been heavily relied upon to eliminate termite degradation because of their low cost and ease of use, resulting in undesirable side effects, including water and food pollution, negative impact on non-target organismsand insect resistance to the pesticide (Verkert *et al*., 2001). Therefore, many researches and studies have resorted to the use of alternatives from the biological control agents that have grown in the pesticide market over the past decades and which are mainly supported by the need to move towards safer and sustainable cultivation. The wet environment favoured by termite supports epidemiology and enhances the potential of biological control (Verma *et al*., 2009; Stow and Beattie, 2008). Due to the success of the biological control agents in termite controlling, B.t. was used because of it is production of toxic proteins against multiple types of termite (Singha *et al*., 2010) as well as the bacteria of bacillus species are among the most widely used in the biological pest control programs. In recent years, B.t. bacteria have received widespread interest from many researchers to use it as alternatives to chemical pesticides or chemical pesticide aids because they are specific families, safe for humans and do not infect beneficial insects, making them more environmentally acceptable (Ginsburg, 2006 and Kumar *et al*., 2008). Additionally, B.t. is one of the most widely used pesticides after chemical pesticides and has been successfully used in the integrated control programs of many insect pests (Diego, Benintende, 2008; Dubovskiy, 2005; Cariso and Conelly, 2009). Due to the economic damage caused by MDS and no applied study for controlling of termite by bacteria B.t., the present study aimed to evaluate the efficiency of different concentrations of commercial and local B.t. strains with dietary medium cellulose and agarin killing of MDS individuals in laboratory conditions.

Materials and Methods

Source of Microcerotermes diversus Silv colony and it is in vitro adaptation

A number of severely infected citrus trees with MDS were selected from one of the orchards in the fields of Al Jadiriya in Baghdad for the purpose of use as preliminary sources to obtain the individuals of MDS to carry out laboratory studies where parts of these trees and blocks of soil surrounding the infected trees and containing a number of different individuals of MDS were taken to the laboratory for the purpose of adaptation to the new environment after the isolating from the field community, cut off and decomposed the infected pieces to remove the individuals of MDS by moving them and roads on them and took blocks of soil located under trees infected and surrounding the tree trunks for the purpose of breeding and adapting individuals on the new environment for 7-14 days before starting the laboratory experiment. After that, boxes with dimensions of (30 cm × 22 cm × 15 cm) with an airtight cover (the four sides of which are coated with aluminum foil) are covered with a metal plate for providing a complete darkness and base filter sheets as a food source for the MDS workers were prepared (Yanagawa *et al*., 2009). A daily inspection of these boxes (which contain the individuals) in the laboratory and monitoring them daily to observe the activity, feeding, and movement of different individuals and build new tunnels for workers and remove unnatural termites in terms of movement and activity. For providing the necessary moisture to sustain the lives of individuals a hand sprayed with distilled water by a small sprinkler was carried out whenever needed.

Cultivation and adaptation of individuals on the food medium Cellulose and agar in laboratory conditions

The obtained pure cellulose was in powder form and produced by the Spanish Company Barcelona, the cellulose was dried and sterilized at 120° C for two days (Timothy *et al*., 2009), and then prepared 36 Petri dish diameter 9 cm and placed in each dish 4 grams of cellulose Dried and sterilized. Amount of 8 ml of 4% wafer solution (4 g of sugar per 100 ml distilled water) was added for the cohesion of the cellulose molecules with each other and leave the dishes until stiffened. A number of workers were transferred to the dishes containing the food medium by using a small soft brush (2 mm), where a small amount of the natural center crumbs of the insect and the tunnels built by the ground were transferred to the breeding boxes. The dishes were then sealed with cellophane chips to provide the necessary dark and left in the conditions of laboratory for conducting experiments in natural laboratory conditions. A daily check is carried out to monitor the activity of individuals, build new tunnels and raise dead and non-natural individuals, while providing the necessary moisture for the insect by hand spraying using a small hand spray whenever necessary, then adaptation was carried out for 7 days (Marsoumi, 2012).

