Interaction Between Entomopathogenic Nematodes and Bacillus thuringiensis as a New Approach for Biological Control of Some Lepidopterous Pests


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Abstract: The obtained data indicate that tested larval instars of Spodoptera littoralis and P. xylostella were susceptible to all tested steinernematid and heterorhabditid nematodes. However, the degree of susceptibility differed in levels according to instar and nematode species. The 2nd larval instar of S. littoralis seemed more susceptible to the tested Bacillus thuringiensis var. aizawai than the 5th one. As well, the 2nd larval instar of P. xylostella was more susceptible than the 3rd one. In other words, younger larval instars appeared more sensitive to B.t.a. than older ones. The combination of S. carpocapsae All and B. thuringiensis var. aizawai at higher concentration levels against 2nd and 5th instar larvae of S. littoralis killed as many larvae as expected assuming independent action of the two mortality agents; thus, yielding an additive interaction. The above mentioned combination against the 2nd and 3rd larval instar of P. xylostella was significantly less effective than expected, assuming independent action and indicating an antagonistic interaction. This also occurred in the combined application of H. bacteriophora HP88 against S. littoralis (2nd and 5th larval instars) and P. xylostella (2nd and 3rd larval instars).

Key words: steinernematid, heterorhabditid, B. Thuringiensis, aizawai, Plutella xylostella, Spodoptera littoralis, Steinernema carpocapsae Heterorhabdittis Bacteriophora, combination, antagonistic.

INTRODUCTION

Entomopathogenic nematodes in the families steinernematidae and heterorhabditidae are soil inhabiting insect pathogens that possess potential as biological control agents[6,18,19]. Most biocontrol agents require days or weeks to kill their hosts, yet nematodes, working with their symbiotic bacteria, kill insects in 24-48 hr. The non-feeding infective Juvenile seeks out insect hosts; when a host has been located, the nematode penetrates into the insect body, usually through natural body openings (mouth, anus, spiracles) or areas of thin cuticle. Once in the body cavity, a symbiotic bacterium (Xenorhabdus for steinernematids and Photorhabdus for heterorhabditids) is released from the nematode, multiplies rapidly and causes rapid insect death. The nematodes feed upon the bacteria and liquefying insect; and mature into adults. Thus, entomopathogenic nematodes are a nematode-bacterium complex.

On the other hand, the entomopathogenic bacterium, Bacillus thuringiensis is also one of the most commonly used biological control agent proving its efficacy against many lepidopterous species with no adverse effect on beneficial species[6,18,19]. Burgerjon and Martouret[3] demonstrated that the susceptibility to Bacillus thuringiensis infection is dependent upon two main factors; firstly, factors related to the insect species such as the degree of the pH in both foregut and midgut and action of proteolytic enzymes. Secondly, factors associated with the bacterial strains; in addition to the role played by the midgut microflora which can not be ignored.

The idea of contaminating nematode infective juveniles with Bacillus thuringiensis may help to provide another pathway for Bacillus thuringiensis spores to reach the insect haemocoel avoiding any other obstacle preventing this process when ingested. In addition, two pathogens inside the insect haemocoel may result better control results. Entomopathogenic nematodes are compatible with B. thuringiensis and can be combined in single application against a complex of foliar and soil pests[14]. Therefore, the present study was carried out to evaluate the possibility of using entomopathogenic nematodes and Bacillus thuringiensis and their combinations as biological control agents.

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against the most destructive pests of cabbage; *Spodoptera littoralis* and *Plutella xylostella*.

**MATERIALS AND METHODS**

1- **Target Insects:** The original stock culture of *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae) was obtained from Federal Biological Research Centre for Agriculture and Forestry Institute for Biological Control in Darmstadt, Germany. The original stock culture of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) was obtained from Bayer AG Pflanzenschutzzentrum (Plant Protection Department) Monheim, Leverkusen, Entomology Lab, Germany and both were maintained at the Applied Entomology Department Lab, Institute for Phyto medicine, Hohenheim University, Stuttgart, Germany.

2- **Tested Nematodes:** Following is tested entomopathogenic nematodes, their geographical origin and their reference: *Heterorhabditis bacteriophora* HP88, Utah, USA, Randy Gaugler, Rutgers University, New Brunswick, NJ, USA. and *Steinernema carpocapsae* All, California, USA. Ramon Georgis, Biosys, Palo Alto, CA, USA.

3- **Tested B.t.:** *Bacillus thuringiensis* var. *aizawai* was used as commercial formulation (Xentari).

