ROLE OF CERTAIN BIO-PESTICIDES IN PEST MANAGEMENT OF SOME ORGANIC VEGETABLES

By

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B.Sc. Agric. Sc. (Pesticides), Ain Shams University, 1999

M. Sc. Agric. Sc. (Pesticides), Ain Shams University, 2005

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ABSTRACT

Tamer Abdalla El-Mashtoly: Role of Certain Biopesticides in Pest Management of Some Organic Vegetables. Unpublished Ph. D. Thesis, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, 2010.

The present work was conducted to evaluate the efficiency of different biocontrol agents, prepared from *Bacillus thuringiensis* against certain insects, (i.e. *B. thuringiensis* serovar *japonensis* strain Buibui toxin to Oriental beetle, *Anomala orientalis* "Waterhouse" and Northern masked chafer, *Cyclocephala borealis* Arrow. *Bacillus thuringiensis* subsp *Kurstaki* and *aizawai* formulated as Dipel® DF and XenTari ®DF respectively, to black cutworm, *Agrotis ipsilon*). Also, to investigate the enhanced toxicity of *Btj, Btk and Bta* with the isolated associated bacteria. Data showed significant differences in LC_{50}'s for both pests, Oriental beetles and Northern masked chafers in autoclaved and non-autoclaved soil. Toxicity of *Btj* was varied tremendously according to the concentration used, soil type, organic matter %, and soil sterility. There were significant differences between Dipel and XenTari in controlling the 2^{nd} and 4^{th} instar of *Agrotis ipsilon*. Two of the isolated and associated hemolymph bacteria (NFD2 and FNFD1) showed significant synergistic effect to the toxicity of all the tested bio-agents versus their recommended pest. Five bacterial strains were isolated from hemolymph and identified using PCR technique with fifteen pairs of universal primers, and then 2% agarose gel electrophoresis was used to check the yielded DNA band. PCR products were purified using spin-column technology recommended for efficient recovery of DNA. The sequencing of both forward and reverse primer in all used primer sets were highly aligned (99-100%). This identity showed that PCR product was completely sequenced. The identification data emphasized that the enhanced bacterial strains were *Bacillus sp* and *Pseudomonas sp* for NFD2 and FNFD1, respectively.

Key words: **Enhancement, Biopesticides, *Bacillus thuringiensis* Associated bacteria, PCR, and DNA Sequencing.**

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I- INTRODUCTION

Over 90 species of naturally occurring, insect-specific (entomopathogenic) bacteria have been isolated from insects, plants, and the soil, but only a few have been studied intensively. Much attention has been given to *Bacillus thuringiensis*, a species that has been developed as a microbial insecticide **(Weeden *et al*., 2007)**.

Bacillus thuringiensis (*Bt*) is a common gram positive, spore-forming, soil bacterium. When resources are limited, vegetative Bt cells undergo sporulation, synthesizing a protein crystal during spore formation. Proteins in these crystals are called Cry (Crystal) endotoxins and have been known for decades to display insecticidal activity against specific insect groups **(Weeden *et al*., 2007)**. This insecticidal activity is primarily due to the production of a toxic protein crystal (the delta endotoxin) which accounts for 20-30% of the dry weight of sporulating cultures **(Fast, 1981)**. Genes responsible for expression of the delta-endotoxin have been reported to occur on large plasmids in many strains of *B. thuringiensis* **(Schnepf & Whitely, 1981; Klier *et al*., 1982 and Gonzalez *et al*., 1982).** The crystal is a protoxin composed of repeating polypeptide subunits, although the number and size of the polypeptides varies in different isolates **(Calabresde & Nickerson, 1980 and Fast 1981).** Cry1A toxins active against lepidopteran pests are synthesized as 130 kDa protoxins. In order to exert their toxic activity, crystal inclusions are ingested by larvae, dissolved and activated in the midgut lumen by proteases to yield a 60 kDa monomer. The activated toxin binds with high affinity (Kd,1 nM) to a cadherin receptor that is located in the apical membrane of midgut epithelial cells **(Vadlamudi, *et al.*, 1995)**. Binding of Cry1A toxin to cadherin induces oligomerization of the toxin, which is essential for membrane insertion**.** Even though insecticidal formulations based on *Bt* toxins have been used for many years, it was the development and commercialization of insect-resistant transgenic *Bt* crops expressing Cry toxins that revolutionized the history of agriculture. Benefits of this technology include high specificity and potency, reduction in chemical pesticide applications, and increased crop yield. Even though Cry toxins have been extensively used

commercially, the specificity of their mode of action are still controversial. This multi-step toxicity process includes ingestion of the Cry protein by a susceptible insect, solubilization, and processing from a protoxin to an activated toxin core in the insect digestive fluid. The toxin core travels across the peritrophic matrix and binds to specific receptors called cadherins on the brush border membrane of the gut cells. Toxin binding to cadherin proteins results in activation of an oncotic cell death pathway and/or formation of toxin oligomers that bind to GPI-anchored proteins and concentrate on regions of the cell membrane called lipid rafts. Accumulation of toxin oligomers results in toxin insertion in the membrane, pore formation, osmotic cell shock, and ultimately insect death. Whether oncosis, pore formation and/or both mechanisms are ultimately responsible for enterocyte death is still controversial **(Jurat-Fuentes, 2008)**.

An isolate of *Bacillus thuringiensis,* designated serovar *japonensis* strain Buibui, obtained from a soil sample collected in the vicinity of Tokushima, Japan exhibits strong larvicidal activity against several scarabaeid pest species **(Ohba *et al.*, 1992)**. The parasporal inclusions are spherical to ovoid in shape, showing a morphological similarity to those produced by the type strain of *B. thuringiensis* serovar *japonensis* **(Ohba and Aizawa, 1986).** The parasporal inclusion of *B. thuringiensis japonensis* is highly toxic to *Anomala cuprea* Hope, *A. rufocuprea* Motschulshy and *Popillia japonica* Newman in qualitative oral toxicity tests **(Ohba *et al.*, 1992).** When fed on bacterial amended humus, scarabaeid larvae were often killed within 1-2 days post feeding and 100% mortality occurred within 5-7 days **(Ohba *et al.*, 1992)**. No toxicity was shown by this isolate against larvae of Lepidoptera, Diptera, Orthoptera, and adults of chrysomelids **(Ohba *et al.*, 1992).**

The toxicity of *B. thuringiensis japonensis* varies significantly among scarabaeid species and life stages. **Suzuki *et al.*, (1992)** showed that 25 µg of 130 kDa protein per g compost was highly toxic (63 to 100% mortality in 21 days) against *Anomala albopilosa* Hope (1st instar), *A. rufocuprea* (3rd instar), *A. daimiana* Harold (1st instar), *A. shonfledti* Ohaus (3rd instar, *Mimela splendens* (Gyllenhal) (1st instar), and *A. orientalis* (2nd