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Research Paper

POTENTIALS OF USING *Bacillus thuringiensis* BACTERIUM AS A BIOPESTICIDE IN STORED-PRODUCTS PROTECTION IN NIGERIA

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Abstract

B. thuringiensis is a bacterium that has wide applications as a biopesticide in pest control on several arable, ornamental and forest crops. and is ideally suited for use on stored grain and seed. It is compatible with most other protectants, fumigants, and seed fungicides, and the deposits on grain remain active indefinitely except under extremely high temperature conditions. Bt is available in dust, wettable powder, and liquid formulations that can be mixed with grain in augers or other handling equipment or applied directly to the surface of grain in storage. During *sporulation, many Bt strains produce crystal proteins (proteinaceous inclusions), called δ-endotoxins, that have insecticidal action. This has led to their use as insecticides, and more recently to genetically modify crops for resistance to pests, using Bt* genes. *A number of insecticides are based on these toxins. Bt is considered ideal for pest management because of its specificity to pests and because of its lack of toxicity to humans or the natural enemies of many crop pests. There are different strains of Bt each with specific toxicity to particular types of insects: Bt aizawai (Bta.) is used against wax moth larvae in honeycombs; Bt israelensis (Bti.) is effective against mosquitoes, blackflies and some midges; Bt kurstaki (Btk.) controls various types of lepidopterous insects, including the gypsy moth and cabbage looper. A new strain, Bt san diego, has been found to be effective against certain beetle species and the boll weevil. More than 150 insects, mostly lepidopterous larvae, are known to be susceptible in some way to Bt. In order to be effective, Bt must be eaten by insects in the immature, feeding stage of development referred to as larvae. It is ineffective against adult insects. Monitoring the target insect population before application ensures that insects are in the vulnerable larval stage, for effective Bt application*.

Key words: *Bacillus thuringiensis, Bacterium, Biopesticide, Endotoxins.*

INTRODUCTION

For over thirty years, pathogenic microorganisms have been studied as potential alternatives to chemical insecticides for controlling insect pests. This interest has been stimulated by the real and perceived problems and dangers associated with the use of chemical pesticides, particularly after the World War II; which includes environmental hazards, applicator safety, destruction of beneficial, organisms, predators and parasites, and pesticide resistance. In view of these problems, insect pathogens have been advanced as alternatives that were believed to be free of most, if not all of these problems because of their relative specificity for a few or sometimes only a single pest species, and because of their natural occurrence in the environment without any observed harmful effects. In addition, many scientists and environmentalists have come to believe that insect pathogens would be free of the resistance problem associated with most man-made chemicals and pests of grain and other stored commodities have not been neglected in this search for pathogens that could be used to mitigate insect damage. *Bacillus thuringiensis* (Bt) is the first microorganism to be approved for use in stored grain protection. It was one of the first biological control agents registered for use against insects in the U.S. and is the most widely used and intensively studied of the microbial insecticides. Bt is a naturally-occurring gram-positive, soil-dwelling, bacterium that produces poisons which cause disease in insects. It makes proteins that are toxic to immature insects (larvae). Apart from soil, Bt also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well on leaf surfaces, aquatic environments, animal feces, insect-rich environments, and flour mills and grain-storage facilities (Madigan and Martinko, 2005; du Rand, 2009).

There are many types of Bt, each targets different insect groups. The target insects include beetles, mosquitoes, black flies, caterpillars, and moths. Among the many recognized Bt subspecies, those commonly used as insecticides include subspecies *kurstaki* (Btk), *israelensis* (Bti) and *aizawa* (Bta).[9]

MODE OF ACTION OF *BACILLUS THURINGIENSIS*

Like certain members of the plant kingdom, such as ferns and mushrooms, *Bacillus thuringiensis* (Bt) forms asexual reproductive cells, called spores, which enable it to survive in adverse conditions. During the process of spore formation, Bt also produces unique crystalline bodies as a companion product. The spores and crystals of Bt must be eaten before they can act as poisons in the target insects. Bt is therefore referred to as a stomach poison. Bt crystals dissolve in response to intestinal conditions of susceptible insect larvae. This paralyzes the cells in the gut, interfering with normal digestion and triggering the insect to stop feeding on host plants. Bt spores can then invade other insect tissue, multiplying in the insect's blood, until the insect dies. Death can occur within a few hours to a few weeks of Bt application, depending on the insect species and the amount of Bt ingested (Ware, 1982).

Upon sporulation, *B. thuringiensis* forms crystals of proteinaceous insecticidal δ-endotoxins (called crystal proteins or cry proteins), which are encoded by *cry* genes (Nor-Am. 1985). Cry toxins have specific activities against insect species of the orders lepidoptera (moths and butterflies), diptera (flies and mosquitoes), coleoptera (beetles), hymenoptera (wasps, bees, ants and sawflies) and nematodes. *B. thuringiensis* therefore serves as an important reservoir of cry toxins for production of biological insecticides and insect-resistant genetically modified crops.