4- **Nematode Treatment:** The tested nematodes were suspended in distilled water to obtain the desired concentrations. Cabbage leaf disks (6 cm diameter) were cut from plants grown in the green house. They were placed in 9 cm diameter × 4.5 cm high plastic boxes lined with filter paper. Leaf disks were sprayed with 1 ml (0.5 ml/side) of different concentrations of a nematode suspension. A handheld aerosol sprayer was used to apply the spray. Sprayed cabbage leaves were left for several minutes to avoid water condensation. From three to ten larvae (according to the test insect and larval instar) with uniform size of each test insect (2nd and 5th larval larvae of *Spodoptera littoralis* and 2nd and 3rd larval larvae of *Plutella xylostella*) were placed on each leaf disk. Five replicates for each test were performed for each tested inoculum level. Insect larvae were allowed to feed on the sprayed leaves for 24 hr before they were transferred to untreated leaves. The untreated leaf disks (control) received 1 ml (0.5 ml/side) distilled water only. The treated and untreated replicates were incubated under constant conditions of 25±1°C and 65-70 % RH. Mortality counts were recorded 24 hr. after treatment. Then, the survivors were transferred into new clean boxes containing fresh untreated cabbage plant leaves and kept under the same conditions. Mortality counts were recorded on the untreated cabbage plant leaves 2, 4, 6 and 8 days for *Spodoptera littoralis* but 2 and 4 days for *Plutella xylostella* after treatment. Percent mortality was calculated for each concentration level. The mortality data were subjected to statistical analysis, where probit analysis was used and probit analysis was used for determining the LC_{50} and slope (b) values as well mortality data of inoculum level (250 IJs /ml) were subjected to probit analysis; and then time-mortality relation was calculated by Linear regression using Excel 2000. The time taken to kill 50% of the insects was determined for each nematode on each tested insect species.

5- **B. Thuringiensis Treatment:** The tested formulation was suspended in water at the desired concentration and then Tween 20 was added as a wetting agent to ensure the proper dispersal of bacteria. Cabbage leaf disks (6 cm diameter) were cut from plants grown in the green house. They were placed in plastic boxes (9 cm diameter × 4.5 cm high) lined with filter paper. Each leaf disk was sprayed with one ml (0.5 ml/side) of each concentration suspension. Concentration levels representing suitable toxicity range were used.

A handheld aerosol sprayer was used to apply the spray. Sprayed cabbage leaves were air dried before being offered to the larvae. From five to ten larvae (according to the test insect and larval instar) with uniform size of each test larval instar (2nd and 5th larval larvae of *Spodoptera littoralis* and 2nd and 3rd larval larvae of *Plutella xylostella*) were placed on each leaf disk. Six and /or five replicates for each test were used for each concentration level. The untreated leaf disks received 1 ml (0.5 ml/side) water plus Tween 20 were used as control. The treated and untreated replicates were incubated under constant conditions of 25±1°C and 65-70 % RH.

Mortality counts were recorded 24 hr. after treatment. Then, the survivors were transferred into new clean boxes containing fresh untreated cabbage plant leaves and kept under the same conditions. Mortality counts were recorded on the untreated cabbage plant leaves 2, 4, 6 and 8 days for *Spodoptera littoralis* but 2 and 4 days for *Plutella xylostella* after treatment. Percent mortality was calculated for each concentration level.

The mortality data were subjected to statistical analysis, where probit analysis was used and probit analysis was used for determining the LC_{50} and slope (b) values as well mortality data of inoculum level (250 IJs /ml) were subjected to probit analysis; and then time-mortality relation was calculated by Linear regression using Excel 2000. The time taken to kill 50% of the insects was determined for each tested insect species at one inoculum level (150 μg /ml).

6- **Combined Treatment:** These experiments were aimed at the check for synergism or antagonism between nematodes and *B. thuringiensis* in combined treatment. The tested nematode strains, *Steinernema carpocapsae* (All) and *Heterorhabditis bacteriophora* (HP88) were suspended in distilled water at the desired...
concentrations. As well, *Bacillus thuringiensis* var. *aizawai* was suspended in water at the desired concentrations; and Tween 20 was added as a wetting agent. Cabbage leaf disks (6 cm diameter) were cut from plants grown in the green house. They were placed in plastic boxes (9 cm diameter × 4.5 cm high) lined with filter paper.

Leaf disks were sprayed with a mixture of the bacterium and each nematode strain suspension separately, half ml each. Sprayed cabbage leaves were left for several minutes to avoid water condensation. Five larvae with uniform size of each tested larval instar (2nd and 5th instar larvae of *Spodoptera littoralis* and 2nd and 3rd instar larvae of *Plutella xylostella* ) were placed on each leaf disk. From three to five replicates were used for each concentration level. The untreated leaf disks received 1 ml water plus Tween 20. The treated and untreated replicates were incubated under constant conditions of 25±1°C and 65-70% RH. Mortality counts were recorded and statistically analysed as mentioned before.

