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Evaluation of acute lethal response of *Corcyra cephalonica* to *Bacillus thuringiensis* Berliner Var. *kurstaki*, strain ABTS-351, an isolate of strain HD-1

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Abstract

Bacillus thuringiensis (*Bt*) is the most successful among other microbial pesticides. The microbe formulations are being implemented in many IPM programs. Among many subspecies, *Bacillus thuringiensis* Berliner Var. *kurstaki* subspecies is specifically used against lepidopteran pests. Its strain ABTS-351 (*Btk* ABTS-351) is the active biocide product in several new *Bt* formulations and is essentially an isolate of strain HD-1. Efficacy screening is imperative due to chances of pest insects developing resistance to *Bt* formulations. This study was conducted to characterize *Btk* ABTS-351 strain for potent insecticidal activity against Rice moth, *Corcyra cephalonica* larvae in local conditions. The LC_{50} value (with 95% confidence limits) of *Bt* on *C. cephalonica* 4th instar larvae for 24 hr was 65.813 (52.946 – 85.689) mg/mL. This value is comparatively higher than those observed in other lepidopteran larvae. The toxicity and mode of action of this formulation can be used efficiently to manage stored grain pests like *C. cephalonica*, and also has the potential to be combined with other compatible IPM control strategies.

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

1. Introduction

Bacillus thuringiensis (*Bt*) is a facultative anaerobic, Gram-positive, spore-forming soil bacterium, commonly used as an effective biopesticide. It is the most successful among other microbial pesticides, occupying more than 90% of the biopesticide market, attaining wide commercial use against major insect pests [1]. The microbe formulations are being fully implemented in many IPM programs. This bacterium is also a key source of genes for genetically modified organisms (GMOs) to provide pest resistance in various crops against various pests [2].

Bt produces one or more inclusion bodies during the sporulation process, which have been found to be toxic for invertebrates, primarily insect species in the orders *Coleoptera*, *Diptera* and *Lepidoptera* [2, 3]. After discovery of 13 *Bt* subspecies by 1977, we now know about approximately 100 subspecies, identified using various criteria such as, phage susceptibility and plasmid profiles along with serotyping [2,4]. *Bt* subspecies synthesize more than one inclusion, adjacent to the endospore during sporulation, composed of different insecticidal crystal proteins (ICP). ICPs are also called δ-endotoxins, and have various crystalline shapes, depending on their ICP composition. These proteinaceous insecticidal endotoxins are also called crystal proteins or *Cry* proteins, and are encoded by the C*ry* and C*yt* genes [2].

Commercial *Bacillus thuringiensis* (*Bt*) biopesticide products contain specific insecticidal crystalline proteins (ICPs) and most often living spores as well as formulating agents, and are processed fermentation products [5]. Different toxins have different spectra of activity, and different strains and serotypes have been developed by different companies. They are commercially available under trade names such as Dipel, Halt, Delfin and Thuricide. Their typical agricultural

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formulations include wettable powders, spray concentrates, liquid concentrates, dusts, baits, and time release rings [6]. *Bacillus thuringiensis* Berliner Var. *kurstaki* subspecies is specifically used against lepidopteran pests. Trade names of its commercial formulations 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, Plantibac, Able, Delfin, CoStar, Steward, Vault, Bactur, Toaro, Toaro, Ct, WOCK Biological (Halt-Bt), etc. The chief strains of this subspecies include HD-1.

Bacillus thuringiensis subsp. *kurstaki*, Serotype 3a3b, Strain ABTS-351 (*Btk* ABTS-351) is the active biocide product in several new *Bt* formulations. *Btk* ABTS-351 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. This strain is registered at the American Type Culture Collection under No. ATCC-SD-1275 [7]. Although *Bt* was discovered and named in 1915 by Berliner [8], who isolated a spore forming bacterium from *Anagasta (Ephestia) kuhniella*, a more potent strain of this variety was isolated from diseased mass-reared pink bollworm, *Pectinophora gossypiella* larvae by Dulmage (1970) who coded it HD-I [9]. Abbott Laboratories obtained the DiPel production strain, HD-1, directly from Dulmage and it was maintained at the lab as lyophilized material. Later in 1986, this isolate was assigned an internal strain identification code, ABTS351 (ie. Abbott BT Strain-351), solely for regulatory purposes, so it is essentially an isolate of HD-1 [10].

Formulations of *Btk* using HD-1 strains have had high activity against various lepidopteran pests in agriculture. However, current formulations based on the isolate ABTS-351 should be screened for their efficacy as few reports showed that few insects developing resistance to *Bt* formulations [11]. Keeping this in view, the aim of this study was to characterize *Btk* ABTS-351 strain for potent insecticidal activity against Rice moth, *Corcyra cephalonica* larvae.

2. Material and methods

Eggs of rice moth, *C. cephalonica* , were obtained from Central Integrated Pest Management Centre, Gorakhpur, UP, India; and then cultured as by 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 ± 10% RH. Active 4th instar larvae were used in this study [12].