Source of *Bacillus thuringiensis*

1. *Bacillus thuringiensis* var. Kurstaki (B.t.k.) in wet powder form containing blackboard 2.5×10^{-6} spore/ml.
2. Local strain (B.ti.) israeliansis *Bacillus thuringiensis* var.
3. Local strain (B.t.k.) *Bacillus thuringiensis* var.

kurstaki isolated from moth larvae dates EP Ephestia and molecularly identified PCR.

Effect of *Bacillus thuringienesis*in the death of MDS workers in laboratory conditions

The study evaluated the efficiency of different concentrations of bacterial strains (commercial and local) in causing death to MDS workers when treating the dietary medium of the cellulose and agar in natural laboratory conditions. A total of 4 cups of sterile and dried cellulose was added to each dish. In addition, 8 mL of sugar was added to the mixture.

First : The commercial product of *Bacillus thuringenesis* was used in three concentrations.

1. Bacteria with a concentration of 5 ml/L (5×10^{-6} spore/ml).

2. Bacteria with a concentration of 10 ml/L (10×10^{-6} spore/ml).

3. Bacteria with a concentration of 20 ml / L (20×10^{-6} spore/ml).

Second : The local strain *Bacillus thuringenesis israelansis* has been used three concentrations.

1. Bacteria at a concentration of 5 ml / L (5×10^{-6} spore/ml).

2. Bacteria with a concentration of 10 ml / L (10×10^{-6} spore/ml).

3. Bacteria with a concentration of 20 ml / L (20×10^{-6} spore/ml).

Third : The local strain *kurstaki Bacillus thuringenesis* var has been used with three concentrations.

1. 5 ml / liter (5×10^{-6} spore/ ml).

2. 10 ml / liter (10×10^{-6} spore/ml).

3. 20 ml / liter (20×10^{-6} spore/ml).

Fourth : The chloro phenocol is used at a concentration of 2 g/liter

Fifth : Fertilizer (Imidachloprid Premise) at a concentration of 5 ml/liter

Sixth : Control treatment (distilled water only).

After drying and hardening of the food medium, 3 ml of each treatment solution was added to the dishes for treatment of the food medium and left for two hours until the saturation of the solution in food medium (Salhi, 2006). Using a soft brush, 100 workers were transferred to the treated dishes under the conditions of complete sterilization and then closed the treated dishes and packaged with cellophane paper to provide complete darkness and left in the laboratory conditions. Daily examination of all treated dishes was conducted for three days and recorded the number of dead individuals at each examination and then the examination carried out every three days in addition to a required humidity was provided by a 7 ml hand spray whenever needed. The experiment was conducted on 10th of December 2017 and corrected the death rate based on the Abbott equation (Abbott, 1925). In order to prove the pathogenicity of the bacteria to the individuals of MDS, a number of dead workers are randomly selected from the various treatments. They were sterilized with alcohol by 70% for 2 minutes and in the minor solution at 10% concentration for 2 minutes in a bottle and put in distilled water for 2 minutes, then transferred to filtration papers for moisture absorption and drying workers. A number of 36 petri dishes were prepared and a nutrient agar (NA) agricultural medium was poured into the dishes as well as 5 dead workers randomly transferred and distributed evenly in the dish where three dishes were used, represented 3 replicates per treatment and then placed in the incubator at 30°C for 24 hours and the growth of bacteria was observed.

Life duration of B. t. strains in various treatments and their efficiency in causing death to the individuals of MDS in laboratory conditions

In order to determine the survival and efficacy of B. t. strains and their efficiency in causing death to the MDS in the subsequent period after the treatment. The dishes were kept at laboratory temperature under complete darkness and the artificial infection was conducted two months after the first treatment. The examination of all the dishes treated with bacteria daily for three days thereafter, the examination was carried out every three days and recorded the number of dead workers at the time of the examination. The first infection was done on 10th of December 2017, the second infection 10th of February 2018 and the third infection on 10th of April 2018 and the fourth on 10th of June 2018 in addition to death rates were corrected according to Abbott equation (Abbott, 1925). The results were statistically analyzed according to the CRD design and were compared using the smallest significant difference in LSD as well as Genstat program was used in the analysis.

