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# SCOPE OF COMMERCIAL FORMULATIONS OF *BACILLUS THURINGIENSIS* BERLINER AS AN ALTERNATIVE TO METHYL BROMIDE AGAINST *TROGODERMA GRANARIUM* EVERTS LARVAE

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**Abstract**

Phasing out of ozone depleting pesticides like Methyl bromide has opened a new window for searching alternative pesticides to control insect and other pests of agriculture storage sector. Besides, concern has also been expressed by small farmers of the developing world that conventional chemical insecticides may occasionally result in poisoning of humans and livestock. Fortunately, *Bacillus thuringiensis* (Bt) has expressed very low mammalian toxicity and is considered as a valuable safe insecticide option for farmers. Different strains of *B. thuringiensis* have been shown to produce a number of insect toxins, which have shown effectiveness against different insect species belonging to the orders Lepidoptera, Diptera and Coleoptera. Due to the high activity, specificity to target pest species, and non-toxicity to human, strains of Bt form the basis for a number of commercial products to be used as pest control agents. The present investigations focus on use of commercial formulations of *Bacillus thuringiensis* as an alternative to methyl bromide for combating *T. granarium* infestation in stored wheat. The results have revealed that liquid formulations yielded better results as compared to the dry formulations. Maximum mortality of the larvae was observed in Ecotech Pro treated grains followed by Dipel ES, whereas, Bactospeine had not provided promising results. Inferences were therefore drawn that insect mortality depended upon ingestion of the Bt spores and toxins. It was also concluded that liquid formulations not only enhanced the moisture contents but also made the grain more palatable for *T. granarium* larvae resulting in more mortality as compared to the dry formulations.

## Introduction

Montreal protocol has deprived the world from Methyl bromide with effect from January 2005 due to its harmful effects on ozone layer (Taylor, 1994; Noling & Becker, 1994, TEAP, 2000a, Fields & White, 2002, EPA, 2006). There is no doubt that this chemical had been serving as an effective and broad-spectrum insecticide, fungicide, acaricide and bactericide since 1930s (Fields & White, 2001). Loss of methyl bromide has seriously affected stored grain industry where insect pests were successfully controlled by this chemical without having abnormal range of residual toxicity and pesticide resistance problems. Although many chemical and non-chemical alternatives have been proposed and researched, each has limitations that prevent it from being a direct replacement for Methyl bromide in all its current uses (Bell *et al*., 1996). Candidate fumigants include phosphine, sulfuryl fluoride, carbonyl sulfide, carbon dioxide, carbon disulfide, ethyl formate, ethylene oxide, hydrogen cyanide, methyl iodide, methyl isothiocyanate, ozone, sulphur dioxide, ethyl or methyl formate, and acetaldehyde (Annis & Waterford, 1996; MBTOC, 1998; TEAP, 2000b). However, phosphine ($PH_3$) has been used extensively as an alternative fumigant in the grain industry to counter infestations of live insects and their eggs (Annis, 2000; Williams & Ryan, 2000). Unfortunately stored

