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Insecticidal activity of *Bacillus thuringinesis* strains against rice grain moth, *Corcyra cephalonica*

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ABSTRACT

This investigation was carried out at Department of Plant Protection, Allahabad Agricultural Institute-Deemed University, Allahabad. Toxicity tests for Bt subsp *kenyae*, Bt subsp. *kurstak*i HD-1 , Bt subsp *sotto*, Bt subsp. *kurstaki* HD-73 and Bt subsp *tolworthi* were performed against second instar larvae of *Corcyra cephalonica* at concentrations ranging from 0.5 to 3.0 per cent. The LC_{50} and LC_{90} values at 72 hrs were 0.8417 and 14.84, 0.384 and 9.549, 0.2367 and 10.04, 0.3480 and 10.31 and 0.1631 and 2.965, respectively.

Key words : *Bacillus thuringiensis*, Toxicity, LC_{50}.

Among the biopesticides based on entomopathogenic micro-organisms the most widely used microbial insecticides are *Bacillus thuringiensis (*Bt*)* which is rod shaped, gram positive bacterium, abundant in soil and other habitats throughout the world. During sporulation Bt produces a parasporal crystal compared of proteins known as delta-endotoxin or Insecticidal Crystal Proteins (ICPs). Following ingestion, solublization and in some cases proteolysis, activation toxins bind to receptors and form pores in the midgut epithelium of susceptible insects (Gill *et al.,* 1992). The process results in disruption of membrane integrity, starvation and ultimately death. Because d-endo-toxins are generally safe to vertebrates (Siegel and Shadduck, 1989) and to beneficial arthropods and are often highly toxic to insect pests at relatively low doses, genes encoding these proteins were among the first to be used in genetic engineering of plant for enhanced insect resistance (Vaeck *et al*., 1987).

Most currently, formulation of Bt used for controlling insect pests are based on the HD-1 strains of the Bt subsp. *kurstaki* because of its high activity against various lepidopteran pests in agriculture (Kees van Frankenhuyzen *et al*., 1992). However, it is not necessary that HD-1strain is the most effective strain for the control of lepidopterans. There are other Bt strains which should be screened for their efficacy as few reports showed that few insects developing resistance to Bt formulations. Keeping this in view some Bt strains are evaluated against a lepidopteran stored grain pest in this investigation

MATERIALS AND METHODS

The present study was conducted in the Department of Plant Protection Allahabad Agricultural Institute Deemed University, Allahabad, Uttar Pradesh.India.

Bacterial strains and culture conditions:

B. thuringiensis strains *viz.*, Bt subsp. *kurstaki* HD1, HD 73, *Bt* subsp. *tolworthi*, Bt subsp. *sotto* and Bt subsp. *kenyae* were obtained from Bacillus Genetic Stock Center (BGSC), Ohio State University, USA. And these strains were cultured on nutrient agar(peptone 20.0g, beef extract 3.0g, Nacl 3.0g and agar 20.0g in 1000ml of distilled water) at 30^0C. Nutrient agar slants containing bacterial strains were also maintained at 4 ^{0}C until use.

Preparation of spore crystal mixture of Bacillus thuringiensis strains:

Pure culture of Bt strains maintained on nutrient agar plates were used by inoculating a loopful in 250 ml sterile nutrient broth kept in 1 litre conical flask. The flask was incubated in an incubator shaker at 150 rpm for 7 days at 30^0C with continuous shaking. After that it was centrifuged at 6000 rpm for 10 min. The resulting pellet containing spore and parasporal protein crystals were washed in 20 ml sterile distilled water and centrifuged at 6000 rpm for 5 minutes and washing was repeated twice. The pellets were resuspended in 10 ml of sterile distilled water and kept at 4 ^{0}C (Carozzi *et al.,* 1991).

Rearing of rice grain moth Corcyra cephalonica:

Rearing of rice grain moth, *C. cephalonica* from eggs to adult stage was undertaken using broken grain of sorghum in wooden rearing cage of 45 x 30 x 15 cm size, covered with wooden lid. Broken sorghum grains were first sterilized at 110 ^{0}C for 2hrs. in a hot air oven and used for mass rearing. The sterilized grains were mixed with dried yeast powder @ 2g/kg, 0.01%. streptomycin, pinch of sulphur dust and 2.5 kg of sorghum was kept in each tray. At the beginning of rearing, 1 cc of *Corcyra cephalonica* eggs were spirinkled and kept for the development. The larvae fed on grain and pupated inside the tray itself. The moths started emerging from the 30^{th} day onwards. The emerged moths were collected and kept for egg laying in oviposition cage provided with honey solution. The moths laid most of the eggs within 3 days after emergence. Moths emergence reduced after 100 days of initial infestation and boxes were reused after cleaning.