Results and Discussion

Effectiveness of bacterial strains *Bacillus thuringiensis* in the death of MDS workers in dietary medium cellulose and agar at the laboratory conditions (first infection)

The effect of bacteria on death is based on the concentration and duration of exposure. The results of

Table 1 : Death percentage of MDS workers in different concentrations of *Bacillus thuringiensis* during different periods in laboratory conditions (first infection).

Treatments		Death percentage (day)														Total average
	Concentration	1	2	3	6	9	12	15	18	21	24	27	30	33	36	39	42	
Commercial strain B.t.k	**5 × 10^{-6}**	0	0	0	0.77	3.1	10.35	22.36	39.92	60.23	73.59	82.38	89.29	94.04	97.02	98.64	100	48.23*
	10 × 10^{-6}	0	0	2.28	4.85	11.71	22.28	38.89	54.56	75.72	84.25	89.86	93.53	96.2	98.37	100	100	54.53*
	20 × 10^{-6}	0.76	2.79	4.32	6.93	15.58	31.03	49.87	64.86	70.42	85.18	90.4	94.64	97.83	100	100	100	57.16*
	Average	0.25	0.93	2.2	4.18	10.13	21.22	37.04	53.11	68.79	81	87.54	92.48	96.02	98.46	99.54	100	53.30*
Local strainB.t.k	**5×10^{-6}**	0	0	1.52	4.87	11.36	23.01	37.56	51.92	64.08	73.71	79.97	89.7	94.87	98.64	100	100	51.32*
	10 × 10^{-6}	0.51	3.81	12.22	25.18	36.89	51.94	63.71	73.23	79.94	85.63	92.22	95.7	97.83	100	100	100	63.67*
	20 × 10^{-6}	4.06	13.99	24.95	38.28	50.66	61.37	70.72	78.82	84.93	89.91	93.84	97.58	100	100	100	100	69.31*
	Average	1.52	5.93	12.89	22.77	32.97	45.44	57.33	67.99	76.31	83.08	88.67	94.32	97.56	99.54	100	100	61.64*
Local strain***B.t.i***	**5×10^{-6}**	0	1.53	4.83	10.99	18.34	32.81	51.82	68.93	78.95	86.5	91.48	95.44	98.65	100	100	100	58.76*
	10 × 10^{-6}	1.01	3.02	6.35	9.04	18.6	29.97	54.82	69.44	80.17	89.41	94.92	97.85	100	100	100	100	59.66*
	20 × 10^{-6}	10.68	25.45	42.5	58.58	71.75	81.33	87.2	91.63	94.96	97.09	98.64	100	100	100	100	100	78.73*
	Average	3.89	10	17.89	26.2	36.23	48.03	64.62	76.66	84.69	91	95.01	97.76	99.55	100	100	100	65.71*
Antibiotic	2 g	0	2.29	6.13	14.47	33.52	57.25	77.02	86.35	90.5	93.63	96.79	98.92	100	100	100	100	66.05-
Pesticide	5 ml	77.4	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	98.58-
L.S.D.		0.87	1.19	3.03	7.07	7.57	9.11	9.68	8.26	8.63	6.55	4.95	2.31	1.38	0.79	0.23	0	

*All the dead individualsbecause of the bacterial infection

table 1 showed that bacterial strains are characterized by their gradual death. The death rate at the beginning of the treatment was very low. After that, gradual increase in all treatments was noted as this increase is slow at the low concentrations of different treatments and high at the highest concentrations with increasing speed of achieving death rates 100% the greater the concentration as the death rate achieved 100% after 36, 39, 42 days for commercial strain Btkurstakias well as 33, 36, 39 days for the local strain B. t. kurstaki and 30, 33, 36 days for the local strain sraeleansis B.tii at concentrations of 5, 10 and 20 x 10^{-6} spore/ml, respectively. The obtained results showed a significant difference between treatments in general average of death rates in the different strains. Moreover, the overall mortality rate of the workers during all readings was 78.73, 61.64 and 53.30% for the local strain B. t.i, local strain B. t. k. and commercial strain B. t. k respectively. Additionally, tests for the development of Bt. (The hypothesis of the kukh) detected that all dead workers in all treatments and different concentrations were the result of bacterialinfection compared to the absence of bacteria in the dead workers treated with pesticide, antibiotic and control (table 1). In the pesticide treatment, the mortality rate was 100% after 2 days of the dietary medium treatment, while the antibiotic was characterized by slow and gradual death in the individuals until 100% died after 30 days. It is clear that the B. t.i strain was the fastest in all concentrations in achieving 100% mortality rate for M. diversus workers. The significant decrease in death rates and the increase in exposure duration may be due to the activity and nutrition of termite functions, as well as the growth of bacterial spores associated with temperature fluctuations in laboratory conditions. Keynan *et al*. (1964), said that bacteria germs are most affected by temperature, In particular its *Bacillus thuringiensis* bacteria.

