Comparative Bioactivity of Several Formulations of *Bacillus thuringiensis* Toxins against Diamondback Moth, *Plutella xylostella* (Linnæus) (Lepidoptera: Yponomeutidae) Using a Diet Incorporation Method¹,²

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**Key words:** diamondback moth, *Plutella xylostella*, *Bacillus thuringiensis*, bioactivity

Insecticidal proteinaceous crystal toxins (δ-endotoxins) of *Bacillus thuringiensis* have been used to control a variety of insect pests belonging to Lepidoptera, Diptera and Coleoptera. In Japan, *B. thuringiensis* formulations have been mostly utilized for controlling diamondback moth, *Plutella xylostella*, which has become a serious problem on cruciferous crops due to its resistance to many chemical insecticides (Hama, 1990).

It is well known that δ-endotoxins of *B. thuringiensis* have species specific activity among insects even in the same order, family or genus. Difference in host spectrum has been reported for a variety of isolates (Beegle and Yamamoto, 1992). An enormous body of literature has revealed that *B. thuringiensis* usually synthesizes multiple δ-endotoxins, the components and ratios of which differ among isolates (Höfte and Whiteley, 1989). Each δ-endotoxin has its own insecticidal spectrum (Höfte et al., 1988). Therefore, the biological potency of *B. thuringiensis* formulations differ depending on the kind of isolate used and manufacturing conditions (Delmage and Rhodes, 1971; Dulmage, 1981). Besides, the choice of bioassay organisms and/or bioassay methods may influence an estimate of potency (Asano et al., 1993).

In the present study, bioactivities of three experimental and four commercial *B. thuringiensis* toxin wettable powder formulations were investigated against late third instar larvae of the diamondback moth, *P. xylostella*, using a diet incorporation method (Asano et al., 1993).

**MATERIALS AND METHODS**

*Insects.* A laboratory colony of the diamondback moth *Plutella xylostella* that had been maintained on an artificial diet was used (Asano et al., 1993). The late third instar larvae were used for bioassays.

*Test materials.* Three experimental (KM202, KM301, and KM302) and four commercial (Bacilex®, Thuricide®, Toarow-CT®, and Dipol®) *B. thuringiensis* wettatable powder formulations, containing crystal toxins of *B. thuringiensis*, were used. All formulations except Bacilex® contain toxins of *B. thuringiensis* serovar kurstaki. Bacilex® contains mixed toxins of *B. thuringiensis* serovar kurstaki and serovar aizawai. KM301, Bacilex®, Thuricide®, and Dipol® are composed of crystal toxins and live spores. Toarow-CT® was composed of crystal toxins and chemically killed spores. KM202 and KM302 contained a single crystal toxin encapsulated in killed cells of *Pseudomonas fluorescens*.

*Test methods.* About 100 mg of each test sample was weighed and suspended in distilled water at a rate of 10 mg/ml. Serial dilution of the suspension to get seven bioassay doses with an 0.6 dilution factor was done by thoroughly mixing 2 ml of solution with 18 ml of diet in liquid form as described in a previous paper (Asano et al., 1993). Twenty-four third instar larvae were used per dose and each dose had three replicates (a total of 72 larvae per dose). Larvae were held for 16 days at 25°C, 60% R.H. in the dark. Numbers of dead larvae, pupae and adults were counted daily during the test period.

Probit analysis (Finney, 1952) was used to estimate LC₅₀ (median lethal concentration), EC₅₀ (median pupation inhibitory concentration), and LT₅₀ (median lethal time) each reading day after larval transfer to diet treated with test materials.

**RESULTS**

1. **Relationship between LC₅₀ and reading days**

   Dead larvae first appeared on the second day and mortality increased rapidly thereafter. The time-

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² A part of this study was presented at the Annual Joint Meeting of the Entomological Society of Japan and the Japanese Society of Applied Entomology and Zoology held at Shinshu University in April of 1993.
mortality curves were similar among *B. thuringiensis* formulations except KM301, in which the mortality increased rapidly in two to four d and thereafter reached a constant level. Relationships between LC$_{50}$ and reading days are shown in Fig. 1. Similar parabola curves were obtained among *B. thuringiensis* formulations except KM301. In the case of KM301, there was a small decline in LC$_{50}$ within two or three d and then almost constant values were reached.