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grain pests such as Khapra beetle *Trogoderma granarium* Everts have shown resistance to this chemical. Price (1984) proposed *active exclusion* as a resistance mechanism, where $PH_3$ is kept out of the cell or away from the target site by some active transport process or by a permeability barrier. Chaudhry (1997) suggested that the reduced uptake of the compound might be either due to the presence of a phosphine-insensitive target site or a membrane-based efflux system that excludes phosphine gas in resistant insects. There is sufficient evidence in the literature that the pest has also shown resistance to some other groups of chemical insecticides. Among the stored grain insect pests, *T. granarium* is considered as one of the most notorious primary insect pests (Hill, 1983; Banks, 1977). In addition to direct loss caused by larval feeding, infestation is often followed by colonization of the secondary insect pests and fungi; consequently leading to deterioration in grain characteristics. Whereas chemicals used for controlling this pest have resulted in toxicity of the treated food grains ultimately creating some sanitary and phytosanitary problems. Biologists are therefore concentrating on controlling this pest with non- chemical alternatives including use of natural products, predators, parasitoids and microbes for the development of successful integrated pest management (IPM) approaches to meet the demands of HACCP (Hazard Analysis and Critical Control Points) (Quiniones, 1992; Copping & Menn, 2000). Luckily, a bacterium *Bacillus thuringiensis* Berliner has shown a promising role in controlling lepidopteron as well as coleopteran pests. The bacterium commonly known as Bt was initially isolated in 1902 in Japan from diseased silkworm larvae. Nine years later, the organism was again isolated from diseased Mediterranean flour moth larvae. The literature reveals that the pathogen was first used as an insecticide in an experiment during 1938 (Worthington, 1991). It took another 20 years to use *Bacillus thuringiensis* as biopesticide on commercial scale in the United States for the first time in 1958 (Ghassemi *et al.,* 1981). Nowadays, Bt is being widely used on food crops, forage crops and forests for Lepidopteran, Coleopteran and Dipteran insect pests control. From taxonomic point of view *B. thuringiensis* belongs to the family Bacillaceae, which has two genera *Clostridium* with 205 species and *Bacillus* with 33 species (Friel, 1981). There are about 19 subspecies in *B. thuringiensis* species, out of which five are being currently used for the control of different insect pests *viz-a-viz kurstaki* is being used for controlling forest pests; *israelensis*, for mosquito control; and *aizawai*, *morrisoni*, and *san diego* subspecies are being used for combating crop pests (Richard, 1992). There are many companies in the world, which are manufacturing commercial formulations of *B. thuringiensis s*uch as Dipel ES, Dipel 2X Bt subsp. *kurstaki* and Vectobac Bt subsp *israelensis* are the product of Abbot Laboratories, Bactospeine (Philips Duphar), Thuricide, Javelin and Teknar (Sandoz), Bathurin 82 and Moskitur (Slu1ovice), Coleoptera Bt *subsp. san diego* Trident (Sandoz), M-One (Mycogen) and Ecotech Pro is the product of Aventis **(**Taborsky, 1992; Thomson, 2005). Typical agricultural formulations include wettable powders, spray concentrates, liquid concentrates, dusts, baits, and time release rings. Conventional Bt products, which utilize naturally occurring Bt strains, account for approximately 90% of the world Microbial Pest Control Agent (MPCA) market. Most Bt products contain insecticidal crystal proteins (ICP) and viable spores, but in some Bt products, the spores are inactivated. Each year, around 13000 tonnes Bt pesticide is produced using aerobic fermentation technology (Anon., 1999). Conventional Bt products have been targeted primarily against lepidopteran pests of agricultural and forest crops. However in recent years, Bt strains active against coleopteran pests have also been marketed. Strains of Bt active against dipteran vectors of bacterial and viral diseases are being used in public health programs. Bt is toxic to the host pests in two ways i.e., by production of endotoxin in the mid gut of the insect pests that dissolves the gut and by germination of the spores, which may lead to infection and ultimate mortality of the insect hosts (Jenkins, 1992). Naturally occurring *B. thuringiensis* is rare and, when found, is predominately in the active or vegetative form and does not normally contribute significantly to insect mortality. However for pest control purposes, Bt is formulated using the dormant or spore form, which contains a crystalline toxin. The bacterium forms asexual reproductive cells, called endospores, which enable it to survive in adverse conditions. During the process of spore formation, Bt also produces unique crystalline bodies which when eaten, along spores act as poison in the target insects. Bt is therefore, referred to as a stomach poison. Bt crystals dissolve in the intestine of susceptible insect larvae. They paralyze the cells in the gut, interfering with the normal digestion and triggering the insect to stop feeding on host grains, plants or part of the plants. Bt spores can then invade other insect tissues, multiplying in the insect's blood, until the insect dies. Death can occur within a few hours to a few weeks after Bt application, depending on the insect species and the amount of Bt ingested. These are also frequently isolated from stored products (Norris, 1969; Burges & Hurst, 1977; DeLucca *et al*., 1982; Donovan *et al*., 1988; Burges & Jarrett, 1990). Similarly, DeLucca *et al*. (1979) described a new serotype, *B. thuringiensis* subsp *colmeri*, isolated from grain dust whereas, Donovan *et al*., (1988) isolated a novel strain of Bt from grain dust which is toxic to coleopteran larvae. Norris (1969) also reported one *B. thuringiensis* strain from stored products. Researchers have used serological and biochemical techniques to differentiate newly isolated *B. thuringiensis* strains obtained from different sources (Bonnefoi & Barjae, 1963; Ohba & Aizawa, 1978; Travers *et al*., 1987). Dulmage *et al*., (1981) studied the toxicity of approximately 320 strains of *B. thuringiensis* in 23 species of insects collected from insects, stored product residues and sericulture surroundings. They concluded that there was a fairly good but limited correlation between insecticidal activity and variety, as defined by serotyping.