Table 2 : Death percentage of MDS workers in different concentrations of *Bacillus thuringiensis* during different periods in laboratory conditions (second infection).

Treatments		Death percentage (day)													Total average
	Concentration	1	2	3	6	9	12	15	18	21	24	27	30	33	36	
Commercial strain B.t.k	**5X10^{-6}**	0	0	1.79	6.19	16.3	34.01	57.25	76.38	86.03	91.49	94.91	97.03	98.64	100	54.28*
	10 x 10^{-6}	0	0.2	1.02	10.32	28.06	52.95	70.83	82.5	90.03	94.46	97.61	98.92	100	100	59.06*
	20 x 10^{-6}	0.51	2.29	7.66	16.53	36	62.69	76.24	83.45	88.9	92.83	96.25	100	100	100	61.62*
	Average	0.17	0.83	3.49	11.01	26.78	49.88	68.1	80.77	88.32	92.92	95.92	98.71	99.54	100	58.32*
Local strainB.t.k	**5X10^{-6}**	0.25	1.02	2.3	8.51	18.64	32.11	48.17	68.76	79.68	87.29	93.31	97.04	100	100	52.64*
	10 x 10^{-6}	1.02	3.57	13.8	27.52	50	75.39	86.38	92.38	95.52	97.86	98.92	100	100	100	67.31*
	20 x 10^{-6}	1.51	7.65	26.41	47.11	70.43	82.36	90.09	93.31	96.83	98.94	100	100	100	100	72.47*
	Average	0.92	4.08	14.17	27.71	46.35	63.28	74.88	84.81	90.67	94.69	97.42	99.01	100	100	64.14*
Local strain *B.t.i*	**5X10^{-6}**	0.76	1.27	5.62	18.59	54.78	61.14	77.54	86.61	92.1	96.02	98.66	100	100	100	63.79*
	10 x 10^{-6}	2.77	7.65	15.55	29.6	48.05	72.47	89.02	94.75	97.11	97.31	100	100	100	100	68.16*
	20 x 10^{-6}	13.19	29.59	38.62	66.18	79.27	89.11	94.77	97.33	98.94	100	100	100	100	100	79.07*
	Average	5.57	12.83	19.93	38.12	60.76	74.24	87.11	92.89	96.05	97.77	99.55	100	100	100	70.34*
Antibiotic	2 g	3.04	102	20.45	36.94	56.58	69.94	80.47	89.53	94.47	97.47	100	100	100	100	68.50 _
Pesticide	5 ml	75.51	100	100	100	100	100	100	100	100	100	100	100	100	100	98.46 _
L.S.D.		0.87	1.05	1.64	10.06	4.27	9.54	3.22	2.48	2.02	1.81	1.43	0.94	0.49	0.23	0

Effectiveness of bacterial strains *Bacillus thuringiensis* in the death of MDS workers in dietary medium cellulose and agar in the laboratory conditions (second infection)

The results of the second infection revealed that, the bacteria were the most efficient in causing death to the workers, after 60 days of the first treatment but slowly except for high concentrations and for all treatments (table 2). Where the high concentration of 20×10^{-6} (spore/ml) was exceeded with speed of the death in MDS workers. The mortality rate was 100% after 24, 27 and 30 days with commercial bacteria B.tk, local B.tk. and local B.ti., respectively (table 2). While the concentration of 5×10^{-6} was the slowest in achieving 100% death of M. diversus workers. The duration of time was 33, 33 and 36 days for commercial bacteria B.tk strains, local B.tk and local B.ti. respectively (table 2). Furthermore, the concentration of 10×10^{-6} has achieved 100% mortality rate after 27, 30 and 33 days for commercial bacteria B.tk, local bacteria B.tk and local bacteria B.ti. Respectively (table 2).