To estimate medial lethal concentration (LC_{50}) value of *Bt* on *C. cephalonica*, commercial formulation based on *Bt* selected for the assay was Dipel DF (*B. thuringiensis* var. *kurstaki*, strain ABTS-351, 32 MIU g-1 [millions of International Units per gram]). After initial pilot experiment for approximate LC_{50} estimation, dilutions were made using 1, 2, 4, 8, 16, 32 and 64 mg of *Bt* per mL of 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 *C. cephalonica* active 4th instar larvae were introduced into the diet in each beaker and kept at 27 ± 2 °C, 70 ± 10% relative humidity and 12:12 L:D photoperiod in the laboratory. Larval mortality was recorded after 24 hours of initial inoculation. The experiment was replicated five times [13]. The LC_{50} for 24 hours (with 95% confidence limits) were calculated by using POLO - Plus 2.0 program (Leora Software, 2005) and Probit Analysis Statistical Method and mortality data of preliminary screening tests and different treatments were subjected to analysis of variance (One Way ANOVA) and mean separation tests were conducted with Tukey's B test using SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software.

3. Results

Probit analysis in SPSS by plotting linear curve between probit mortality of *C. cephalonica* 4th instar larvae exposed for 24 hr against log concentration of Dipel DF yielded lethal concentration values (Fig. 1). The LC_{50} value (with 95% confidence limits) of *Bt* on *C. cephalonica* 4th instar larvae for 24 hr was 65.813 (52.946 – 85.689) mg/mL. Whereas, for 24 hours exposure LC_5, LC_{10}, LC_{25} and LC_{95} values were 9.495 (5.570-13.756), 14.565 (9.418-19.933), 29.773 (22.058-38.043), and 457.279 (296.529-863.567) mg/mL respectively.

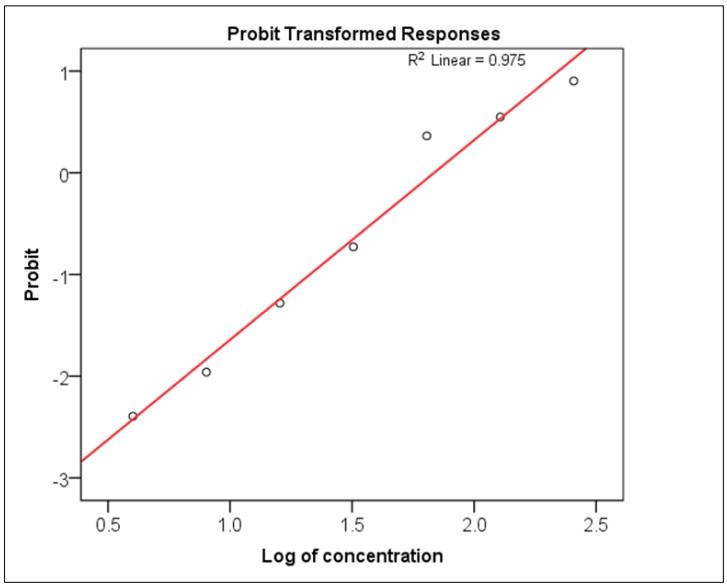

Figure 1 Graph showing linear curve between probit mortality of *Corcyra cephalonica* exposed for 24hr against log concentration of *Bacillus thuringiensis* subsp. kurstaki, Serotype 3a3b, Strain ABTS-351 (Dipel DF)

4. Discussion

The insecticidal efficacy of *Btk* ABTS-351 was studied on *C. cephalonica*. 4th instar larvae are the most active ones, causing tremendous damage to stored food material partly by eating and partly by the formation of silken webbings [14]. This makes the stored food or commodity unfit for human consumption. This *Bt* strain being the same as HD-1, cause mortality, reduces survivorship, and an anti-feeding effect on the larvae, with the potential to control pest population in grain storage sites. *C. cephalonica* infected with *Bt,* tends to continuously replace *Bt* toxin-damaged midgut cells with newly developed cells found at the basal portion of the epithelium, as a mechanism to resist *Bt* toxin action [15]. This may be the reason for a comparatively higher LC_{50} value than other lepidopteran larvae. *Bt* being more lethal in early and active instars (1st to 4th), a combined treatment employing other biocontrol agents targeting other stages will be significantly more effective. This is based on the fact that *Bt* is safe for non-target organisms. Thus, the toxicity of this formulation to lepidopteran pest larvae may efficiently manage stored grain pests like *C. cephalonica*, and has the potential to synergistically complement combined compatible IPM control strategies in other pest control scenarios [16].

5. Conclusion

The toxicity and mode of action of this formulation can be used efficiently to manage stored grain pests like *C. cephalonica*. Proper integration of more than one compatible biocontrol agents has shown better results. Since, *Bt* is safe for non-target organisms, it has the potential to be combined with other compatible IPM control strategies for much efficient pest control.

Compliance with ethical standards

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Disclosure of conflict of interest

We state no conflict of interest.

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