The present study involves use of some commercial formulations of *Bacillus thuringiensis* against different strains of khapra beetle *Trogoderma granarium*. Mortality of the pest in response to the pathogen has been analyzed and discussed in detail to formulate a strategy for using such safe biological pesticides as an alternative to Methyl bromide and other hazardous chemical insecticides in general for stored grain insect pests.

## Materials and Methods

**Collection of insects:** Live adults and larvae of *T. granarium* were collected from wheat godowns, bins, silos etc. located in Rawalpindi and Lahore districts of the Punjab province. Only those godowns were selected having history of *T. granarium* infestation as well as phosphine fumigation for at least 3 years.

**Rearing of insects:** The collected insects were brought to the Entomology Laboratory of the University of Arid Agriculture, Rawalpindi and reared on wheat grains medium under optimum conditions. The insect pests collected from Rawalpindi and Lahore districts were labeled as RWP-05 and LHR-05, respectively. Identification of the stocks was verified by detailed morphological comparison with type specimens (Burges, 1957) kept in the laboratory. The insect stocks were cultured in properly labeled 500 ml glass jars having 200 g wheat with 14% moisture contents. Mouths of the jars were closed by muslin cloth to avoid interbreeding of the strains. The glass jars were placed in the Memmert incubator at 30ºC. Humidity inside the incubator was maintained at 70±2% by placing saturated salt solution of Sodium chloride contained in a dish (Winston & Bates, 1960).

**Table 1. Commercial formulations of *B. thuringiensis* used in the experiment.**

| S. No. | Generic name & Active ingredient | Potency | Trade name | Formulation type | Source |
|---|---|---|---|---|---|
| 1. | *Bacillus thuringiensis* subsp kurstaki strain EG 2348, transconjugant Bt *kurstaki* x Bt *aizawi* | 24,000 iu/mg | Ecotech Pro 7.5 | SC | Ecogen |
| 2. | *Bacillus thuringiensis* subsp *kurstaki* strain SA-11 | 16000 iuAK/mg | Bactospeine 20% | WP | Biochem product |
| 3. | *Bacillus thuringiensis* subsp *kurstaki* HD-1 | 17,600 iu/mg | Dipel ES 3.2% | WP | Abbot Laboratories |