The local strain B.t.i. was the most efficient in achieving the death of MDS workers where the overall rate of death of workers during all readings was 70.34% followed by local strain B.tk which achieved 64.14% and finally the commercial strain B.tk. Table 2 showed a significant difference between treatments in the general death rate. Additionally, the pesticide treatment was characterized by the rapidity of killing and the antibiotic achieved 100% death in the same period of time in the first infection. The speed of the killing of bacteria increases with concentrations, due to the increase in the number of bacteria and toxins produced by crystals of bacteria and their effect on the death of workers. In this regard, Corn forth *et al.* (2015) noted that the increase in the percentage of casualties occurs when they are high concentrations. Furthermore, Wright and Cornelinus (2012) reported that the efficacy of *Bacillus thuringiensis* in the death rate of MDS depends on the concentration of the bacteria. The use of the

Table 3 : Death percentage of MDS workers in different concentrations of *Bacillus thuringiensis* during different periods in laboratory conditions (Third infection).

Treatments	Concentration	Death percentage (day)												Total average
		1	2	3	6	9	12	15	18	21	24	27	30	33	
Commercial strain B.t.k	**5X10^{-6}**	0	0	0.5	1.78	9.7	25.1	47.16	70.98	83.83	90.59	95.79	98.41	100	47.98*
	10 x 10^{-6}	0.5	5.55	15.51	30.17	52.92	78.06	93.29	97.92	100	100	100	100	100	67.22*
	20 x 10^{-6}	3.28	13.63	29.52	57.79	75.95	86.62	95.35	100	100	100	100	100	100	74.01*
	Average	1.26	6.39	15.17	29.91	46.19	63.26	78.6	89.63	94.61	96.86	98.59	99.47	100	63.07*
Local strain B.t.k	**5X10^{-6}**	2.77	9.84	26.46	47.02	63.42	77.11	87.87	93.98	90.91	100	100	100	100	69.18*
	10 x 10^{-6}	6.56	21.21	42.99	59.94	73.96	86.62	95.07	100	100	100	100	100	100	75.87*
	20 x 10^{-6}	11.11	27.77	49.6	77.29	89.25	96.15	100	100	100	100	100	100	100	80.82*
	Average	7.88	18.71	39.03	61.06	75.51	86.86	93.89	97.77	99.28	100	100	100	100	75.38*
Local strain *B.t.i*	**5X10^{-6}**	7.32	23.23	47.06	64.02	79.28	88.42	95.31	100	100	100	100	100	100	77.29*
	10 x 10^{-6}	11.36	33.07	56.99	80.61	93.09	97.68	100	100	100	100	100	100	100	82.52*
	20 x 10^{-6}	25.25	54.54	76.63	92.58	97.19	100	100	100	100	100	100	100	100	88.16*
	Average	14.64	36.94	60.22	79.07	89.85	95.36	98.43	100	100	100	100	100	100	82.65*
Antibiotic	2 g	0	0.25	1.26	7.1	21.22	38.36	58.48	73.82	82.84	90.82	95.28	98.15	100	51.35_
Pesticide	5 ml	78.78	100	100	100	100	100	100	100	100	100	100	100	100	98.36_
L.S.D.			1.65	1.94	2.12	3.06	2.72	2.72	1.74	1.17	6.15	0.78	0.59	0.35	0

concentration 10^{-6} spore/ml caused a high mortality rate within three days of the treatment and thus the proportion of killing is directly proportional to the concentrations used.

