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Evaluation of the Larvicidal Activity of *Bacillus thuringiensis* Compared with Chemical Insecticides Against the forth instar Larvae of the *Culex* Mosquito

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Abstract

The aim of the study at the possibility of finding an integrated control program, consisting of the biological preparation made from the biocontrol (*Bacillus thuringiensis*) in combination with chemical pesticides (gentapride, metkol, methoprene) used to control Culex.

Each of the biological preparations made from Bacteria and chemical pesticides showed good efficiency in controlling, with a highly significant superiority to the chemical pesticide treatment, in which the death rate ranged between 100 and 88.66 %, compared to 73, 60, and 30% for the treatment of the biological preparation. And the chemical pesticide Metical had a death rate of 100% compared to (75, 66, 37%) for the treatment of the biological preparation. The chemical pesticide methane, in which the death rate ranged between (100, 90.66%), compared to (70.66, 55, 32%) for the treatment of the biological preparation.

The study also showed a compatibility between the biological preparation and the chemical pesticide by showing the bacteria Bacillus thuringiensis had a good ability to tolerate the chemical pesticide when they were both present in the culture medium (N.A), Nutrient agar, both individually at half and a quarter of the recommended dose. In addition to maintaining the mixture's efficiency in controlling Culex mosquitoes, the results showed superiority for the integration of the biological preparation, giving the highest death rate

The results of laboratory experiments showed that all concentrations of the treatments showed good efficacy in controlling the insect. They all affected the fourth larval stage of mosquitoes.

Keywords: *Bacillus thuringiensis*, *Culex* mosquitoes, larvae, biological control

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1-Introduction

Chemical control is the most common method for controlling harmful insects, but despite the success achieved by pesticides in this field, their use was accompanied by the emergence of a number of problems, represented by the emergence of strains resistant to the action of pesticides, in addition tothe large environmental damage resulting from the widespread use of pesticides. (1)

The most widely used biological agents are bacteria, especially *Bacillus thuringiensis*, due to their easy reproduction and growth on cheap and available culture media. (2,3).

Since the use of a biocide in the field is largely ineffective compared to the laboratory (protoxin), in addition to the climatic changes affecting the growth and reproduction of the vital factor (4). The direct use of these factors gives limited success at the field

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level. Because of this, the researchers resorted to the use of the method of integration between chemical and biological control, which enhances the opportunity to reach effective resistance to agricultural pests. This method also leads to curbing the characteristic of resistance and reducing the risks of environmental pollution (5,6).

Because of the seriousness of this insect and the possibility of its spread to larger areas, we decided to study this subject and lay scientific foundations to combat it.

2-Materials and methods of work

2 – 1. The microorganism used in the study:

Used a microorganism isolate *B. thuringiensis* that was obtained from the Department of Biology - College of Science - University of Kufa, which consists mainly of bacteria and their carrier (calcium carbonate, CaCo3). Dry powder is a commercially available preparation containing bacterial spores and protein crystals along with an inert carrier. It is usually named according to its trade name, such as WP (Wettable Powder) = Wettable powder. WG or WDG = Water-dispersible granules. The carrier in the powder may be an inert material such as clay, talc, Limestone, chalk, marble, or others (7).

2-1-1- insects used in the study

Fourth instar isolates of *Culex* mosquito larvae were obtained from the Animal Research Department of Life Sciences - College of Science - University of Kufa.

2-1-2- Chemical pesticides:-

The chemical pesticides (metical, methoprene, gentapride) were obtained from the agricultural offices. They are chemical insecticides used to control many types of harmful insects.

2-1-3- The vital preparation through:-

The biological preparation was obtained from the College of Science, which consists of bacteria and the carrier material (calcium carbonate).

2-2- Laboratory experiments:-

2-2-1- Evaluation of the efficacy of the bio-solution against the fourth larval stage of mosquitoes:-

A laboratory experiment was conducted to test the efficacy of the biological preparation against the fourth instar of the larval mosquito (8).