***Bacillus thuringiensis* formulations:** Commercial Bt formulations used in the experiment are presented in Table 1. These included**, Ecotech Pro 7.5** (24,000 international units [iu]/mg formulation, 3% [ai] *B. thuringiensis* subsp. *Kurstaki*, strain *EG 2348, transconjugant* Bt *kurstaki* x Bt *aizawi* [Ecogen]; **Bactospeine**16000 iuAK/mg 3% [ai] containing viable spores of *B. thuringiensis* subsp. *Kurstaki* [Biochem product] and Dipel ES 17,600 international units [iu]/mg formulation, 3.2% [ai] spores of *B. thuringiensis* subsp. *Kurstaki,* [Abbott Laboratories, North Chicago, IL]. Dipel is based on *B. thuringiensis* subsp. *kurstaki* HD-1 (17,600 iu/mg [wettable powder]), which produces Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, and vegetative insecticidal protein (Schnepf *et al*., 1998). Ecotech pro was used in liquid form at concentrations of 24,000 iu/mg, 12000 iu/mg and 6000 iu/mg. The bio-insecticide was applied on Petri dish as well as mixed with grains used as diet in the medium for larvae. Dipel ES was also applied in liquid form using three levels viz-a-viz 17,600, 8800 and 4400 iu/mg. The dilutions were applied on the petridishes as well as mixed with grains fed to the larvae as their diet. Bactospeine was used in powder form at concentrations of 16000, 8000 and 4000 iu/mg, respectively by adding fine powdered milk made in dry dilutions. The powder was however sterilized before use. All biopesticides were applied at constant doses of 1, 0.5 and 0.25 gram or ml/kg wheat seeds resulting 100, 50 and 25% of the original labeled concentrations in iu/mg Bt spores.

**Bioassay:** For carrying out bioassay, 30 uniform sized larvae of *T. granarium* (3 week old) from each strain were taken from the jars and placed in the Petri plates already having grains and treated with different formulations of *Bacillus thuringiensis*. For each treatment four replicates were used in completely randomized design. The Petri plates were placed in the incubator at 28°C and 70% relative humidity (RH). The mortality data was recorded after 24, 48, 72, 120 and 240 hours, respectively. Mortality in control was also noted to correct the mortality data according to Abbot's formula (Abbot, 1925).

**Data analysis:** Statistical analysis was carried out in multi-factorial completely randomized designs (CRD) in MSTATC package (MSTATC, 1999) and the means were compared by Duncan's Multiple Range tests at 95% level of confidence (Gomez & Gomez, 1984). Mortality data was also subjected to probit analysis to determine LC-50 and LC-99 values at 95% fudicial limits (Finny, 1987).

## Results and Discussion

Statistical analysis of the data (Table 2) has shown highly significant differences among efficacy of different commercial formulations of *B. thuringiensis* against *T. granarium* larvae. The ANOVA table has further revealed highly significant effect of times factor and host strain on larval mortality. Interaction between times factor as well as Bt formulations have also been found significant which means that time played a key role in mortality of the test insects. Duncan's Multiple Range test further indicated that the *Trogoderma* strain LHR-05 was comparatively more susceptible than RWP-05 with the average mortalities of 54.16 and 46.85%, respectively (Table 3).

Comparison of mean mortalities of the *T. granarium* strains through DMR test (Table 4) revealed that performance of the commercial formulations was statistically different from one another. Ecotech Pro 1.0 indicated maximum (100 %) mortality, followed by Ecotech pro 0.5, Ecotech pro 0.25, Dipel ES 1.0, Dipel ES 0.50, Bactospeine 1.0, Dipel ES 0.25, Bactospeine 0.50 and Bactospeine 0.25 with mean mortality of 100.00, 94.44, 91.25, 48.54, 37.56, 29.30, 28.95, 15.27 and 9. 231%, respectively (Table 3 and Fig. 1). Dulmage and Collaborators (1981) demonstrated that *B. thuringiensis* strains active against lepidopteran larvae differ considerably in their potency and insecticidal spectra. Apparently, the highest mortality rates indicated by Ecotech Pro seems to be a product of its potency i.e., 24000 iu/mg which was greater as compared to the other two formulations Dipel ES and Bactospeine having potencies of 17,600 and 16,000 i.u/mg, respectively. The other possible reason for the highest performance of Ecotech pro may be its active ingredient i.e., the strain EG 2348 which is reportedly a hybrid of subsp Bt *kurstaki* and Bt *aizawi*. The active ingredient, in case of the other two formulations Dipel ES and Bactospeine have been reported as HD-1 and SA-11 strains of Bt *kurstaki*, respectively. The results are supported by the findings of some other workers who have revealed that potency, strain as well as subspecies of *B. thuringiensis* determine the specificity and toxicity of the pathogen against different insect groups (Françoise *et al*., 1987; Dong *et al*., 2004). Discovery of strains potent in Diptera (Goldberg & Margalit, 1977) and Coleoptera (Krieg *et al*., 1983) also demonstrated that spectrum of potential uses of Bt is even wider than initially believed. Further investigations are required to determine efficacy of different strains of Bt against coleopteron pests. Besides, lesser efficacy of powder formulations may also be correlated to hypothesis that inert material in liquid formulation of Ecotech Pro might be more effective against *T. granarium* larvae than crystal toxins as well as spores of *B. thuringiensis*. This hypothesis needs detailed probe into analysis as well as toxicity of inert material present in the commercial formulations against *T. granarium* larvae.