Then, synergistic, antagonistic or additive interaction between nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) and *B. thuringiensis* var. *aizawai* were checked. Benz equation\(^{20}\) was used to calculate the expected mortality in the combined treatment using the observed mortality in the separate treatments and then compared to the observed percentage mortality in the combined treatment.

\[
\text{Expected mortality} = \frac{\text{WG}_A + \text{WG}_B - (\frac{\text{WG}_A}{10} \times \text{WG}_B)}{10} \\
\text{A&B = Treatments.}
\]

When observed mortality in joined action is > expected mortality, that means, synergistic interaction occurred between the two pathogens.

Observed mortality < expected mortality, i.e. antagonistic interaction.
Observed mortality = expected mortality, i.e. additive interaction.

### 7- Statistical Analysis:
Mortality data were subjected to probit analysis in order to determine the LC\(_{50}\) and slope (b) values\(^{28}\). Time-mortality relation was calculated by Linear regression, using Excel 2000; and the time taken to kill 50% of the insects (LT\(_{50}\)) was determined.

### RESULTS AND DISCUSSIONS

**Nematode Treatment:** With regard to the 2nd larval instar of *S. littoralis*, data given (Table 1) show that the lowest dosage (50 Ijs/ml) of *S. carpocapsae* All caused 100 % mortality. LC\(_{50}\) value < 13 Ijs/ml and lethal time of 50 % mortality (LT\(_{50}\)) < 24 hours at concentration 250 Ijs/ml; but mortality due to *H. bacteriophora* HP88 infection ranged between 16.7 and 100 % using concentrations between 50 and 2000 Ijs/ml within 5 days. LC\(_{50}\) value was 110.69 Ijs/ml; LT\(_{50}\) value was 63.9 hours. With respect to the 5th larval instar of the same insect species; *S. carpocapsae* All at the lowest dosage (50 Ijs/ml) and the highest dosage (250 Ijs/ml) caused mortality percentages 86.7 and 100 %, respectively, within 5 days. Value of LC\(_{50}\) was 13.36 Ijs/ml. LT\(_{50}\) recorded 31.8 hours; while mortality percentages due to *H. bacteriophora* HP88 ranged between 33.3 and 100 % using concentrations between 50 and 1000 Ijs/ml within 5 days. LC\(_{50}\) value was 72.92 Ijs/ml. LT\(_{50}\) value was 92.3 hours.

As shown in (Table 1), *Steinernema carpocapsae* All was more virulent and faster in action to the 2nd larval instar of *P. xylostella* than the 3rd one. Mortality of 2nd instar larvae was between 68.0 and 100 % using concentrations between 50 and 250 Ijs/ml. After five days, the LC\(_{50}\) value was 33.79 Ijs/ml; while LT\(_{50}\) was 20.5 hours at 250 Ijs/ml. The 3rd instar larvae showed mortality percentages between 60.0 and 100 % for same concentrations. LC\(_{50}\) value was 44.33 Ijs/ml. LT\(_{50}\) recorded 23.0 hours. Also, *Heterorhabditis bacteriophora* HP88 was more virulent and faster in action to the 2nd instar larvae of *P. xylostella* than the 3rd ones. Mortality among 2nd instar larvae was between 32.0 and 76.0 % at concentrations between 50 and 500 Ijs/ml. After 5 days, the LC\(_{50}\) value was 114.84 Ijs/ml; while LT\(_{50}\) value was 56.3 hours at 250 Ijs/ml. The 3rd instar larvae showed mortality percentages between 20.0 and 84.0 % at concentrations between 50 and 2000 Ijs/ml. LC\(_{50}\) value was 254.61 Ijs/ml. LT\(_{50}\) value was 74.2 hours.

**B. Thuringiensis Treatment:** Data presented (Table 2) showed that the lowest concentration (50 µg/ml) did not induce any mortality to the 2nd larval instar of *S.littoralis* throughout the period of experiment; as well, the low concentrations (50 and 100 µg /ml) to the 5th larval instar. It was also noticed that *B. thuringiensis* var. *aizawai* had no effect within 24 hours on both 2nd and 5th instar larvae of *S. littoralis*. After 2 days, the larval mortality percentages ranged from 26.7-70.0% and 10.0-16.7% in the 2nd and 5th instar larvae, respectively, at concentrations 150-600 µg/ml. After 8 days, the mortality percentages increased to reach 63.3 -100% and 33.3 -76.7%, respectively, at the same concentrations. The highest concentration (600µg/ml) caused 100 and 73.3 % mortality within 4 days in both 2nd and 5th larval instars, respectively. Values of LC\(_{50}\) were 123.39 and
Table 1: Efficacy of S. carposcaceae All and H. bacteriophora HP88 against (2nd & 5th) and (2nd & 3rd) instar larvae of Spodoptera littoralis and Plutella xylostella, respectively.