Where the biological solution was prepared by dissolving 10 ml of the biological preparation with a liter of distilled water, after good mixing, it was distributed on plastic dishes, at a rate of 100 ml of the biological solution, in three dishes, in addition to three dishes of the solution as a control treatment.

The larvae were distributed on the dishes so that each dish contains 20 larvae at a rate of 3 replications for the treatment of the biological preparation, and the same for the treatment of the control, with the addition of food to all dishes

The percentage of larval death was calculated after 4 days of treatment.

2-2-2- Evaluation of the efficacy of chemical pesticides against the fourth larval stage of mosquitoes:-

In this experiment, three types of chemical insecticides (Metical, Methoprene, and Gentapride) were used to test their efficacy against the fourth larval stage of mosquitoes, at a concentration of 0.5 pesticide per liter of water. This concentration was used for other pesticides as well, where the larvae were distributed on dishes, and concentrations were prepared. Of the aforementioned pesticides, the concentrations were distributed on dishes, with three replicates made for each concentration, in addition to making three dishes from the control treatment (water only without pesticide). The mortality rate was calculated after four days of treatment (9).

2-2-3-Compatibility test between chemical pesticides and biological preparation (bio-solution):-

A concentration of half the dose of the biological product was prepared, as well as the preparation of half the dose of the previously mentioned chemical pesticides; then they were mixed together and distributed on dishes in the amount of 100 ml ofthe overlap mixture(10), noting that 20 caterpillars were placed in each dish with sufficient food for their reproduction and growth.

After that, the concentration of a quarter of the dose for the biological preparation and a quarter of the dose for the three previously mentioned pesticides was prepared and distributed in the same way as before. The percentage of mortality was calculated by recording the number of dead insects after four days of treatment.

2-3-Statistical analysis

All laboratory experiments were analyzed, and the averages were compared according to the calculation of the least significant difference (L.S.D) at the level ofprobability 0.05 (11). As for the values of the percentage of mortality for homicides according to the Abbott equation (12), known as the (Schneider and Orell) equation, as follows:-

$$Perishable\ Rate\% = \frac{loss\ in\ non\ treatment\% - loss\ in\ treatment\%}{100 - Loss\ in\ non\ treatment\%}\%$$

3-Results

- Estimating the density of *Bacillus thuringiensis* in one gram of biopesticide. Theresults showed that the density of bacteria was 251×10^6 CFU/g.

- Evaluation of bacterial density against the fourth larval instar of mosquitoes

Table No. (1) The effect of *B. thuringiensis* against the fourth larval instar ofmosquitoes:-

treatment	perishability rate
Bacteria *B.thuringiensis*	40
control	4.3
L.S.D=0.35	

- Evaluation of the efficiency of chemical pesticides against the fourth larval stage of mosquitoes, as the study indicated that all used pesticides had a very high effect,as they all caused 100% death, with significant differences from the control
- treatment, which amounted to 4.3. This confirms the effectiveness of these pesticides in controlling this insect.

Table No. (2) Compatibility test between chemical pesticides and bacteria *B.thuringiensis*:-

Chemical pesticide	Concentration (gm or ml)	The density of bacteria per colony-forming unit/g	The logarithm of the colony formation unit
Gentapride	0.25	$24 * 10^6$	7.38
	0.16	$74 * 10^6$	7.86
Methoprene	0.25	$58 * 10^6$	7.76
	0.16	$61 * 10^6$	7.78
Metkol	0.25	$33 * 10^6$	7.51
	0.16	$54 * 10^6$	7.73
The control	0	$214 * 10^6$	8.33
L.S.D=3.36			

The effect of the chemical pesticide methoprene and biocontrol and their interaction in controlling the fourth larval instar:-

