

Temporal effect on insecticidal activity of *Bacillus thuringiensis* Berliner Var. *kurstaki*, strain ABTS-351, against *Corcyra cephalonica*

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Abstract

Bacillus thuringiensis (*Bt*) formulations are the most successful biopesticides now regularly used in many IPM programs against lepidopteran pests. Newer formulations like DiPel contain strain ABTS-351 as active toxin product, which is essentially an isolate of HD-1. Insecticidal activity assay of Dipel DF was performed to characterize the strain for its insecticidal efficacy against Rice moth, *Corcyra cephalonica* larvae; and the effects of duration of exposure. The obtained mortality curves for the three exposure durations were typically sigmoid as expected. Longer duration of exposure increased the mortality. Lethal concentration values, obtained through Probit analysis for 24, 48 and 72 hrs exposure against log concentration of *Bt*, yielded LC₅₀ value (with 95% confidence limits) of *Bt* on *C. cephalonica* 4th instar larvae as 65.813 (52.946 – 85.689), 36.311 (29.953 – 45.704) and 17.745 (15.350 – 20.742) mg/mL respectively. The insecticidal activity was found to be comparatively much efficient at lower concentrations with longer exposures. This feature is the potential key to a more efficient biocontrol strategy, allowing other compatible biocontrol agents to be combined for a far more effective, or even synergistically, control over pest.

Keywords: *Bacillus thuringiensis*; ABTS-351; *Corcyra cephalonica*; Lethal response

1. Introduction

Bacillus thuringiensis (*Bt*) formulations are the most successful biopesticides available. The microbe is a facultative anaerobic, Gram-positive, spore-forming bacterium, commonly found in soil. The formulations are now regularly used in many IPM programs [1]. *Bt* subspecies synthesize more than one inclusion, adjacent to the endospore during sporulation, composed of different insecticidal crystal proteins (ICP). These inclusion bodies have been found to be toxic for invertebrates, primarily insect species belonging to orders *Coleoptera*, *Diptera* and *Lepidoptera* [1,2]. ICPs are composed of proteinaceous δ -endotoxins and have various crystalline shapes, thereby also called crystal proteins or *Cry* proteins, and are encoded by the *Cry* and *Cyt* genes [3].

Bacillus thuringiensis Berliner Var. *kurstaki* subspecies has high specificity against lepidopteran pests. Commercial formulations contain specific insecticidal crystalline proteins (ICPs) and most often living spores as well as formulating agents, and are processed fermentation products [1]. Some commercially available popular product trade names are Mvp, Dipel, Biobit, Foray, Condor, Cutlass, Crymax, Lepinox, Javelin, Thuricide, Bactospeine, Futura, Bernan Bt, Bactis, Biospor, Larvo Bt, Bt, Sporoine, M-peril, SOK, Plantibac, Able, Delfin, CoStar, Steward, Vault, Bactur, Toaro, Toaro, Ct, WOCK Biological (Halt-Bt), etc [3,4].

Although *Bt* was discovered in infected *Anagasta (Ephestia) kuhniella* and named in 1915 by Berliner, a more potent strain of this variety was isolated from diseased mass-reared pink bollworm, *Pectinophora gossypiella* larvae by

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Dulmage (1970) [5]. He coded it HD-I and was later obtained by Abbott Laboratories for their DiPel production. Later this isolate was assigned an internal strain identification code, ABTS351 (ie. Abbott BT Strain-351) in 1986. Thus, this strain is essentially an isolate of HD-1. *Bacillus thuringiensis* subsp. *kurstaki*, Serotype 3a3b, Strain ABTS-351 (*Btk* ABTS-351) is the active toxin product in several new *Bt* formulations. This strain originates from a natural wild strain of the organism and has not been genetically modified nor is the result of a spontaneous or an induced mutation [6,7].