Effectiveness of bacterial strains *Bacillus thuringiensis* in the death of MDS workers in the dietary medium cellulose and agar at laboratory conditions (third infection)

The results of the third infection (after 120 days of treatment) is shown in table 3. The bacteria have maintained their efficiency and effectiveness in the injury and death of workers in all treatments and all concentrations have been characterized by bacteria in this infection by achieving death and a rapid rate compared to previous hostilities, with a death rate of 100% after 18, 21 and 33 days of treatment commercial strain B.tk, after 15, 18, 24 days of treatment for local strain B.tk and after 12, 15, 18 days of treatment for the local strain B.ti. at concentrations 5, 10 and 20 × 10^{-6} (spore/mL) respectively. The pesticide permise was also characterized by a speed of 100% mortality after 2 days of treatment when compared with the gradual effect of killing in all concentrations of bacteria and various treatments. Table 3 revealed that the high concentration of all strains of bacteria, which significantly differed from the rest of the concentrations and in all treatments and was superior in its efficiency and speed of death. This superiority is attributed to the increase of bacteria spores and their toxic effect associated with temperature and activity of workers in movement. Weidner (1987) demonstrated that an increase in the activity of MDS was noted by increasing the temperature and humidity. The results in table 3 indicated the superiority of B.t. israelensis on other strains of bacteria, where the overall mortality rate of the workers during the various readings was 82.65% while the general mortality rate of the workers was 75.38 for the local strain B.t.k and 63.07% for the commercial strain B.t.k. The statistical analysis showed that there was a significant difference between treatments in regard to the overall death rate (table 3). The

Table 4 : Death percentage of MDS workers in different concentrations of *Bacillus thuringiensis* during different periods in laboratory conditions (forth infection).

Treatments		Death percentage (day)													Total average
	Concentration	1	2	3	6	9	12	15	18	21	24	27	30	33	36	39	
Commercial strain B.t.k	**5X10^{-6}**	0	0	0	1.27	3.87	16.94	32.54	52.43	67.85	77.53	86.5	92.33	95.99	98.66	100	48.39*
	10 x 10^{-6}	0	0	2.02	12.52	25.76	45.86	62.27	76.74	85.7	91.49	95.51	97.86	100	100	100	59.71*
	20 x 10^{-6}	1.01	1.01	2.02	7.92	21.22	41.47	64.68	79.24	87.49	93.16	97.36	100	100	100	100	59.77*
	Average	0.33	0.33	1.34	7.23	16.95	34.75	53.16	69.47	80.34	87.39	93.12	96.73	98.66	99.55	100	55.95*
Local strain B.t.k	**5X10^{-6}**	0	7.32	12.37	23.93	44.74	67	78.8	86.03	83.05	94.47	97.08	98.93	100	100	100	66.24*
	10 x 10^{-6}	0.5	2.27	7.82	38.46	39.64	62.02	74.22	83.64	89.68	93.96	97.35	100	100	100	100	65.98*
	20 x 10^{-6}	4.54	22.72	43.43	60.86	72.62	81.65	88.88	93.5	96.61	98.68	100	100	100	100	100	77.56*
	Average	1.69	10.77	21.2	41.08	52.33	70.22	80.63	87.72	89.78	95.7	98.14	99.64	100	100	100	69.92*
Local strain***B.t.i***	**5X10^{-6}**	0.25	4.79	14.14	26.1	48.59	70.57	86.2	92.74	96.08	98.55	100	100	100	100	100	69.20*
	10 x 10^{-6}	4.04	19.7	43.76	63.43	76.47	85.18	91.21	95.33	97.82	100	100	100	100	100	100	78.46*
	20 x 10^{-6}	16.16	37.62	61.61	82.1	88.48	93.29	96.12	98.44	100	100	100	100	100	100	100	84.92*
	Average	6.81	20.7	39.83	57.21	71.18	83,01	91.17	95.5	97.96	99.51	100	100	100	100	100	77.52*
Antibiotic	2 g	14.14	35.35	60.34	80.04	89.24	92.52	95.85	98.32	100	100	100	100	100	100	100	84.38-
Pesticide	5 ml	71.96	100	100	100	100	100	100	100	100	100	100	100	100	100	100	98.13-
L.S.D.		1.05	2.1	3.12	15.91	4.16	4.87	4.67	3.96	7.73	1.3	1	0.81	0.46	0.22	0	

appropriate temperatures on April reflected the activity of the workers and thus increased nutrition and speed of access of blackboards to the digestive system and the formation of toxic crystals and deaths. Schumann *et al*. (2014) pointed out that the gradual killing of bacteria treated with bacteria is due to the mechanism of the effect of toxic bacteria from the beginning of the insect swallow the bacteria containing the crystalline name and the melting of poison within the space of the central gastrointestinal tract, which leads to damage to epithelial cells and the death of the target insect.