2. Relationship between EC$_{50}$ and reading days

Rates of pupation were examined daily in treatment and control groups after larval transfer. In controls, 89–96% pupation occurred in three d. However, pupation in treated groups was observed one to two d later at doses giving about 50% mortality compared with that in controls. Unlike LC$_{50}$ estimates, the relationships between EC$_{50}$ and reading days became constant after five to seven d in all treatments (Fig. 2), although the rates tested were quite different among formulations. All pupae in controls emerged four d after pupation (seven d after larval transfer). Although adult emergence in treatments was also one to three d later than that in controls, the actual pupal periods did not differ.

3. Comparison of LC$_{50}$ and EC$_{50}$

The observed difference in the relationships between LC$_{50}$ or EC$_{50}$ and reading days should be considered in evaluating bioactivities of *B. thuringiensis* formulations. When the bioactivities were compared based on LC$_{50}$ values (Fig. 1), the reading day after larval transfer will be more important, for example, A and B in Fig. 1. However, if EC$_{50}$ is used instead of LC$_{50}$, the problems from reading days will not be incorporated, especially after the values reach constant levels. Table 1 shows LC$_{50}$ and EC$_{50}$ values of *B. thuringiensis* formulations on 7 and 14 reading days. LC$_{50}$ values at day 14 were 1.3–2.3 times smaller than that of day seven. KM301 was an exception, and showed the same LC$_{50}$ on both days. In contrast, EC$_{50}$ values on days 7 and 14 were similar and close to LC$_{50}$ values on day 14.
Table 1. Estimates of LC₅₀ and EC₅₀ values for different *Bacillus thuringiensis* formulations against third instar larvae of *Plutella xylostella* in diet incorporation assays

<table>
<thead>
<tr>
<th>Formulation</th>
<th>LC₅₀ (µg/g diet) at Day 7</th>
<th>EC₅₀ (µg/g diet) at Day 7</th>
<th>Ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LC₅₀ (µg/g diet) at Day 14</th>
<th>EC₅₀ (µg/g diet) at Day 14</th>
<th>Ratio&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM202</td>
<td>0.25</td>
<td>0.16</td>
<td>1.6</td>
<td>0.14</td>
<td>0.15</td>
<td>0.93</td>
</tr>
<tr>
<td>KM301</td>
<td>0.73</td>
<td>0.73</td>
<td>1.0</td>
<td>0.64</td>
<td>0.64</td>
<td>1.0</td>
</tr>
<tr>
<td>KM302</td>
<td>0.55</td>
<td>0.24</td>
<td>2.3</td>
<td>0.20</td>
<td>0.22</td>
<td>0.91</td>
</tr>
<tr>
<td>Bacilex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>6.6</td>
<td>5.0</td>
<td>1.3</td>
<td>4.4</td>
<td>4.7</td>
<td>0.94</td>
</tr>
<tr>
<td>Thuricide&lt;sup&gt;®&lt;/sup&gt;</td>
<td>7.2</td>
<td>4.5</td>
<td>1.6</td>
<td>4.1</td>
<td>4.3</td>
<td>0.95</td>
</tr>
<tr>
<td>Toarow-CT&lt;sup&gt;®&lt;/sup&gt;</td>
<td>2.8</td>
<td>1.6</td>
<td>1.8</td>
<td>1.2</td>
<td>1.3</td>
<td>0.92</td>
</tr>
<tr>
<td>Dipol&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1.9</td>
<td>1.5</td>
<td>1.3</td>
<td>1.4</td>
<td>1.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratio = LC₅₀ or EC₅₀ (day 7)/LC₅₀ or EC₅₀ (day 14).

Table 2. Estimates of LT₅₀ values for different *Bacillus thuringiensis* formulations against third instar larvae of *Plutella xylostella*

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Conc. (µg/g diet)</th>
<th>% Mortality at days</th>
<th>LT₅₀ (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 14</td>
<td></td>
</tr>
<tr>
<td>KM202</td>
<td>0.26</td>
<td>0 0 4 9 21 34 51 89</td>
<td>7.4</td>
</tr>
<tr>
<td>KM301</td>
<td>1.4</td>
<td>0 65 92 96 97 97 97</td>
<td>2.4</td>
</tr>
<tr>
<td>KM302</td>
<td>0.43</td>
<td>0 0 3 20 24 34 41 90</td>
<td>7.8</td>
</tr>
<tr>
<td>Bacilex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>9.6</td>
<td>0 14 30 48 55 66 75 94</td>
<td>5.4</td>
</tr>
<tr>
<td>Thuricide&lt;sup&gt;®&lt;/sup&gt;</td>
<td>16</td>
<td>0 56 68 72 77 82 84 94</td>
<td>3.6</td>
</tr>
<tr>
<td>Toarow-CT&lt;sup&gt;®&lt;/sup&gt;</td>
<td>2.9</td>
<td>0 0 1 22 43 58 64 100</td>
<td>6.7</td>
</tr>
<tr>
<td>Dipol&lt;sup&gt;®&lt;/sup&gt;</td>
<td>9.6</td>
<td>0 20 61 74 75 88 89 94</td>
<td>3.8</td>
</tr>
</tbody>
</table>