Btk strain HD-1 has long been used in formulations against various lepidopteran pests in agriculture. However, several recent formulations are based on the isolate ABTS-351. Proper detailed screening of its insecticidal efficacy at local level on local susceptible pests are very important as there are few incidents of insects developing resistance to *Bt* formulations [8]. The insecticidal efficacy is a function of the duration of exposure of the susceptible pest to *Bt*. This is important because the persistence of *Bt* formulations in different pest control scenarios vary and is subjected to local conditions. This study aims towards this direction, to characterize *Btk* ABTS-351 strain for its insecticidal efficacy against Rice moth, *Corcyra cephalonica* larvae; and the effect of various exposure durations.

2. Material and methods

C. cephalonica eggs were obtained from Central Integrated Pest Management Centre, Gorakhpur, UP, India; and then cultured according to Deepak (2004) in coarsely ground mixed diet of four cereal diets *viz.*, rice (*Oryza sativa*. Linn.), jowar (*Sorghum vulgare*. Pers.), maize (*Zea mays*. Linn.) and wheat (*Triticum aestivum*. Linn.); at 27 ± 2 °C and $70 \pm 10\%$ RH. Healthy and active 4th instar larvae were used in this study [3,9].

Commercial formulation based on *Bt* selected for the insecticidal activity assay was Dipel DF (*B. thuringiensis* var. *kurstaki*, strain ABTS-351, 32 MIU g⁻¹ [millions of International Units per gram]). Dilutions of 1, 2, 4, 8, 16, 32 and 64 mg of *Bt* per mL were prepared in distilled water. 1mL distilled water was used as control. These were mixed thoroughly with artificial mixed cereal diet at the rate of 0.2mL/g and allowed to dry. 10g of the treated diets were introduced onto 250mL Borosil glass beakers. Twenty healthy and active *C. cephalonica* 4th instar larvae were introduced into the *Bt*-treated diet in each beaker and kept at 27 ± 2 °C, $70 \pm 10\%$ relative humidity and 12:12 L:D photoperiod in BOD chamber. Larval mortality was recorded after 24, 48 and 72 hours of initial exposure [10]. The experiment was replicated five times. The LC values for 24, 48 and 72 hours (with 95% confidence limits) were calculated by using POLO - Plus 2.0 program (Leora Software, 2005) and Probit Analysis Statistical Method using SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software. Mortality data of screening tests under different exposure duration were also subjected to analysis of variance (One Way ANOVA), and mean separation tests were conducted with Tukey's B test [4].

3. Results

The mortality curves of *C. cephalonica* for *Btk*, Serotype 3a3b, Strain ABTS-351 (Dipel DF) for the three exposure durations were typically sigmoid as expected (Fig. 1). Longer duration increased the mortality. Lethal concentration values were obtained through Probit analysis in SPSS by plotting linear curve between probit mortality of *C. cephalonica* 4th instar larvae exposed for 24, 48 and 72 hrs against log concentration of Dipel DF. The LC₅₀ value (with 95% confidence limits) of *Bt* on *C. cephalonica* 4th instar larvae for 24, 48 and 72 hours were 65.813 (52.946 – 85.689), 36.311 (29.953 – 45.704) and 17.745 (15.350 – 20.742) mg/mL respectively. Comparatively, the insecticidal activity was found to be much efficient at lower concentrations if given longer exposures. Linear curve between probit mortality of *Corcyra cephalonica* against log concentration of *Bacillus thuringiensis* exposed for 24, 48 and 72 hrs; also confirmed this observation (Fig. 2). The linear relationship tended to be more curved (J-shaped) for 72 hrs long exposure.

