



**TOXICOLOGICAL AND BIOCHEMICAL STUDIES ON SOME NON-
CONVENTIONAL INSECTICIDES AGAINST THE COTTON LEAFWORM
SPODOPTERA LITTORALIS (BOISD.)**

By

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5. SUMMARY

Laboratory experiments were conducted to evaluate the efficacy of four bioagents; two entomoparasitic nematodes (*Heterorhabditis bacteriophora* and *Steinernema carpocapsae*), the entomopathogenic fungi; *Beauveria bassiana* (Biopower, 1.4% WP) and the entomopathogenic bacteria; *Bacillus thuringiensis* (*B.t*) (Biocade, 5.5%) separately or combined by sequential treatments against the freshly molted 3rd instar larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera : Noctuidae).

The obtained results could be summarized as follows:

1. Toxicological studies:

1.1. Toxicity response of *S. littoralis* 3rd instar larvae to each of *H.*

***bacteriophora*, *S. carpocapsae*, *Bacillus thuringiensis* and *Beauveria bassiana* separately:**

The susceptibility of *S. littoralis* 3rd instar larvae to the tested pathogens, *H. bacteriophora*, *S. carpocapsae*, *Bacillus thuringiensis* (Biocade , 5.5% WP) (*B.t*) and *Beauveria bassiana* (Biopower, 1.4% WP). The LC₂₅ values were 18.15 IJs/ml, 18.34 IJs/ml, 1.26 g/L and 6.95 g/L, respectively. Also, the LC₅₀ values of the four pathogens were; 53.33 IJs/ml, 81,41 IJs/ml, 4.66 g/L and 20.08 g/L, respectively 72h post treatment.

1.2. Mortality Percentages among treated *S. littoralis* 3rd instar larvae by the tested bioagents :

Obtained data on the susceptibility of treatments by using the highest concentration from the tested bioagents against *S. littoralis* 3rd instar larvae after 72h of infection proved that *H. bacteriophora* was

the highest effective against *S.littoralis* 3rd instar larvae compared with all other tested bioagents. The mortality percentage after treatment with *H. bacteriophora* was 84.61, while those for *S. carpocapsae*, *B. thuringiensis* and *B. bassiana* were 78.96 ,76.12 and 40.76 respectively, where :

- Nematodes were applied at 200 IJs/ml.
- B.t. product (Biocide) was applied at 15 gm/l.
- Fungal product was applied at 10 gm/l.

1.3. Median lethal time values (LT₅₀'s)of the tested bioagents at the highest concentration against *S. littoralis* 3rd instar larvae:

The LT₅₀ values of the tested bioagents were tabulated with their corresponding slopes after 3,5,7 and 10 days from treatment by the tested bioagents at their highest concentrations resulted that the nematode *H .bacteriophora* caused the highest mortality in shortest time, the LT₅₀ value was 1.18 days. While LT₅₀ values for the other three bioagents were 1.70, 2.07 and 9.52 days for *S. carpocapsae*, *B. thuringiensis* and *B. bassiana* treatments, respectively.

1.4. Co-Toxicity factors and final effect of binary sequential of entomopathogenic agents against the 3rd instar larvae of *S. littoralis* using sequential method:

To determine the interaction between entomopathogenic nematode, *B.t.* (Biocide, 5.5% WP), and fungus, *B .bassiana* (Biopower) using sequential application, each pathogen at LC₂₅ level was applied firstly to *S. littoralis* 3rd instar larvae, then after 24hr the other pathogen was applied at LC₂₅ too. Data after 72 hrs from treatment indicated that the combined effect of the tested bioagents as sequential treatment differed significantly between the infection with each pathogen used separately. In the present study, all combinations of

nematode with fungi showed an increase in the host mortality and gave potentiation effect. The highest potentiative effect was observed with the combination of *B. bassiana* + *H. bacteriophora* (+87.70), that was followed by *H. bacteriophora*+*B. bassiana* (79.16) and *B. bassiana*+*S. carpocapsae*(+73.90) mixtures, *B. bassiana*+ *B. thuringiensis*(+66.16), *B. thuringiensis*+*B. bassiana* (+50.00), then *S. carpocapsae*+*B. bassiana* (+58.33) mixtures. In the present study, some of binary mixtures of nematode with bacteria showed additive effect, such as (*H. bacteriophora* + *B. thuringiensis*) (+16.66), (*S. carpocapsae*+ *B. thuringiensis*) (+1.05). While some other combinations of nematode with bacteria showed antagonistic effect, such as in case of (*B. thuringiensis*+ (*H. bacteriophora*) (-25) and (*B. thuringiensis* + *S. carpocapsae*) (-35.48).

2. Biological studies:

Obtained data showed the effect of LC₅₀ values of all the tested bioagents on *S. littoralis* 3rd instar larvae at different time intervals (2, 3, 5, 7, 9 and 12 days) post treatment. Data clearly indicated that the mortality rate among treated 3rd instar larvae of *S. littoralis* increased gradually with increasing time of exposure. The most effective bioagent was *H. bacteriophora* which caused (84%) mortality followed by; *S. carpocapsae* (80%), *B. thuringiensis* (74%) and *B. bassiana* (66%) after 12 days post treatment. There were significant differences between the tested bioagents compared with the control. On the other hand, in case of treatment with the binary mixtures of bioagents (*H. bacteriophora*+ *B. bassiana*), (*H. bacteriophora*+ *B. thuringiensis*), (*S. carpocapsae*+ *B. bassiana*), (*S. carpocapsae*+ *B. thuringiensis*), (*B. thuringiensis*+ *H. bacteriophora*), (*B. thuringiensis*+ *S. carpocapsae*), (*B. thuringiensis*+ *B. bassiana*), (*B. bassiana*+ *H. bacteriophora*), (*B. bassiana* + *S. carpocapsae*) and (*B. bassiana*+*B. thuringiensis*), the corrected

accumulative larval mortality percentages were 88, 74, 86, 70, 70, 60, 82, 90, 90 and 86%, respectively after 12 days post treatments.

