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INSECTICIDAL ACTIVITY OF SOME *BACILLUS THURINGIENSIS* STRAINS AGAINST *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA : TENEBRIONIDAE)

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Abstract: The biotoxicity analysis of crystal protein of some *Bacillus thuringiensis* strains has been carried out against the larvae of red flour beetle, *Tribolium castaneum* (Herbst). Seven isolates found highly active against *T. castaneum*. The most toxic isolate SG 31.11 has calculated LC_{50} value of 0.2 µg/mg of artificial diet.

Key words: *Bacillus thuringiensis*, stored grain pest, *Tribolium castaneum*, biotoxicity, insecticidal activity.

INTRODUCTION

Tribolium castaneum is a serious pest of stored grains throughout the world. It not only affects the quantity but also the quality of stored grains. The quantitative estimation of the loss incurred by red flour beetle is difficult because this insect is found in flour mills, godowns, and warehouses with other associated stored grain pest complex. To control the infestation of this insect, many synthetic pesticides have been used for several years now. However, these pesticides produce several adverse effects, which include accumulation of lethal chemicals in food chain and environment, lack of selectivity towards beneficial insects and evolution of resistance. These factors have directed the attention of scientists from traditional chemical pesticides to biopesticides.

Microbial control of insect pest of crops using entomopathogens is an ecologically sound pest management strategy. Although insect viruses and fungal pathogens are used as microbial control agents, but *Bacillus thuringiensis* Berliner (Bt) appears to have the greatest potential for this purpose. This gram-positive, spore forming crystalliferous bacterium synthesizes a proteinaceous parasporal crystalline inclusion (δ-endotoxin) during the sporulation phase. These crystalline proteins are highly specific against different insect orders, and non-target organisms like parasitoids, predators and vertebrates are not affected by their use (Aronson *et al*., 1986; Whiteley and Schnepf, 1986). A promising variety of crystal proteins (cry proteins) have been recognized in different Bt strains. Of these crystal proteins CryIII are reported to be toxic against coleoptera (Herrnstadt *et al*., 1986). This study presents our initial efforts to assess the potential of Bt

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strains, isolated from different environmental samples, as a biological control agent of *T. castaneum*.

MATERIALS AND METHODS

Bacterial culture and isolation of crystal proteins

The strains of *B. thuringiensis* used in the present study, were very kindly supplied by the Culture Collection Laboratory, Centre of Excellence in Molecular Biology, Punjab University, Lahore. These samples were collected from different areas of Pakistan. Most of the strains selected for the study, were isolated from wheat grain, wheat dust, pulse dust, soil and dead insects *E. coli* clone of CryIII was obtained from the Donald Dean Lab., and HD1, from ATCC.

B. thuringiensis cells were grown on petri plates of T3 medium, it contained 3g of tryptone, 2g of Tryptose, 1.5g of yeast extract, 0.005g of $MnCl_2.2H_2O$ 2.5 ml of 1M potassium phosphate (pH 6.9) and 15g of agar/liter.

Cultures were streaked on petri plates and incubated at 30±1°C for 3 to 5 days until sporulation took place. Cells were harvested by washing twice with autoclaved distilled water centrifugation at 7,000 rpm for 10 min., at 4°C. The pellet was resuspended in 50 mM sodium carbonate, pH 9.5 containing 10 mM dithiothreitol at 37°C for overnight. After centrifugation at 7,000 rpm for 10 min., at 4°C, the supernatant was collected and the concentration of soluble crystal proteins (protoxin) was quantified by the microassay method of Biorad using bovine serum albumen (Sigma) as a standard. The solubilized protoxin was activated by treating its 20 µg with 1 µg of trypsin. Solutions were mixed well and incubated at 37°C for 3 hours.

Insect rearing and toxicity assay

Tribolium castaneum were reared on a diet containing semolina and 10% yeast extract. We maintained 20 individual matting pairs in glass jar containing 250g of diet and covered it with muslin cloth. The rearing jar was placed in the insectory set at 30±1°C, a photoperiod of 16.8 (L:D) and a relative humidity of 50±5.