Probit analysis of the mortality data is shown (Table 5). Ecotech Pro has proved to be one of the most effective formulations with LC50 and LC 99 values of 0.049 X 1.6x $10^4$ and 0.925 X 1.6x $10^4$ iu/mg spores, respectively followed by Dipel ES with LC50 and LC 99 values of 1.123 X 1.76x $10^4$ and 31.782 X 1.76x $10^4$ iu/mg spores, respectively. Bactospeine represented least mortality as compared to former formulations with $LC_{50}$ values of 2.667 X 1.6x $10^4$ iu/mg and LC99 values of 21.093 X 1.6x $10^4$ iu/mg spores, respectively.

**Table 2. ANOVA showing effect of Bt formulations, host strain and time of exposure on mortality of *T. granarium* larvae.**

| S.O.V. | d.f. | Sum of square | Mean square | F-value | Prob |
|---|---|---|---|---|---|
| **BT formulations** | 8 | 483397.327 | 60424.666 | 15463.9412 | 0.0000 |
| ***Trogoderma* strains** | 1 | 5778.389 | 5778.389 | 1478.8112 | 0.0000 |
| Interaction (AxB) | 8 | 4044.268 | 505.534 | 129.3766 | 0.0000 |
| **Time of Exposure** | 5 | 57606.068 | 11521.214 | 2948.5206 | 0.0000 |
| AxC | 40 | 22779.325 | 569.483 | 145.7427 | 0.0000 |
| BxC | 5 | 519.171 | 103.834 | 26.5734 | 0.0000 |
| AxBxC | 40 | 8614.117 | 215.353 | 55.1133 | 0.0000 |
| **Error** | 324 | 1266.016 | 3.907 | | |
| **Total** | **431** | **584004.681** | | | |

Coefficient of variation: 3.91%

**Table 3. Duncan's Multiple Range Test showing susceptibility of *T. granarium* larvae to commercial formulations of *Bacillus thuringiensis* Ber.**

LSD value = 0.3742
s = 0.1345 at alpha = 0.050
$\bar{x}$

| Original order | | | Ranked order | | |
|---|---|---|---|---|---|
| RWP-05 | = | 46.85 B | LHR-05 | = | 54.16 A |
| LHR-05 | = | 54.16 A | RWP-05 | = | 46.85 B |

**Table 4. D.M.R. Test showing comparison of different Bt formulations in relation to mortality of *T. granarium* larvae.**

LSD value = 0.7938
s = 0.2853 at alpha = 0.050
$\bar{x}$

| Original order | | | Ranked order | | |
|---|---|---|---|---|---|
| Ecotech Pro 1.0 | = | 100.0 A | Ecotech Pro 1.0 | = | 100.0 A |
| Ecotech pro 0.5 | = | 94.44 B | Ecotech pro 0.5 | = | 94.44 B |
| Ecotech pro 0.25 | = | 91.25 C | Ecotech pro 0.25 | = | 91.25 C |
| Bactspeine 1.0 | = | 29.30 F | Dipel ES 1.0 | = | 48.54 D |
| Bactospeine 0.50 | = | 15.27 G | Dipel ES 0.50 | = | 37.56 E |
| Bactospeine 0.25 | = | 9.231 H | Bactspeine 1.0 | = | 29.30 F |
| Dipel ES 1.0 | = | 48.54 D | Dipel ES 0.25 | = | 28.95 F |
| Dipel ES 0.50 | = | 37.56 E | Bactospeine 0.50 | = | 15.27 G |
| Dipel ES 0.25 | = | 28.95 F | Bactospeine 0.25 | = | 9.231 H |

