NOVEL BIOCHEMICAL AVENUES FOR ENHANCING

*BACILLUS THURINGIENSIS* ENDOTOXIN POTENCY
AGAINST *SPODOPTERA LITTORALIS* [LEP. : NOCTUIDAE]

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Simple biochemical means were used to enhance the endotoxin effect of *Bacillus thuringiensis* Berliner through optimization of conditions present in the insect gut required for release of intoxicating fragments from nonpoisonous intact endotoxin. This was achieved through the addition of either alkaline compounds naturally occurring in the insect midgut, general proteolytic activators or mildly toxic compounds of inorganic (e.g. borax) and organic (e.g. tannic acid) nature along with endotoxin in the diet. A marked increase in the potency of *B.t.* var. *entomocidus* HD-635 and *aizawai* HD-133 against *Spodoptera littoralis* (Boisduval) was obtained in the presence of 0.5 % concentration of potassium carbonate. On the other hand, sodium carbonate was less effective at the tested concentrations. Among the tested proteolytic activators, the effect of magnesium ion was proportional to the concentration of the incorporated salt. Thus the potencies of δ-endotoxin preparations of HD-635 and HD-133 were increased 2-3 times after the addition of 1 % concentration of magnesium chloride. A lesser effect was noted at 0.5 %, whereas virtually the potency did not change at 0.25 % concentration of the salt. Similar trends were noted with the incorporation of calcium chloride along with the endotoxin in the insect diet. The addition of sodium borate or tannic acid was highly effective in enhancing the potency of the tested spore-δ-endotoxin preparations with 2-4 fold increase.

It is now an accepted fact that the intact δ-endotoxin of *Bacillus thuringiensis* Berliner is only effective against target insects through the oral route (Heimpel & Angus, 1959, 1960) with the intoxication of the susceptible insect follows rapidly after ingestion of the crystal. Although there is no common theory that explains the mode of action of the endotoxin, there is general agreement that toxicity arises when subunits of the crystal attack the lining of the midgut causing paralysis via leakage of the alkaline gut contents into the hemocoel (Couch & Ross, 1980). Thus the crystal δ-endotoxin protein is not a contact poison. Instead it must be ingested, solubilized and activated by larval gut enzymes. The highly selective toxicity for lepidopterous larvae at least partly derives from the highly alkaline pH of the midgut of these insects due to the presence of high levels of potassium carbonate. Alkaline proteases are assumed to solubilize the crystal and render it susceptible to proteolysis by gut enzymes (Lecadet & Martouret, 1965).
Although the literature explaining such a mechanism of toxicity is voluminous, no detailed reports are available that explore the possible enhancement of the endotoxin effect through biochemical means and/or additives that furnish ideal conditions for breakdown of the intact crystal into the toxic subunits actually involved in the intoxication mechanism of the insect larvae. In this concern, however Charles & Wallis (1964) found that the mortality of the larvae of the gypsy moth *Porthetria dispar* (L.) increased after feeding on lettuce leaves treated with combination of boric acid and *B. thuringiensis* var. *thuringiensis* with no explanation of the mode of action of this additive. Smirnoff (1974) observed that the addition of chitinase considerably increased the septicemia enterotoxins provoked by *B. thuringiensis* against the spruce budworm *Choristoneura fumiferana* Clemens. Also Burges (1977) reported that P-amino-salysilic acid increased the potency of *B. thuringiensis* vs. the wax moth *Galleria mellonella* L..

In the present work, new approaches have been followed in this direction to introduce new simple biochemical means to enhance the endotoxin effect through optimization of conditions present in the insect gut required for release of intoxicating fragments.

**MATERIAL AND METHODS**

In the present study, 3 types of materials were evaluated as potential enhancers of the \( \delta \)-endotoxin by incorporating them into insect diet as follows:

a - Addition of alkaline compounds naturally occurring in the insect midgut in order to augment their concentration and directly affect the solubility of the crystalline protein. e.g.-sodium carbonate and calcium carbonate.

b - Addition of general proteolytic activators, notably the divalent cations (Dixon & Webb, 1964). These metal ions, e.g., Ca\(^{++}\) and Mg\(^{++}\) serve as co-factors in proteolytic processes and hopefully would facilitate the enzymatic breakdown of the endotoxin in the larval midgut.

c - Addition of mildly toxic inorganic compounds (e.g., sodium borate or tannic acid) along with the endotoxin in the diet to see if any possible synergistic effects might lead to improvement of endotoxin efficacy. Sodium borate is a feeble antiseptic that has slightly toxic effect; tannic acid or tannins are natural compounds that occur in the bark and fruits of many plants, and they are also feeble antiseptics but essentially astringents.

In selecting the forementioned additives the following requirements were taken into consideration:

- The compounds must be non-toxic to man (with the exception of borax) in order to be feasible for field application if the combination proved to be successful.
- The compound should have no harmful effect on the plants at the concentration tested.
- The compound must be commonly available and of low price so that it would be practical to use them.
- The compound must be bio-degradable in order to assure no accumulative effect by repeated application.

The *B.t.* strains used in the present study were vars. *entomocidus* HD-635 and *aizawai* HD-133. These were prepared through acetone precipitation procedure from fermentation flasks of culture grown in fodder yeast BM media (Salama *et al.*, 1983).

The assay procedure proposed by Dulmage *et al* (1971) was adopted. The standard HD-1 – S-1980 (Potency of 16,000 IU/mg) has been used throughout the experimental work. By this