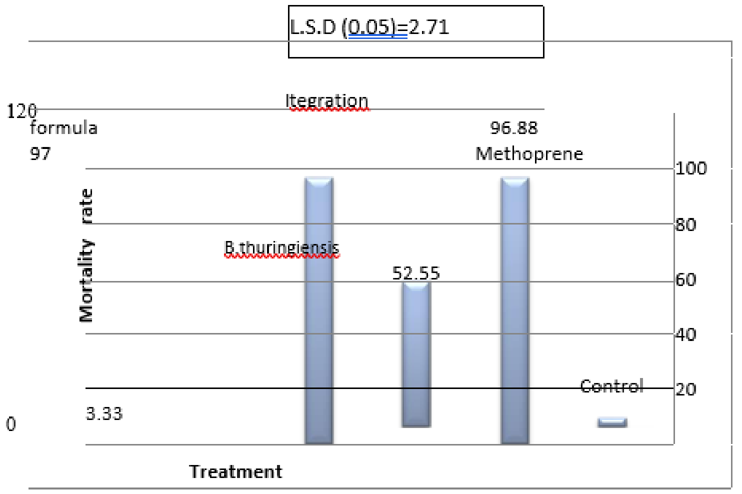

Figure No. (1) The effect of the chemical pesticide methane and bacteria and their interaction in controlling the fourth larval stage of the mosquito insect

Table No. (3) Effect of chemical concentrations of methoprene and *B. thuringiensis* pollen and their interaction on the control of the fourth larval instar of the mosquitoinsect

Treatment	the concentration	perishability rate%
Chemical Insecticide methoprene	0.5 gm/liter	100
	0.25 gm/liter	100
	0.16 gm/liter	90.66
bacteria *B.thuringiensis*	10 ml/liter	70.66
	5 ml/liter	55
	2.5 ml/liter	32
Integration formula	5 ml of bacteria +0.25 ofInsecticide	100
	2.5 ml of bacteria +0.16 ofInsecticide	94
control	0	3.33

L.S.D(0.05)=3.73

The effect of the chemical pesticide Metkoll and *the B. thuringiensis* vaccine andtheir interaction in the control of the fourth larval instar of the mosquito insect:-

In Figure 2, the results showed that the statistical analysis showed that the chemical pesticide and the complementary formula showed death rates that reached100% and with highly significant differences that differed from the treatment of bacterial vaccine and control, which amounted to (52,5)%, respectively. While the death rates increased to 100% in the integration formula, which indicatesthe compatibility between the pesticide and the bacteria

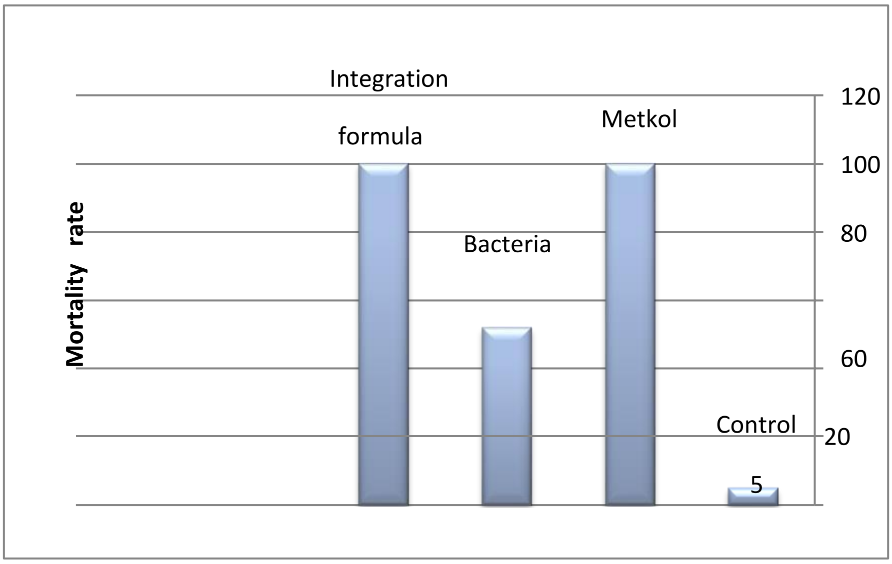

Figure No. (2) The effect of the chemical pesticide Metkol on bacteria and theirinteraction in controlling the fourth larval stage of the mosquito insect