<table>
<thead>
<tr>
<th>Conc. (IJs/ml)</th>
<th>Spodoptera littoralis</th>
<th>Plutella xylostella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd</td>
<td>5th</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>HP88</td>
<td>HP88</td>
</tr>
<tr>
<td>50</td>
<td>100.0</td>
<td>16.7</td>
</tr>
<tr>
<td>100</td>
<td>100.0</td>
<td>50.0</td>
</tr>
<tr>
<td>250</td>
<td>100.0</td>
<td>83.4</td>
</tr>
<tr>
<td>500</td>
<td>-</td>
<td>94.4</td>
</tr>
<tr>
<td>1000</td>
<td>-</td>
<td>94.5</td>
</tr>
<tr>
<td>2000</td>
<td>-</td>
<td>100.0</td>
</tr>
<tr>
<td>LC_{50} (IJs/ml)</td>
<td>&lt;13</td>
<td>110.69</td>
</tr>
<tr>
<td>LT_{50} (hours)*</td>
<td>&lt;24</td>
<td>63.9</td>
</tr>
</tbody>
</table>

All = Steinernema carposcaceae All; HP88 = Heterorhabditis bacteriophora HP88
Control mortality was zero % throughout the period of experiment. (-) not tested.

(* ) LT_{50} at concentration 250 IJs/ml.

Table 2: Susceptibility of 2nd and 5th instar larvae of Spodoptera littoralis to different concentrations of Bacillus thuringiensis var. aizawai.

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd</td>
<td>5th</td>
<td>2nd</td>
<td>5th</td>
<td>2nd</td>
</tr>
<tr>
<td>50</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>150</td>
<td>0.0</td>
<td>0.0</td>
<td>26.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>300</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
</tr>
<tr>
<td>600</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Control mortality was zero % throughout the period of experiment.
LT_{50} at concentration 150 µg/ml.

249.01 µg/ml; while LT_{50} values recorded 123.9 and 185.6 hours, respectively, indicating that the 5th instar larva was much less susceptible than the 2nd one to B. t.a.

The obtained (Table 3), revealed that most of larval mortalities of P. xylostella occurred after 24 hours; then increased slightly and gradually till the 4th day. Mortality readings; however, remained almost constant from the 1st to the 5th day at high concentrations. For both larval instars, mortality was concentration dependent. The lower concentrations (1-5 µg/ml) showed mortality percentages ranging from 4.0-28.0% and 0.0-12.0% within 24 hours; increased to 20.0-60.0% and 20.0-56.0% after 4 days for the 2nd and 3rd larval instars, respectively. On the other hand, the higher concentrations (10-150 µg/ml) showed mortality percentage ranging from 28.0-92.0% and 16.0-88.0% within 24 hours. After 2 days, the mortality increased to reach 80.0-96.0% and 64.0-92.0% for both 2nd and 3rd larval instars of P. xylostella, respectively, with no more mortality till the 4th day. LC_{50} values were 4.59 and 6.20 µg/ml; LT_{50} values were 9.3 and 12.9 hours, respectively, at 150 µg/ml, also indicating that the younger larval instar was more susceptible than the older one.

In general, for both species the results reveal that most of larval mortalities occurred after 48 hours. Mortality then increased slightly and gradually. In some cases, however, mortality readings remained almost unchanged. This indicates that young larvae which escaped from the rapid death by endotoxins, later on suffered from starvation due to the possible paralysis of
the gut muscles, one of the effects known for B.t. Furthermore, surviving larvae that fed on treated food appeared dwarfed and much smaller in size than those of the untreated control. Therefore, longer exposure and higher amount of ingested contaminated food are needed to show more effect. Felke and langenbruch[7] recorded that larvae of P. rapae, P. brassicae and P. xylostella showed significant mortality, fed less and grew more slowly than larvae of untreated control group.