Effectiveness of bacterial strains *Bacillus thuringiensis* in the death of MDS workers in the dietary medium cellulose and agar at laboratory conditions (Infection IV)

For the fourth infection, a slight decrease in the efficiency of the bacterial strains and delayed the achievement of 100% mortality rate for the workers was revealed (table 4). The duration of the death was 30, 33 and 39 days for the commercial strain B.t.k and 27, 30 and 33 days for local strain B.tk as well as 27, 24, 21 days for the local strain B.ti. For concentrations 5, 10 and 20 × 10^{-6} spore/ml, respectively. It can be attributed that the low-efficiency of bacterial strains in death events was attributed to the low number of bacterial germs that were the primary source of infection and the loss of vitality of the bacteria due to environmental conditions during the period. Hunsberger (2000) and Lacey (2007) reported that B.t bacteria was affected by surrounding environmental conditions. The local bacterial strain B.ti. was superior to other treatments and the overall rate of death of MDS workers was 77.52% with a significant difference in the other treatments, which was 69.92 and 55.95% for the local and commercial strain B.t.k., respectively . The pesticide premise quickly characterized the killing of 100% of the workers after two days of treatment compared to the gradual death in all other treatments and in all concentrations (table 4).

Conclusion

It can be concluded that the strains of

B.t. proved its efficiency in causing death of MDS workers with all its concentrations, but the high concentration was more efficient and faster in killing MDS workers and the local strain B.t.i. was the first to cause death to the workers followed by the local strain B.t.k. Finally, the commercial strain B.t.k. Moreover, the local strains isolated from the natural environment of bacteria were more efficient than commercial strains due to the adaptability of these local strains to the surrounding environmental conditions and their resistance to the harm conditions in the environment in which they were located. Based on this, the strains of Bt. are an important and effective biological agent in controlling the MDS through the use in termite baitstation and the elimination of MDS colonies in case of disease spread.

References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomo*l., **18** : 265 - 267.

AL-Jassany, R. F. (1996). Evaluation of some physical masures and chlorofit 48% TC in building protection against termite infestation *Microcerotermes diversus* Silvestri (Isoptera : Termtidae). *Ph.D. Thesis*, College of Agriculture. Baghdad University. .

Al-Marsoomy, M. D. (2012). Evaluation the efficacy of some insect growth regulators to control subterranean termite *Microcerotermes divesrsus* (Silvestr) (Isoptera :Termtidae). *Mast.D*. College of Agriculture Baghdad University. pp.111.

Al-Salehi, M. A. (2006). Evaluation the efficacy of the entomopathogenics and insect growth regulators for control subterranean termite *Microcerotermes divesrsus* (Silvestr) (Isoptera :Termtidae). *Ph.D. Thesis*, College of Agriculture Baghdad University. pp.176.

Arab, A., A. M. Costa -Leonardo, F. E. Casarin, A. C. Guaraldo and R. C. Chave (2005). Foraging activity and demo graphic patterns of towtermite Species (Isoptera: Rhinotermitidae) living in Urban I and scapes in Southeastern Brazil. *Eur. J. Entomol.*, **102(169)** : 691-697.

Bignell, D. E., Y. Roisin and N. Lo. (2010). Biology of termites: a modern synthesis (Ist ed). Dordrecht: Spring. ISBN 978 - 90 - 481 - 3977 - 4.

Connelly, C. R. and D. B. Carlson (2009). Florida coordinating council on mosquito control: the state to the mission as defined by mosquito controllers , regulator, and environmental manager. University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory.p: 225.

Cornforth, D. M., A. Matthews, S. P. Brown and B. Raymond (2015). Bacterial cooperation causes system– atic errors in pathogen risk assessment due to the failure of the independent action hypo thesis. *Plos. Pathog*., **11(4)** : e1004775.

Diegoh, S. and G. B. Benintende (2008). *Bacillus thuringiensis*: generalidades, un acercamiento a su empleo enel biocontrol de insectos lepidopteros que son plagasagriclas revista. *Argentina de Microbiologia*, **40** : 124-140 (Abst in English).