Table (4): The effect of the concentrations of the chemical pesticide metkol and the bacterial *B.thuringiensis* and their interaction on the control of the fourth larval instar of the mosquito insect

Treatment	the concentration	perishability rate%
Chemical Insecticide Metkoll	0.5 gm/liter	100
	0.25 gm/liter	100
	0.16 gm/liter	100
bacteria *B.thuringiensis*	10 ml/liter	75
	5 ml/liter	66
	2.5 ml/liter	37
Integration formula	5 ml of bacteria +0.25 ofInsecticide	100
	2.5 ml of bacteria +0.16 ofInsecticide	100
Control	0	5

L.S.D(0.05)=3.73

Whereas the integration formula caused a mortality rate of 100% compared to thedoses of the chemical pesticide, the results of the treatment of the bacterial vaccine showed a mortality rate of 75% after four days of treatment.

The effect of concentrations of the chemical pesticide gentapride and B.thuringiensis vaccine and their interaction in the control of the fourth larvalinstar of the mosquito insect:-

In Figure 3 it is noted that there are high effects of laboratory treatments in the fourth larval stage, with high significant differences between the treatments, as the treatment of the chemical pesticide Gentapride reached 96.22% after four days of treatment, while it was followed by the treatment of the integration formula and it was 91%, which had differences Highly significant in relation to the treatment of the bacteria individually (13), which amounted to 54%, in addition to that the percentage of loss for the control treatments was 0%, so it is preferable to use the integration formula in control in order to reduce environmental pollution that results from chemical pesticides.

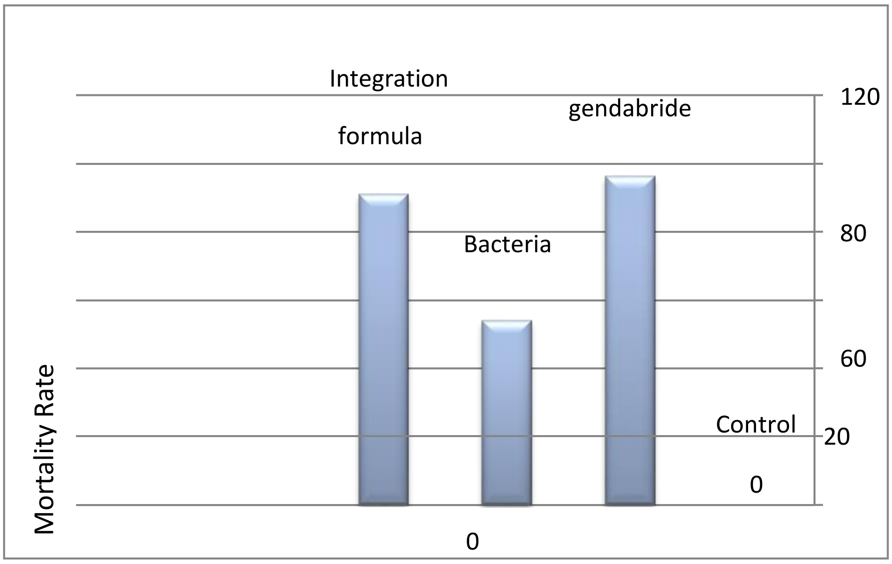

Figure No. (3) The effect of the chemical pesticide gendabride on bacteria and their interaction in controlling the fourth larval stage of the mosquito insect. L.S.D(0.05)= 3.72

Table (5): The effect of the concentrations of the chemical pesticide gendabride and the bacterial vaccine *B.thuringiensis* and their interaction on the control of the fourth larval instar of the mosquito insect