All the tested bioagents caused reductions in the 6th larval instar weights in all bioagents (separately or in sequential). The 6th instar larval weight recorded (0.17, 0.16, 0.18 and 0.19 g/larva.), days after treatment by the LC₅₀ value of the tested bioagents, respectively compared to the untreated ones (0.36g/larva) and its sequences (0.16, 0.17, 0.16, 0.18, 0.17, 0.16, 0.17, 0.16, 0.16 and 0.17g/larva, respectively) compared of the untreated ones (0.38 g/larva).

Also, results showed a reduction in succeeded pupation percentages, after larval treatment by either of the bioagent separately (16, 20, 26 and 34% respectively) compared to these resulted from 92% among the untreated ones and their sequences (12, 26, 14, 30, 30, 34, 18, 10, 10% and 14%, respectively) compared to the untreated one, (92%).

Pupal weight was, also, decreased after all treatments with bioagents separately (0.16, 0.17, 0.20 and 0.19 g/pupa) compared to the untreated larvae (0.38 g/pupa), and their sequences of bioagents (0.16, 0.17, 0.16, 0.17, 0.17, 0.16, 0.17, 0.16, 0.16 and 0.17g/pupa, respectively) compared of untreated larvae (0.39 g/pupa). Adults' emergence percentages after treatment with LC₅₀ values of the tested bioagents separately were also reduced. Those recorded (16, 18, 22 and 28%, respectively for single treatments) compared to (92%) from the untreated pupae. Sequences of bioagents gave (10, 22, 10, 26, 24, 30, 12, 10, 12 and 8% emergence, respectively) compared to those from untreated larvae (92%).

3. Biochemical studies:

This part dealt with evaluation the effect of the LC₅₀ values of the four tested bioagents (separately or in sequential using the previously mentioned sequential treatments) on some biochemical

parameters of *S. littoralis* larval body homogenate 72h after treatment of the 3rd instar larvae. Total protein content and the activity of the enzymes; alkaline and acid phosphatases (ALP and ACP), Glutamate-oxaloacetate transaminase (GOT) and glutamatepyruvate transaminase (GPT).

3. 1.Total protein content:

3.1.1.Effect of treatment with LC₅₀ values of each tested bio-agent applied alone on total protein content:

The results, indicated significant share reductions in total protein content of *S. littoralis* 3rd instar larvae, after 72h post-infection with each bio-agent separately compared to control. The highest decrease was recorded in case of infection by *H. bacteriophora*, followed by *S. carpocapsae*, *B. thuringiensis* and *B. bassiana* with percentages of change -53.70, -40.73,-35.57 and -30.12%, than control.

3.1.2.Effect of treatment with binary sequences of the tested bioagents on total protein content:

The results indicated significant reductions in the total protein content of *S. littoralis* larvae treated with the previously indicated binary sequences of the tested bioagents after 72h compared with the control with percentages of change (-90.70,-91.10,-90.82,-90.94,-11.88,-11.88,-91.19,-90.31,-90.07 and -90.27), respectively compared with control.

3.2.Transaminase activities (GOT & GPT):

3.2.1. Effect of treatment with LC₅₀ of each tested bio-agent separately:

Results of Transaminase activities (GOT & GPT) indicated that infection by *H. bacteriophora*, *S. carpocapsae*, *B. thuringiensis* and *B. bassiana* , significantly, decreased the activity of GOT of 3rd instar

larvae of *S. littoralis* by percentages of change (-36.89, -29.60,-20.04 and -14.37%) and GPT (-50.98 , -44.48 , -18.38 and -32.60%), respectively as comparison with the control.

3.2.2. Effect of treatment with binary sequences of the tested bioagents on GOT and GPT:

Obtained results showed significant reductions in GOT and GPT activities of *S. littoralis* 3rd instar larvae treated with the mentioned binary sequences of bioagents than the control by percentages of change (39.04, -4.86, -23.39, -4.64, 3.95, -6.62, -30.23, -28.73, -34.18 and 23.13%) but it increases in case of sequences (*B. thuringiensis*+*H. bacteriophora*) (3.95), and (-35.43, -5.55,-25.51,-2.45,1.10, -0.025, -15.16, -39.96, -33.12 and-23.03%), respectively, but it increased in case of sequences (*B. thuringiensis*+*H. bacteriophora*) (1.10 % than control).

3.3. Alkaline and acid phosphatase activities:

3.2.1. Effect of treatment with LC₅₀ of each tested bio-agent separately:

The activity of alkaline and acid phosphatases, significantly, increased in the larvae treated with *H. bacteriophora*, *S. carpocapsae*, *B. thuringiensis* and *B. bassiana* by percentages of change (188.7, 303.9, 28.8 and 29.3%) and (81.33, 140.80 , 11.42 and 39.41%), respectively as compared to the control.

3.3.2. Effect of treatment with binary sequences of the tested bioagents:

The activity of Alkaline and acid phosphatases of *S. littoralis* 3rd instar larvae after treatment with the aforementioned binary sequences of the tested bioagents, showed significant increases compared with the control by (62.97, 10.97, 10.97, 21.52, 5.16, -5.16, 48.11, 72.65, 68.46 and 65.87%) but it decreased in case of sequences (*B. thuringiensis*+*S. carpocapsae*) (-5.16), and (72.20, 9.74, 9.74, 31.52, -6.13, -13.11, 39.71, 57.28, 57.14 and 31.52%, respectively).