Dubovskiy, I. (2005). The activity of antioxidants in mid gut of larvae *Galleria mellonella* infected by *Bacillus thuringiensis* 38th Annual Meeting of the Society for Invertebrate pathology. P 70.

Evans, T. A. B., T. Forschler and K. J. Grace (2013). Biology of invasive termites :A worldwide review. *Annual Review of Entomology*, **58** : 455-474.

Ginsburg, C. (2006). Aerial spraying of Bacillus *thuringiensis* kustaki (Btk). *Journal of Pesticide Reform*, **26 (2)** : 13-16.

Hunsberger, A. (2000). B.T (*Bacillus thurgiensis*), a microbial insecticide urban horticulture agent. Miami. Dade county and Entomologisst : 1- 6.

Keynan, A. Z., H. O. Halvorson and J. W. Hastings (1964). Activation og Bacterial Endospores. *Journal of Bavteriology*, **88** : 313 – 318.

Kumar, S., A. Chandra and K. C. Pandey (2008). *Bacillus thuringiensis* (B.T) trans gentic crop : An environment friendly insect - pest management strategy. *J. Environ. Biol.* , **29** : 641 -653.

Lacey, L. A. (2007). *Bacillus thuringiensis* serovariety is raelensis and *Bacillus sphaericus* for mosquito control . *Journal of the American Mosquito control Association*, **23(Sp2)** : 133-163.

Marcus, A. S. (2008). Retkinking museums adutt education for k- teachers. *Journal of Museum Education*, **33 (1)** : 55– 78.

Mahapatro, G. K. and K. Sreedevi (2014). Indigenous approaches for the management of termite and white grub in uplandrice current. *Biofica*, **8(1)** : 97-108.

Miller, D. M. (2010). Signs of subterranean termite infestation. J. Virginia Cooperative extension publication 444-501.

Peralta, C. and L. Palma (2017). Is the insect world over coming the efficacy of *Bacillus thuringinsis*: *Toxins* **9 (1)** : 39.

Verma, M., S. Sharma and R. Pasad (2009). Biological alternatives for termite Biodeteri or Biodegradation. Journal article: *International Bacterioration and Biodegradation*, **63** : 959 – 97.

Raychoudhury, R., C. Y. Sen and Y. Sun (2013). Comprative etatrans criptomic signatures of wood and paper feeding in the gut of the ternite Reticulitermes flavipes (Isoptera : Rhinotrmitidae). *Insect Molecular Biology*, **22(2)** : 155.

Schumann, R., N. Knaak and L. M. Fiuzal (2014). Mode of action and specificity *of Bacillus thuringiensis* toxin in the control of caterpillars and stink bugs in soybean culture, ISRN Microbiology. Hindawi publishing Corporation Article ID 135675, pp 12.

Singha, D. and B. Singhaand Dutta (2010). *In vitro* pathogenicity

of *Bacillus thuringiensis* against tea termite's. *J. Biol. Control*, **24** : 279 -281.

Stow, A. and A. Beattie (2008). Chemical and genetic defenses against disease in insect societies. *Bran Behav*. Immunol., **22** : 1009-1013.

Timothy, M. J. and C. C. Corbin (2009). Effect of cellulose concentration the feeding preferences of termite Reticulitermes flavipes (Isoptera : Rhinotermitidae). *Sociobiology*, **53(3)** : 775-794.

Verkert, R. H. J. and A. F. Pravery (2001). The uktermite eradication programme Justification and implementation. *Sociobiology*, 37-351-360.

Weidner, H. (1987). Advances in Applied Terminology 1976-1980. *Plant Research and Development*, **26** : 8-113.

Wright, M. S. and M. L. Cornelinus (2012). Mortality and repellent effects of microbial pathogens on *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *J. Bmc Microbiology*, **12** : 291-398.

Yanagawa, A., F. Yokohari and S. Shimizn (2009). The role of antcnnac in removing entomo pathogenic fungi from cuticle of the termite, *coptotermes formosanus*. *Journal of Insect Science*, **6** : 1- 9.