When insects ingest cry toxin crystals, their alkaline digestive tracts denature the insoluble crystals, making them soluble and thus amenable to being cut with proteases found in the insect gut, which liberate the toxin from the crystal (Sittig, 1980). The Cry toxin is then inserted into the insect gut cell membrane, paralyzing the digestive tract and forming a pore.[14] The insect stops eating and starves to death. Live Bt bacteria may also colonize the insect and further contribute to death (Sittig, 1980; Worthing, 1983; Meister, 1992).

Larvae affected by Bt become inactive, stop feeding, and may regurgitate or have watery excrement. The head capsule may appear to be overly large for the body size. The larva becomes flaccid and dies, usually within days or a week. The body contents turn

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brownish-black as they decompose. Other bacteria may turn the host body red or yellow (Hoffmann, 1993).

Action of Bacillus thuringiensis var. kurstaki on caterpillars

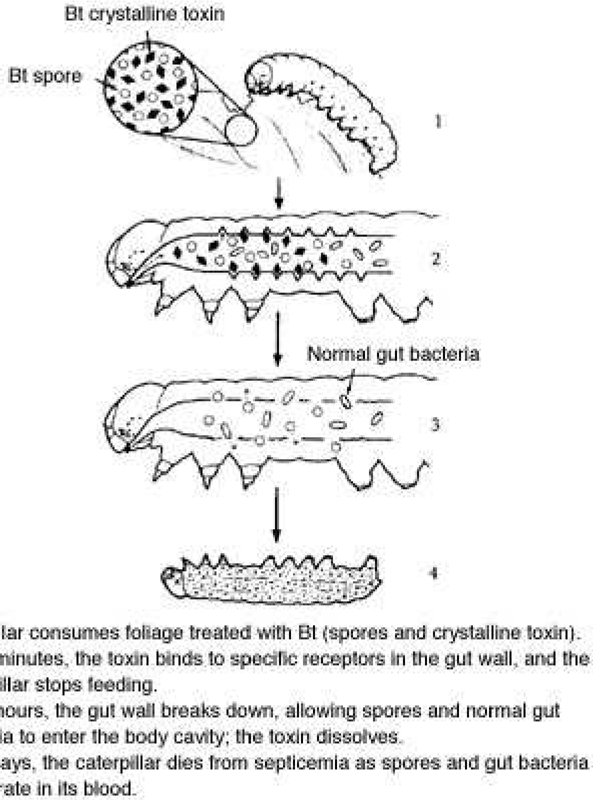

Diagram Courtesy of Abbott Laboratories Toxicology Files, 1982.

USES OF *BACILLUS THURINGIENSIS* (BT)

The crystalline insecticidal δ-endotoxin and the β-exotoxin of *Bacillus thuringiensis* (Bt), is one that is widely used in agriculture. Spores and crystalline insecticidal proteins produced by *B. thuringiensis* have been used to control insect pests since the 1920s and are often applied as liquid sprays. They are now used as specific insecticides under trade names such as DiPel and Thuricide. Because of their specificity, these pesticides are regarded as environmentally friendly, with little or no effect on humans, wildlife, pollinators, and most other beneficial insects, and are used in organic farming (Wikipaedia, 2015). Laboratory and pilot studies have shown that Bt applied to the surface layer of stored grain will provide up to 95% control of infestations of *Plodia interpunctella* and *Cadra cautella*. *Sitotroga cerealella* is somewhat less susceptible. The Laboratory studies indicated that a top-dressing treatment of the 10-cm deep surface layer of grain with Bt at a dosage of Ca. 125 mg/kg was an effective preventive measure (McGaughey, 1976). Such treatments were effective against both the almond moth and Indianmeal moth on a wide range of commodities, but showed only limited potential against the Angoumois grain moth, *Sitotroga cerealella* (Olivier), another insecticide resistant pest, because the larvae of that species feed inside the grain kernels (McGaughey, 1976; Nwanze et al., 1975; McGaughey and Kinsinger, 1978). Applications were most effective against all species when made prior to infestation of the grain because the early larval instars were much more susceptible than the later instars (McGaughey, 1978c).

Work by Dulmage (1981) and others show that Bt isolates differ extensively in their host spectrum and potency. This suggests that there is almost unlimited potential for identifying Bt isolates and producing formulations which will overcome this as well as future cases of Bt resistance. This resistance phenomenon also provides a new approach for investigating the mechanisms of Bt toxicity in insect larvae. Knowledge of the mechanism of this resistance could lead eventually to an understanding of the mechanisms controlling the host specificity of the organism. Once this is achieved there are almost limitless possibilities for using both conventional genetic techniques and genetic engineering technology to produce Bt toxins with activity against a broader range of pest species, includ1ng the coleopteran pests of stored grain. The potential for using Bt for controlling coleopteran pests has already been demonstrated with the recent reports on *B. thuringiensis* subsp. *tenebrionis* by Krieg et al. (1983) in West Germany, and on a similar if not identical Coleoptera-active isolate by Herrnstadt et al (1986) in the U.S.
So far Bt has been used only on grain, but there is also great potential for using existing Bt preparations for controlling pests in the wide range of raw and processed commodities in which lepidopteran pests predominate. Bt spores are readily killed in cooking cereal products and have no effect on baking properties of flour (McGaughey et al., 1980).