The 5th instar larvae of S. littoralis seem to be much less susceptible to the tested bacteria at concentrations that have previously proved to be effective against the 2nd larval instar. As well, the 2nd larval instar of P. xylostella was more susceptible than the 3rd one. These results confirm the fact that the younger larval instar of S. littoralis should be the target of bacterial control, and the fact that increasing the bacterial concentration is necessary to obtain satisfactory larval mortality. Hassan *et al.*[11] proved that Helicoverpa armigera first instar larvae were more susceptible to various B.t. preparations. Felke and langenbruch[7] added that within one species, older and therefore also bigger larvae showed a higher tolerance against B.t. toxin than younger ones.

**Combined Treatment:** Mortality percentages among 2nd instar larvae of S. littoralis due to the combined application of S. carpocapsae All and B. thuringiensis var. aizawai were between 52.0 and 96.0% at concentrations between (50 IJs + 50 µg/ml) and (250 IJs + 150 µg/ml) within 2 days post-treatment (Table 4). After 4 days, the mortality increased to reach 88.0 and 100% for the same concentrations; where mortality readings remained unchanged till the 8th day. The higher concentration (500 IJs + 300 µg/ml) caused 100% mortality within 2 days. It was also noticed that the combined application was faster in action, it induced mortality percentages between 24.0 and 68.0% after 24 hours at concentrations between 100 IJs + 100 µg/ml and 1000 IJs/ ml + 600 µg/ml, respectively.

LC50 values were 4.52 IJs /ml and 12.71 µg/ml and lethal time of 50 % (LT50) recorded 24.7 hours; while the calculated LC50 values for S. carpocapsae All and B.t.a. due to the independent action of the two pathogenic agents to 2nd larval instar of S. littoralis were < 13 IJs/ml and 123.39 µg/ml and LT50 values were < 24 and 123.9 hours , respectively, indicating a considerable improvement in the efficacy of B.t.a. applied in combination with S. carpocapsae All nematode over that of B.t.a. used alone; while it did not induce any increase in the efficacy of S. carpocapsae All nematode applied in combination with B.t.a. over that of nematode used alone. Moreover, the combined application (i.e. observed mortality) at the lower concentrations (50 IJs/ ml + 50µg/ml and 100 IJs/ml + 100µg/ml) was less effective than the expected mortality, indicating an antagonistic interaction. However, the combined application at the higher concentrations killed as many larvae as expected assuming independent action of the two mortality agents; yielding an additive interaction (Table 4).

On the other hand, the percentage of mortality due to the combined application of H. bacteriophora HP88 and B. thuringiensis var. aizawai was between 13.3 and 40.0% at concentrations between (50 IJs/ml +50 µg/ml) and (1000 IJs/ml + 600 µg/ml) within 48 hours (Table 4). After 6 days, the mortality remained constant (13.3) at lower concentration, but increased to reach 86.7 % at the highest concentration (1000 IJs/ml +600 µg/ml); with no more mortality till the 8th day. It was also noticed that the combined application showed no
Table 4: Expected and observed percentage of mortality of 2nd larval instar of *S. littoralis* treated with *S. carpocapsae* All and *H. bacteriophora* HP88 each combined with *B. thuringiensis* var. *aizawai* at different concentrations.

<table>
<thead>
<tr>
<th>Conc. (lJ/ml+µg/ml)</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
<th>antagonist</th>
<th>additive</th>
<th>Synergistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>50+50</td>
<td>0.0</td>
<td>0.0</td>
<td>52.0</td>
<td>13.3</td>
<td>88.0</td>
<td>13.3</td>
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<tr>
<td>100+100</td>
<td>24.0</td>
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<td>92.0</td>
<td>20.0</td>
<td>92.0</td>
<td>26.7</td>
<td>92.0</td>
<td>26.7</td>
</tr>
<tr>
<td>250+150</td>
<td>36.0</td>
<td>0.0</td>
<td>96.0</td>
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<tr>
<td>500+300</td>
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<td>33.3</td>
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<td>53.3</td>
<td>100</td>
<td>60.0</td>
</tr>
<tr>
<td>1000+600</td>
<td>68.0</td>
<td>0.0</td>
<td>100</td>
<td>40.0</td>
<td>100</td>
<td>73.3</td>
<td>100</td>
<td>86.7</td>
</tr>
</tbody>
</table>

Control mortality was zero % throughout the period of experiment.

LT<sub>50</sub> at concentration 250 lJ/ml +150 µg/ml.

Table 5: Expected and observed percentage of mortality of 5<sup>th</sup> larval instar of *S. littoralis* treated with *S. carpocapsae* All and *H. bacteriophora* HP88 each combined with *B. thuringiensis* var. *aizawai* at different concentrations.