Bioassay of* Bt *strains against C. cephalonica:

The bioassay of the Bt strains were studied under laboratory conditions on *Corcyra* larvae. Different concentrations *viz.*, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml were prepared for each bacterial strain. Toxicity assays were performed against the young 2^{nd} star larvae in sterilized Petri plates (90 mm diameter) containing 5 g of diet. Bacterial strains suspension were diet incorporated at different concentrations. Ten larvae were tested per replicate with at least three replicates per treatment. A control treated only with distilled water was also maintained. Petri plates were incubated at 30^0C for 72 hours. The mortality count of larvae was recorded at 72 hrs after treatment. The mortality data of each strain was subjected to probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Bio-assay of B. thuringinesis strains against Corcyra cephalonica:

The spore crystal suspension of *B. thuringiensis* strains were examined for their toxicity against second instar larvae of *C. cephalonica* using diet incorporation method. The results are presented in Table 1.

Dose mortality response of* Bt *subsp. kenyae against C.cephalonica:

The results showed that mortality of *C. cephalonica* ranged from 25 to 90 per cent at 72 hours after treatment. The LC_{50} and LC_{90} values were 0.8417 and 14.84, respectively. Jaquet *et al.* (1987) found that larvae of a Lepidoptera, *Heliothis virescens* were succeptible to Bt subsp. *kenyae*. Obeidat *et al.* (2004) also observed that Bt *kenyae* was highly toxic to a lepidopteran storage pest, *Ephestia kuehniella* and the LC_{50} values were 6.97. But the findings of Vasquez *et al.* (1995) showed that Bt subsp. *kenyae* was approximetly 20 per cent less toxic than Bt *subsp. kurstaki* against moth. This finding is in conformity with the finding of the present investigation.

Dose mortality response of* Bt *subsp. kurstaki HD-1 against C. cephalonica:

It was observed that mortality of *C. cephalonica* ranged from 40 to 100 per cent at 72 hours after treatment. The LC_{50} and LC_{90} values were 0.3845 and 9.549, respectively. Asano *et al.* (1994) reported supernatant of the growth medium *of* Bt *subsp. kurstaki* HD-1 culture was found to be toxic to *Plutella xylostella*, *Bombyx mori* and *Spodoptera litura*, rather than the pellets. Vasquez *et al.* (1995) showed that protoxin from Bt subs*p kurstaki* HD-1 elicited the lowest LT_{50} values namely 56.44 and 65.9 hours for *Helicoverpa zea* and *S.absoluta*, respectively. Porcar *et al.* (2000) also showed that Bt *subsp kurstaki* had high insecticidal activity to *Spodoptera* spp.

Dose mortality response of* Bt *subsp. sotto against C. cephalonica:

The results showed that mortality of *C. cephalonica* ranged from 50 to 95 per cent at 72 hours after treatment. The LC_{50} and LC_{90} values were 0.2367 and 10.04, respectively. Ohgushi *et al.* (2003) found that Bt *sotto* whose parasporal inclusions were toxic to Diptera only and non-toxic to Lepidoptera. It has been generally accepted that strain belonging to serovar *sotto* also synthesis the Cry 1 proteins especially toxic to insects of the order Lepidoptera. In this investigation it was found that this strain was highly toxic to *C.cephalonica* .

Dose mortality response of* Bt *subsp. HD-73 against C. cephalonica:

It was observed that mortality of *C. cephalonica* ranged from 50 to 90 per cent at 72 hours after treatment. The LC_{50} and LC_{90} values were 0.3480 and 10.31, respectively. Saravanan (2005) also showed that Bt *kurstaki*, HD-73 was highly toxic to lepidopteran insects.

Table 1: Toxicity of *Bacillus thuringinesis* strains against *corcyra cephalonica*

Sr. No.	Bt strain	Heterogenicity	Slope($\pm$se)	Regression equation	$LC_{50(\%)}$
1.	Bt subsp *kenyae*	$X^2_{(7)=5.07}$	1.55$\pm$0.085	Y=5.11+55x	0.8417
2.	Bt subsp *kurstaki* HD1	$X^2_{(7)=8.47}$	0.92$\pm$0.01	Y=5.38+92x	0.3845
3.	Bt subsp *kurstaki* HD73	$X^2_{(7)=4.78}$	0.87$\pm$0.018	Y=5.40+0.87x	0.3480
4.	Bt subsp *sotto*	$X^2_{(7)=5.14}$	0.79$\pm$O.I7	Y=5.49+0.79x	0.2367
5.	Bt subsp *tolworthi*	$X^2_{(7)=9.366}$	1.02$\pm$0.019	Y=5.80+1.02x	0.1631

Dose mortality response of Bt *subsp. tolworthi against C. cephalonica:*

The results showed that mortality of *C. cephalonica* ranged from 50 to 100 per cent at 72 hours after treatment. The LC_{50} and LC_{90} values were 0.1631 and 2.965, respectively. Effectiveness of Bt *tolworthi* against lepidopteran insects namely, *Plutella xylostella*, *Manduca sexta* and *Helicoverpa zea* was reported by Lambert *et al.* (1996).Saravanan (2005) also found that this strain was effective against *Achaea janata*, *Plutella xylostella*, *Spodoptera litura* and *Pieris brassicae*.

The results obtained from the present investigation may help to understand the toxicity of different Bt strains. They can be again evaluated against some more insect pests for their broad spectrum of activity. And they can be used as alternatives in case of resistance development in insects to some of the Bt formulations available.

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