Treatment	the concentraton	perishability rate%
Chemical Insecticide gendabride	0.5 gm/liter	100
	0.25 gm / liter	100
	0.16 gm/liter	88.66
bacteria B.thuringiensis	10 ml / liter	73
	5 ml / liter	60
	2.5 ml / liter	30
Integration formula	5 ml of bacteria +0.25 ofInsecticide	100
	2.5 ml of bacteria +0.16 ofInsecticide	80
control	0	0

L.S.D(0.05)=3.73

4-discussion

The results presented in Table 1 indicate that the bacteria showed good efficiency in controlling the fourth larval stage of the mosquito insect, resulting in adeath rate of 40% after four days of treatment, compared to the control treatment, which amounted to 4.3%. These results confirm the effectiveness of the bacteria in controlling this insect.

Testing the extent of compatibility between chemical pesticides and biocontrol. Itis clear from the results of Table No. 2 that the effect of half the dose and a quarter of the recommended dose of the pesticides used in the test did not affect the growth and density of bacteria, meaning that the bacteria showed a high tolerance for these chemical pesticides, as the density of bacteria ranged between 94×10^6 and 33×10^6 compared to 214×10^6 in the control treatment

The reason for the lack of effect of the bacteria may be attributed to the inability of the bacteria cells to absorb these pesticides and then the failure to collect them inside the bacteria cells at an effective concentration, or that the bacteria can destroy the molecules of the chemical pesticide or resist the action of the pesticide (14).

In Figure 1, it indicates the presence of the highest mortality rate is indicated for the treatment (integration of the chemical pesticide Methoprene with the bacteria), if it reached 97%, followed by the pesticide treatment alone, which amounted to 96.88, with a large significant difference compared to the treatment of bacteria, which amounted to 52.55%, while the control treatment reached 3.33%

The results of the statistical analysis showed that the use of the pesticide treatment alone or the bacteria alone gave a lower mortality rate for the fourth instar larvae, while the integration formula gave the highest mortality rate, although it waswithout a significant difference from the methoprene pesticide treatment. Therefore, it is recommended to use the integration formula to control this insect, and the high mortality rate returns as a result of a kind of synergy between the bacteria and the methoprene pesticide in increasing the death rate.

In Table 3, the results showed that the interaction between the high concentrations of the used treatments played an important role in increasing the efficiency of bacteria against the fourth larval stage. Also, the interaction of methoprene concentrations with bacterial concentrations had a significant effect against the insect. It is obvious thatincreasing the concentration leads to an increase in the efficiency of the bacterial inoculum, and this applies to all Chemical and biological pesticides, but the importance of the time period after treatment is necessary in biological pesticides more than chemical pesticides because the microorganism needs a longer period of time to become effective in the pest (15).

The results showed in Table 4 that all the concentrations of the pesticide (full dose, half the dose, and a quarter of the dose) had a very high impact, and all of them caused a death rate of 100%. Among them, the pesticidesmay affect the cellular oxidation processes necessary in the process of respiration, thus forming toxic complexes that can bind to enzymes and vital compounds in the cell of the organism (16).

In Table 4, this apparent discrepancy is attributed to the fact that the chemical insecticide Metkol is highly toxic and potent on its own against insects. It possesses the capability to fully eradicate mosquito larvae (100%) even at its lowest concentration (0.16 gm/liter), without requiring any kinetic or biological support from the bacteria. Consequently, the complete mortality rate (100%) observed in the integrated formulation in Table 4 is driven by the dominant lethal efficacy of Metkol itself, which statistically and biologically masked any reduction in bacterial density caused by the chemical inhibition recorded in Table 2

We noticed in Table 5 that all concentrations of laboratory treatments have affected well in achieving death rates that ranged between (30-100)%. 1 gm of gentapride with 5 ml of the bacterial , while the rest of the concentrations caused a high mortality rate compared to the control treatment, which was 0%.