<table>
<thead>
<tr>
<th>Conc. (lJ/ml+µg/ml)</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
<th>antagonist</th>
<th>additive</th>
<th>Synergistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>50+150</td>
<td>13.3</td>
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<td>66.6</td>
<td>20.0</td>
<td>73.3</td>
<td>33.3</td>
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</tr>
<tr>
<td>100+300</td>
<td>20.0</td>
<td>0.0</td>
<td>86.7</td>
<td>26.7</td>
<td>86.7</td>
<td>46.7</td>
<td>86.7</td>
<td>46.7</td>
</tr>
<tr>
<td>250+600</td>
<td>60.0</td>
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<td>93.3</td>
<td>46.7</td>
<td>100</td>
<td>80.0</td>
<td>100</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Control mortality was zero % throughout the period of experiment.

LT<sub>50</sub> at concentration 250 lJ/ml +600 µg/ml.

effect within the first 24 hours.

Values of LC<sub>50</sub> were 251.94 lJ/ml and 179.95 µg/ml and LT<sub>50</sub> was 89.9 hours; while LC<sub>50</sub> values of *H. bacteriophora* HP88 and *B.t.a.* calculated from the results of independent treatments recorded 110.69 lJ/ml and 123.39 µg/ml, respectively. LT<sub>50</sub> values recorded 63.9 and 123.9 hours, respectively. Comparing these values, it could be concluded that an apparent decrease in efficacy of the combination treatment was evident from that of nematode and bacteria when each was used alone. Moreover, the observed mortality was less than the expected mortality, indicating an antagonistic interaction (Table 4).

With respect to the 5<sup>th</sup> instar larvae of *S. littoralis*, data (Table 5) show that the combined application of *S. carpocapsae* All and *B. thuringiensis* var. *aizawai* caused mortality percentages between 66.6 and 93.3 % at concentrations between (50 lJ/ml+150 µg/ml) and (250 lJ/ml+ 600 µg/ml) after 2 days post-treatment. After 4 days, the mortality increased to reach 73.3 and 100% at the same concentrations; with no more mortality till the 8<sup>th</sup> day. As well, it was noticed that the combined application was faster in action, it caused mortality percentages between 13.3 and 60.0% within 24 hours at the above mentioned concentrations.
Table 6: Expected and observed percentage of mortality of 2nd larval instar of *P. xylostella* treated with *S. carpocapsae* All and *H. bacteriophora* HP88 each combined with *B. thuringiensis* var. *aizawai* at different concentrations.

<table>
<thead>
<tr>
<th>Conc. (ljs/ml + µg/ml)</th>
<th>Cumulative mean mortality % at the indicated days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td>All + B.t.</td>
</tr>
<tr>
<td>50 + 1</td>
<td>0.0</td>
</tr>
<tr>
<td>100 + 3</td>
<td>0.0</td>
</tr>
<tr>
<td>250 + 5</td>
<td>0.0</td>
</tr>
<tr>
<td>500 + 10</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Control mortality was zero % throughout the period of experiment. LT50 at concentration 250 ljs/ml + 5 µg/ml.

Values of LC50 were 27.27 ljs/ml and 86.76 µg/ml and LT50 was 18.0 hours. The calculated LC50 values for *S. carpocapsae* All and *B. thuringiensis* var. *aizawai* due to the independent action of the two pathogenic agents to the 5th larval instar of *S. littoralis* were 13.36 ljs/ml and 249.01 µg/ml and LC50 values were 31.8 and 185.6 hours, respectively. Comparing these values, it could be concluded that the data show an improvement in the efficacy of *B. thuringiensis* applied in combination with *S. carpocapsae* All over that of *B. thuringiensis* used alone; while it did not indicate any improvement in the medium lethal concentrations (LC50) of nematode applied in combination with *B. thuringiensis* over that of nematode used alone. In addition, the combined application at the lower concentrations was less effective than the expected mortality indicating an antagonistic interaction; but at the highest concentration, it killed as many larvae as expected assuming independent action of the two agents; showing an additive interaction (Table 5).

On using the combination of *H. bacteriophora* HP88 and *B. thuringiensis* var. *aizawai*, data obtained (Table 5) showed mortality percentages ranging between 20.0 and 46.7% within 2 days at concentrations between (50 ljs/ml + 1µg/ml) and (250 ljs/ml + 600 µg/ml). After 4 days, the mortality increased to reach 33.3 and 80.0% at the same concentrations, respectively; with no more mortality till the 8th day. The combined application had no effect within 24 hours.