According to LC₅₀ and EC₅₀ on days 7 and 14 in Table 1, KM202 was the most potent and Bacilex<sup>®</sup> and Thuricide<sup>®</sup> were the least among formulations tested. There was a 30-fold difference between the most and least active formulations.

4. Comparison of LT₅₀

Larval mortality usually occurred from day two of feeding with diet treated with *B. thuringiensis* formulations (Table 2). As mentioned above, the mortality increased with time. However, LT₅₀ values differed among treatment rates and formulations. For example, the LT₅₀ values of KM202 at 2, 1.2, 0.72, 0.43, 0.26 and 0.16 µg were 4.2, 4.2, 4.8, 5.2, 7.4 and 8.1 d, respectively. The LT₅₀ values of KM301 at 4, 2.4, 1.4, 0.86, 0.52 and 0.31 µg were 2.1, 2.1, 2.4, 3.8, 4.9 and 4.3 d, respectively (data not shown). These results indicated that the higher doses with faster LT₅₀ had more rapid lethal action. Other formulations also showed similar relationships between doses and LT₅₀ values, except for low mortality at lower doses.

We then compared LT₅₀ values of formulations at a dose giving 90–100% mortality after 14 d of the treated diet (Table 2). The LT₅₀ values differed among formulations, with a range of 2.4–7.8 d. The results indicate that KM301 had the fastest and KM302 slowest lethal action, respectively.

DISCUSSION

This study showed that LC₅₀ values changed greatly over the duration of the bioassays, and that the changes in LC₅₀ values over time differed among formulations. When the bioactivities of seven *B. thuringiensis* formulations were evaluated on the basis of LC₅₀ values, elapsed time should be taken into consideration. On the other hand, the relationships between EC₅₀ and time were different from LC₅₀ estimates, since EC₅₀ estimates became constant after seven days. Therefore, the comparative toxicity of *B. thuringiensis* formulations using EC₅₀ instead of LC₅₀ was hardly influenced by elapsed time.

The LT₅₀ estimates based on median lethal time
differed among rates and formulations. Some formulations showed rapid lethal action and others showed slow but strong growth inhibition. Interestingly, rapid lethal action was always correlated with weak inhibition on larval growth, and slow lethal action with strong inhibition. *B. thuringiensis* formulations containing live spores usually had rapid lethal action. Ones containing dead spores or no spores showed slow lethal action with strong growth inhibition. The mechanisms by which live spores accelerate the lethal toxin formulations are not known. However, similar results have been observed using *Hypantria cunea* (Miyamoto and Aizawa, 1982).

Heimpel and Angus (1959) classified lepidopterous larvae susceptible to *B. thuringiensis* into three types. Larvae of the types I and II are killed by crystals alone, but larvae of the type III are killed only when fed with both spores and crystals. This classification indicates that the intoxication impact of the spore with δ-endotoxins varies by insect species. Moreover, with *Choristoneura fumiferana*, the effect of spores was not consistent among researchers (Bregele and Yamamoto, 1992).

The role of spores in plant protection is not clear. This study suggests that formulations with and without spores can be equally effective in terms of plant protection. At effective concentrations, formulations with spores would have lethal action, while formulations without spores first cease feeding and then cause mortality.

The results of this study clearly demonstrate that LT₅₀ estimates vary considerably depending on whether *B. thuringiensis* toxins come from sporocrystal products, inactivated spore products or single-toxin-based products. Rate of intoxication and onset of mortality must be taken into account when estimating the bioactivity of *B. thuringiensis*-based products on insects in the laboratory. Failure to take differences in LT₅₀ values into account may lead to errors in estimating biocontrol potential of newer biologically manipulated toxins derived from *B. thuringiensis*.

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REFERENCES