The tested insecticides (methoprene, gentapride, and metcol) differ significantly from one another in their chemical structure, active groups, and mode of action. The study deliberately used insecticides with diverse chemical backgrounds and structures to examine and evaluate which of these chemical groups possesses the best biocompatibility with the bacterial inoculum. This is crucial for determining the optimal choice and the most efficient formulation for use in combined chemo-biological control programs

Conclusions

1- The treatment of bacteria alone showed moderate ability in controlling the fourth instar larvae of the mosquito insect.

2- The used chemical pesticides showed very high efficiency in controlling thefourth larval stage of mosquitoes.

3- The possibility of using bacteria alongside chemical alkali without being affectedby pesticides.

4- The treatments of the integration formula showed a greater effect than thetreatment of bacteria alone under laboratory conditions.

Recommendations

In light of the foregoing, we recommend using the formula of integration between bacteria and one of the chemical pesticides when controlling the insect.

Conflict of interest: NIL

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References:

1-Tomlin C. The pesticide Manual. 11th ed. Bracknell, Berkshire: British Crop Protection Council; 1997.

2-Marco J, Manuel B. Environmental Puzzles: Is Bacillus thuringiensis a True Insect Pathogen? Bt Res.2012;3:1-2.

3-Heimpel AM. A critical review of Bacillus thuringiensis var. israelensis Berliner and other crystalliferous bacteria. Annu Rev Entomol. 1967;12:287-322.

4-Angus TA. A bacterial toxin paralysing silkworm larvae. Nature. 1954;173:545-6. University of California, Berkeley. Engineering - Electrical Engineering and Computer Sciences College of Engineering. Berkeley: University of California; 2001.

5-Shoaib N, Siddiqui PJA, Ali A. Acute Toxic Effects of Organophosphate Pesticides on Killifish Fish (Aphanius dispar) Juveniles. Pakistan J Zool. 2012;44:569-72.

6-Heimpel AM, Angus TA. The taxonomy of insect pathogens related to Bacillus cereus frank land and Frankland. Can J Microbiol. 1958;4:531–41.

7-Weeden CR, Shelton AM, Li Y, Hoffman MP, editors. Biological control: a guide to natural enemies in North America [Internet]. 2002 [accessed 2003 May 14]. Available from: [Insert URL here].

8-Bulla LA, Kramer KJ, Davidson LI. Characterization of the entomocidal parasporal crystal of Bacillus thuringiensis. J Bacteriol. 1977;130:375-83.

9-Schnepf E, Crickmore N, Van RJ, Lereclus D, Baum J, Feitelson J, et al. Bacillus thuringiensis and its pesticidal crystal proteins. Microbiol Mol Biol Rev. 1998;62:775–806.

10-Mohsen ZH, Ouda NA, Mehdi NS, Zaiya HH, Al-Chalabi BM. Toxicity of Various Larvicides and Formulation against Larva pupae of Culex quinquefasciatus and other non target arthropods. In: Proc Sci Conf Baghdad. 1989;2:113-15.

11-Al-Rawi KM, Khalafullah AM. Design and analysis of agricultural experiments. University of Mosul: Dar Al Kutub For Printing & Publishing; 2000.

12-Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925;18:265–7.

13-Sanchez-Yañez JM, Rico JL, Ulíbarri G. Bacillus thuringiensis (Bt) is more than a special agent for biological control of pests. J Appl Biotechnol Bioeng. 2022;9(2):33-39.

14-Bauer LS, Londono DK. Effects of Bacillus thuringiensis SDS-502 on Adult Emeraldash borer. In: Research Forum on Invasive Species. GTR-NRS-P,75; 2010.

15-Yudinaa LN, Bergman J. Synthesis and alkylation of indolo carbazoles. Huddinge: Karolinska Institute, Novum Research Park / Department of Organic Chemistry; 2003.

16-Faust PG. The δ-endotoxin of Bacillus thuringiensis DI. A rapid method for separating parasporal bodies from spores. J Invertebr Pathol. 1972;20:130-49.