Values of LC50 were 94.76 ljs/ml and 269.08 µg/ml and LT50 recorded 52.1 hours. Whereas, LC50 values of *H. bacteriophora* HP88 and *B. thuringiensis* var. *aizawai* calculated from the results of independent treatments to the 5th larval instar of *S. littoralis* were 72.92 ljs/ml and 249.01 µg/ml; and LT50 values recorded 92.3 and 185.6 hours, respectively. The data did not show any improvement in the LC50 of the combined application over that of nematode and bacteria when each was used alone; and the observed mortality was less than the expected mortality, showing an antagonistic interaction (Table 5).

Concerning the 2nd larval instar of *Plutella xylostella*, the obtained data (Table 6) show that combined application of *S. carpocapsae* All and *B. thuringiensis* var. *aizawai* induced mortality percentages between 20.0 and 46.7% at concentrations between (50 ljs/ml + 1µg/ml) and (500 ljs/ml + 10 µg/ml) within 2 days with maximum levels of infection (40.0-80.0%) occurring after 4 days. The combination did not show any effect within 24 hours. LC50 values were 93.17 ljs/ml and 2.11 µg/ml and LT50 was 82.3 hours; while the calculated LC50 values of *S. carpocapsae* All and *B. thuringiensis* var. *aizawai* introduced mortality percentages between 20.0 and 46.7% at each concentration (50 ljs/ml + 1µg/ml) and (500 ljs/ml + 10 µg/ml) within 2 days with maximum levels of infection (40.0-80.0%) occurring after 4 days. The combined application did not show any effect within 24 hours. LC50 values were 93.17 ljs/ml and 2.11 µg/ml and LT50 was 82.3 hours; while the calculated LC50 values of *S. carpocapsae* All and *B. thuringiensis* var. *aizawai* introduced mortality percentages between 20.0 and 46.7% at each concentration (50 ljs/ml + 1µg/ml) and (500 ljs/ml + 10 µg/ml) within 2 days with maximum levels of infection (40.0-80.0%) occurring after 4 days. The combination did not show any effect within 24 hours.
Table 7: Expected and observed percentage of mortality of 3rd larval instar of P. xylostella treated with S. carpocapsae All and H. bacteriophora HP88 each combined with B. thuringiensis var. aizawai at different concentrations.

<table>
<thead>
<tr>
<th>Conc. (IJS/ml+μg/ml)</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>LC₅₀ (μg/ml)</th>
<th>LT₅₀ (hours)</th>
<th>antagonistic</th>
<th>additive</th>
<th>Synergistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>All + HP88 + B.t.a.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>All + HP88 + B.t.a.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>All + HP88 + B.t.a.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>All + HP88 + B.t.a.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Control mortality was zero % throughout the period of experiment. LT₅₀ at concentration 250 IJS/ml +5 μg/ml.

This also occurred on using the combination of H. bacteriophora HP88 and B. thuringiensis H. bacteriophora HP88 and B.t.a. against the same larval instar of P. xylostella (Table 6). In this case, the lowest concentrations (50 ljs/ml +1 μg/ml and (100 ljs/ml +3 μg/ml) had no effect within 2 days; while they showed mortality percentages 13.3 and 20.0% after 4 days. The higher concentrations (250 ljs/ml +5 μg/ml and (500 ljs/ml +10 μg/ml) caused 13.3 and 33.3% mortality within 2 days, increased to 40.0 and 60.0%, respectively, after 4 days. It was also observed that the combination had no effect within 24 hours. Values of LC₅₀ were 354.90 ljs/ml and 7.68 μg/ml and LT₅₀ recorded 55.0 hours; while LC₅₀ values of H. bacteriophora HP88 and B.t.a. calculated from the results of independent treatments were 114.84 ljs/ml and 4.59 μg/ml and LT₅₀ values were 56.3 and 9.3 hours, respectively.

Regarding the 3rd larval instar of P. xylostella, data (Table 7) revealed that the combined application of S. carpocapsae All and H. bacteriophora HP88 each with B. thuringiensis var. aizawai showed no effect within 24 hours except at the concentration level of (500 ljs/ml +10 μg/ml) where the combination of S. carpocapsae All and B.t.a. induced 13.3% mortality. After 2 days, the combined application of S. carpocapsae All and B.t.a. caused mortality percentages ranging from 20.0 to 53.3% at concentrations between 50 ljs/ml +1μg/ml and 500 ljs/ml +10 μg/ml. After 4 days, the mortality increased to reach 40.0 and 80.0% at the same concentrations.

On the other hand, the lowest concentration (50 ljs/ml+1 μg/ml) of the other combination (H. bacteriophora HP88 and B.t.a. did not induce any mortality throughout the period of experiment (4 days). After 4 days, the mortality percentages ranged between 13.3 and 46.7% at concentrations between 100 ljs/ml +3 μg/ml and 500 ljs/ml +10 μg/ml. The highest concentration (500 ljs/ml +10 μg/ml) caused 20.0% mortality within 2 days.