**Table 5. Concentrations of the Bt formulations required to produce 50 and 99% mortality of *Trogoderma granarium* larvae at 28ºC and 70% R.H.**

| Formulation | LC 50 (95% fudicial limit) | | | LC 99 (95% fudicial limit) | | | Slope |
|---|---|---|---|---|---|---|---|
| | Estimated* | Lower | Upper | Estimated | Lower | Upper | |
| **Ecotech Pro** 24000 iu/mg | 0.049 | 0.001 | 0.114 | 0.952 | 0.593 | 8.624 | 1.799824 ± 0.629955 |
| **Dipel ES** 17,600 iu/mg | 1.123 | 0.716 | 7.269 | 560.563 | 31.782 | 3125021696.00 | 0.862151 ± 0.303192 |
| **Bactospeine** 16000 iu/mg | 2.667 | 1.478 | 16.831 | 150.050 | 21.093 | 98373.141 | .329158 ± 0.364378 |

*Estimated values represent the percent of the original formulations in international units/mg

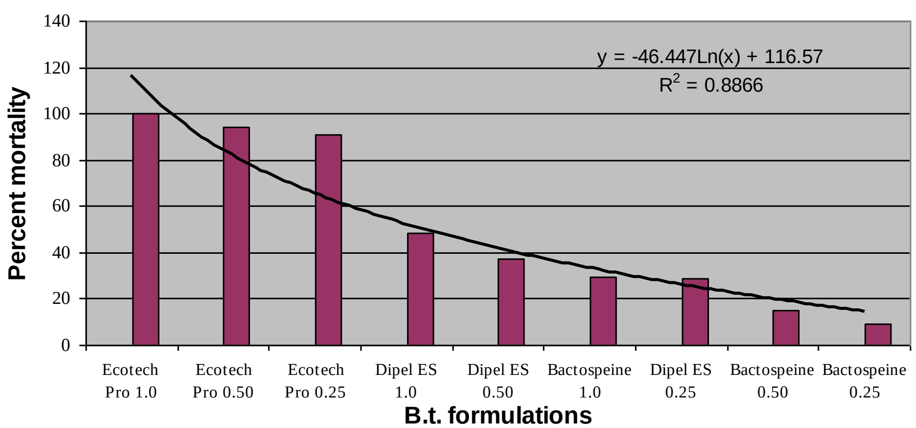


Fig. 1. Comparative efficacy of different B.t formulations against *T. granarium* larvae.

Promising potential of *Bacillus thuringiensis* against *T. granarium* is supported by the previous findings, which have revealed successful control of lepidopterous pests in cruciferous crops (Kennedy & Oatman, 1976; Wyman & Oatman, 1977). Successful control of Diamond back moth in Indonesia by Bt application is another evidence that it can be a potential alternative to chemical pesticides (Sastrodihardjo, 1986). Schesser (1976) also used commercial formulations of *B. thuringiensis* viz., Dipel ES, Thuricide and Bactospeine against Indian meal moth, *Plodia interpunctella,* with mortality ranging 40 to 98%. Higher mortalities from liquid formulations have revealed that insect pests prefer to feed on grains with high moisture contents. Hence, grain moisture also seems a contributing factor in pest control especially when we intend to apply insect pathogens, which also require moisture for their survival, growth and production of toxins.

## Conclusions

The present investigations have revealed that *B. thuringiensis* formulations have potential to become an alternative to Methyl bromide. However, extensive research is required to evaluate effect of different Bt subspecies as well strains against different orders and species of the stored grain pests. Future research work may also focus on the efficacy of inert material used in Bt formulations as well as importance of liquid or dust bases pertaining to palatability and intake of pathogens by the insect pests.

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