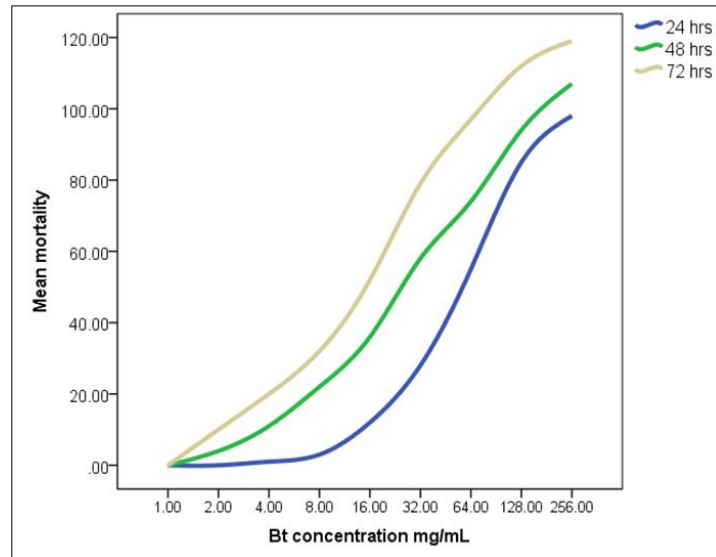


Figure 1 The curve between observed mortality of *Corcyra cephalonica* per 120 individuals, exposed for 24, 48 and 72 hrs against concentration of *Bacillus thuringiensis* subsp. *kurstaki*, Serotype 3a3b, Strain ABTS-351 (Dipel DF)

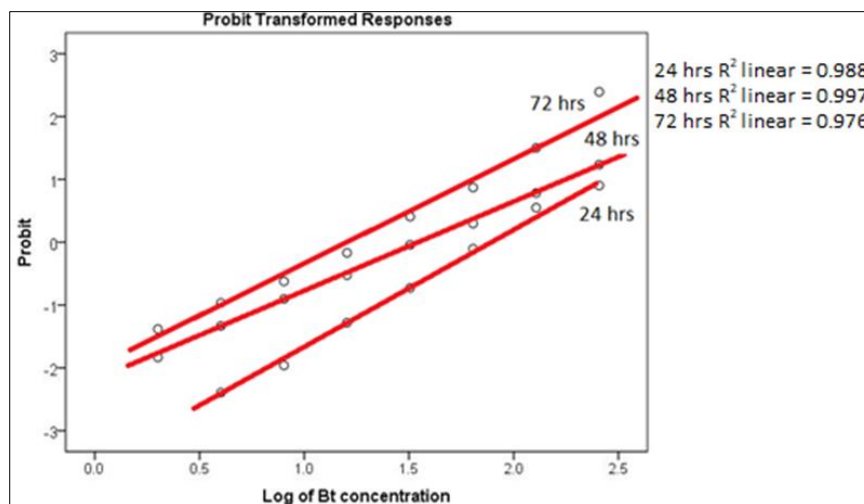


Figure 2 Linear curve between probit mortality of *Corcyra cephalonica* exposed for 24, 48 and 72 hrs against log concentration of *Bacillus thuringiensis* subsp. *kurstaki*, Serotype 3a3b, Strain ABTS-351 (Dipel DF)

4. Discussion

The mortality curves of *C. cephalonica* for *Btk*, Serotype 3a3b, Strain ABTS-351 (Dipel DF) for the three exposure durations clearly indicated the significant effect of insecticide concentrations and exposure duration. One important observation is that at lower sublethal concentrations the longer duration of exposure is comparatively more efficient at pest control. In any combined biocontrol strategy this feature should play an important role leading to a more efficient, or even a synergistic effect on pest control. For example, combining with biocontrol agents like parasitoids, the *Bt* will attack more susceptible deep-dwelling earlier instars, in low concentration and long exposure scenario. On the other hand, suitable compatible parasitoids will seek out and attack later instars [11], because of low preference for early instars due to age-dependent mechanisms which parasitoids use to discriminate between different instars of the same host. Such combined control will allow for an even lesser amount of *Bt* formulations to be used, thus moving leaps forward towards the goals of IPM.

5. Conclusion

The mode of action and higher efficiency with prolonged exposure makes this formulation much suitable to manage stored grain pests. Lower dosage requirement and the safety margins may allow integration of other compatible biocontrol agents for a comprehensive control. *Bt* is safe for non-target organisms so it can be well integrated in other IPM control strategies for much efficient pest control.

Compliance with ethical standards

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Disclosure of conflict of interest

Author state no conflict of interest.

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