Values of LC₅₀ of the combination of S. carpocapsae All and B.t.a. were 105.38 ljs/ml and 2.35 μg/ml and LT₅₀ was 78.2 hours; while the calculated LC₅₀ values due to the independent action of the two pathogenic agents to the 3rd larval instar of P. xylostella were 44.33 ljs/ml and 6.20 μg/ml; and LT₅₀ values were 23.0 and 12.9 hours, respectively. Comparing these values, it could be concluded that there was no improvement in the efficiency of the combination treatment over that of nematode and bacteria when each was used alone. Moreover, the observed mortality was less than the expected mortality, indicating an antagonistic interaction.

As well, on using the combination of H. bacteriophora HP88 and B. thuringiensis var. aizawai against the same larval instar of P. xylostella (Table 7) the values of LC₅₀ were 541.81 ljs/ml and 10.31μg/ml and LT₅₀ was > 96 hours. LC₅₀ values due to the independent action of the two pathogenic agents to the 3rd larval instar of P. xylostella were 254.61 ljs/ml and 6.20μg/ml; and LT₅₀ values recorded 74.2 and 12.9 hours, respectively.
The obtained results did not show synergism between nematodes and \textit{B. thuringiensis} against the tested larval instars of \textit{S. littoralis} and \textit{P. xylostella}. The combination of \textit{S. carpocapsae} All and \textit{B. thuringiensis} var. \textit{aizawai} at higher concentration levels against 2\textsuperscript{nd} and 5\textsuperscript{th} instar larvae of \textit{S. littoralis} killed as many larvae as expected assuming independent action of the 2 mortality agents; indicating an additive interaction. Baur et al.\cite{16} reported that in the laboratory and in the field experiments, combining nematode and \textit{B. thuringiensis} yielded an additive increase in mortality of larvae of \textit{P. xylostella} susceptible to \textit{B.t.} on the other hand, the obtained data of other treatments did not indicate any improvement in the efficiency of the combination treatments over that of nematodes and bacteria when each was used alone. Moreover, results indicate that the combined application of \textit{S. carpocapsae} All and \textit{B.t.a.} against the 2\textsuperscript{nd} and 3\textsuperscript{rd} instar larvae of \textit{P. xylostella} was significantly less effective than expected assuming independent action, indicating an antagonistic interaction; as well, the combined application of \textit{H. bacteriophora} HP88 and \textit{B.t.a.} against the tested larval instars of \textit{S. littoralis} and \textit{P. xylostella}.

According to Molyneux et al.\cite{16} antagonistic effect was detected between \textit{B.t.a.} and \textit{Xenorhabdus nematophilus} or \textit{Photorhabdus luminescence}, the symbiotic bacteria associated with \textit{S. carpocapsae} and \textit{H. bacteriophora}, respectively. The cause of larval death was always due to one bacterium alone; the one which could start its action first. The growth of \textit{B.t.} or the symbiotic bacteria (\textit{Xenorhabdus sp.} or \textit{Photorhabdus sp.}) when well established in the haemocoeel completely prevents the growth of the other. Nematodes can only successfully develop when \textit{Xenorhabdus} or \textit{Photorhabdus} spp. inhibits all other microorganisms in insect’s haemocoel. Molyneux et al.\cite{16} attributed the failure of nematode to reproduce inside the cadavers to mass contamination with other microorganisms brought into the haemocoel with the infective juveniles that the symbiotic bacteria could not overcome. This may be the case in the obtained results. Also a possible antagonism between the two bacteria is present if both started to act together. Moreover, the effect of \textit{B.t.} exotoxin on the nematode juveniles themselves should be put into consideration. In this concern, Krieg\cite{15} reported that from observations on simultaneous infection with more than one pathogen, it is concluded that they may or may not have synergistic or antagonistic effects as expressed by changes in mortality of insect hosts. The effect depends not only on the nature of the microorganisms participating in the process, but also on other parameters such as their virulence, the relative dose applied and the sequence of time of infection.

Contradictory results to that obtained in the present work were recorded by El-Bishry\cite{5} and El-Bishry and Bekheit\cite{5} who reported that better mortality rates against larvae of \textit{Agrotis ipsilon} resulted due to mixing infective juveniles of the nematode, \textit{S. carpocapsae} with \textit{B. thuringiensis} Dipel 2x. The \textit{LC}_{50} value was reduced from 56.25 IJs in case of nematode treatment alone to 10.79 IJs in combination with \textit{B.t.}

\textbf{REFERENCES}