Larvicidal test was carried out with third instar of *T. castaneum* by incorporating suspension containing two-fold serial dilutions of activated proteins into the artificial diet and maintained according to the rearing conditions. Control bioassays were performed with solubilization buffer. Thirty larvae were used for each experiment and each experiment was duplicated. Mortality was counted after five days. LC_{50} and its 95% confidence limits were calculated with probit analysis (Raymond, 1985).

RESULTS AND DISCUSSION

During the present study, different strains of Bt were checked for larvicidal activity against the third instar larvae of *T. castaneum*. Most of the strains were collected from the province of Punjab, some from NWFP and few from Sindh. The main objective to collect the strains from different areas of country is to cover the heterogeneity exist among the populations of Bt. The sources of isolation for most of the strains selected for study were stored grain, and their dust (Table I). It was assumed that we could find novel and highly active strains by screening the host material available to *T. castaneum* in the natural environment.

Table I: Larvicidal activity of *Bacillus thuringiensis* samples collected from different sources and localities.

Isolate	Collection place	Source	LC_{50}*	LC_{50} 95% Confidence Limits	
				Lowest	Highest
CryIIIA	-	*E. coli* clone	0.65	0.40	2.42
SG 31.11	Shakargarh	Wheat grain	0.32	0.21	0.50
Hfz 24.8	Hafizabad	Wheat dust	0.60	0.34	0.79
GU 29.2	Gujranwala	Wheat dust	0.41	0.26	0.63
MR 1.7	Murid-K	Wheat dust	0.33	0.26	0.52
JR 6.3	Chitral	Soil	0.35	0.20	0.72
Hfz 26.8	Hafizabad	Wheat dust	0.30	0.21	0.47
Hfz 2.1	Hafizabad	Wheat dust	0.27	0.16	0.43
Gu 9.1	Gujranwala	Pulse dust	0.38	0.26	0.80
Gu 9.2	Gujranwala	Pulse dust	0.47	0.31	1.02
HM 10.4	Gujrat	Soil	0.32	0.20	0.59

*LC_{50}, concentration at which 50% of larvae were killed.

Of the total samples 1 isolate showed varying level of toxicity against *T. castaneum* larvae in the screening tests. CryIII A protein, obtained from *E. coli* cloned with CryIII gene, showed LC_{50} value as 0.65 µg/mg of diet.

Isolates SG 31.11, Hfz 24.8, HM 10.4, MR 1.7, JR 6.3, Hfz 26.8 and Hfz 2.1 were most toxic with LC_{50} values of approximately 0.3 µg/mg of diet; LC_{50} value of other strains ranged from 0.4 to 0.7 µg/mg of diet (Table I).

The active protein of SG 31.11 was sequenced and data showed that it resemble with a novel CryIII proteins reported by Sato *et al.* (1994). The toxic proteins of other strains were not characterized, so it is difficult to tell whether they are novel proteins or have any homology with existing Cry proteins.

The present study had shown that potential candidate Bt to use in the *T. castaneum* control program could be isolated from a variety of source materials. However, a qualitative survey of the most active isolates is necessary for further evaluation.

REFERENCES

ARONSON, A.I., BECKMAN, W. AND DUNN, P., 1986. *Bacillus thuringiensis* and related insect pathogens. *Microbiol. Rev.*, **50**: 1-24.

RAYMOND, M., 1985. Presentation d'un programme d'analysis log probit pour micro-ordinateur Cah. ORSTROM. Ser. *Entomol. Mer. Parasitol.*, **22**: 117-121.

SATO, R., TAKEUCHI, K., OGIWARA, K., MINAME, M., KAJE, Y., SUZUKI, M., HORI, H., ASANO, S., MORBA AND INAHANA, H., 1994. Cloning, heterologous expression and localization of a novel crystal protein gene from *Bacillus thuringiensis* server japonersis. *Curr. Microbiol.*, **28**: 15-19.

WHITELEY, H.R. AND SCHNEPF, H.E., 1986. The molecular biology of parasporal crystal body formation in *Bacillus thuringiensis*. *Ann. Rev. Microbiol.*, **40**: 549-576.

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