USE OF BT GENES IN GENETIC ENGINEERING OF PLANTS FOR PEST CONTROL
Bt-corn is a type of genetically modified organism, termed GMO. A GMO is a plant or animal that has been genetically modified through the addition of a small amount of genetic material from other organisms through molecular techniques. Currently, the GMOs on the market today have been given genetic traits to provide protection from pests, tolerance to pesticides, or improve its quality. Examples of GMO field crops include Bt-potatoes, Bt-corn, Bt-sweet corn, Roundup Ready soybeans, Roundup Ready Corn, and Liberty Link corn. Bt delta endotoxin is highly effective at controlling Lepidoptera larvae, caterpillars. It is during the larval stage when most of the damage by European corn borer occurs. The protein is very selective, generally not harming insects in other orders (such as beetles, flies, bees and wasps). For this reason, GMOs that have the Bt gene are compatible with biological control programs because they harm insect predators and parasitoids much less than broad-spectrum insecticides. The Bt endotoxin is considered safe for humans, other mammals, fish, birds, and the environment because of its selectivity. These products have an excellent safety record and can be used on many crops until the day of harvest.

TOXICOLOGICAL EFFECTS OF *BACILLUS THURINGIENSIS* ON HUMANS AND OTHER NON-TARGET ORGANISMS
A wide range of studies have been conducted on test animals and man, using several routes of exposure to *B. thuringiensis*. The results of these tests suggest that the use of Bt products has no negative effects on non-target organisms. No complaints were made after eighteen humans that ate one gram (g) of commercial Bt preparation daily for five days, on alternate days; while some inhaled extra 100 milligrams (mg) of the powder daily, in addition to the dietary dosage (Hayes, 1982). Also, humans who ate one g/day of Btk for three consecutive days were not poisoned or infected (US, EPA, 1986). B.t. did not have acute toxicity in other tests conducted on birds, dogs, guinea pigs, mice, rats, humans, or other animals. When rats were injected with B.t.k., no toxic or virus-like effects were seen. No oral toxicity was found in rats, mice or Japanese quail fed

protein crystals from B.t. var. *israelensis* (Roe, et. al., 1991). No toxic effects were observed in rats that had a B.t. formulation put directly into their lungs, at rates of 5 mg/kg of body weight (Abbott Laboratories, 1982).

Bt toxins present in Peanut leaves (image on the right hand side) protected it from extensive damage caused by Cornstalk Borer larvae (Left-side image).

While B.t. interferes with insect digestion, it does not persist in the digestive systems of mammals that ingest it. When placed in the eyes of rabbits, Bt var. *israelensis* was still present after 1 week, but there was no infection or other harmful effect to the eye. When injected into the gut of mice, Bt var. *israelensis* was detected in the spleen and heart blood for as long as 80 days, but there were no infections (Spiegel and Shadduck, 1990). B.t. crystals however have caused deaths in test animals when they were injected directly into the abdominal cavity. This suggests that B.t. can be toxic to mammals, but that when exposure is through normal routes of exposure (oral, dermal or inhalation), metabolism or elimination of the toxin prevents poisoning in mammals (Roe, et al.,1991). B.t. is not toxic to birds (Agriculture Canada. 1982; Meister, 1992). It biodegrades and does not persist in the digestive systems of birds (NCAMP, 1986). B.t. has not been reported as having harmful effects in fish (Agriculture Canada. 1982). Rainbow trout and bluegills exposed for 96 hours to B.t. technical material, at concentrations of 560 and 1,000 parts per million (ppm), did not show adverse effects. A small marine fish (*Anguilla anguilla*) was not negatively affected by exposure to 1,000-2,000 times the level of B.t. expected during spray programs. Field observations of populations of brook trout, common white suckers and smallmouth bass did not reveal adverse effects one month after aerial application of the B.t. formulation (Abbott Laboratories, 1982). Applications of labeled rates of formulated B.t. have not been toxic to beneficial or predator insects (Abbott Laboratories, 1982). Treatment of honeycombs with B.t. var. *aizawai* will not have a detrimental effect upon bees, nor on the honey produced (Berg, 1986). Normal exposure rates do not cause harm to honey bees.

CONCLUSION

The full potential for using microbial insecticides for protecting stored commodities is far from being fully realized, but it has demonstrated that microbial insecticides are a viable alternative to the broad spectrum chemicals that we have relied upon for over 50 years. Furthermore, the work has demonstrated that microorganisms are compatible with other technologies in our pest management systems and that they are cost-competitive with our traditional measures. It has also shown that our storage facilities provide an ideal environment for using microorganisms, an environment where the organisms will remain viable and provide long term insect control**.** B.t. products should be stored in a cool, dry place. Some loss of effectiveness can be expected in products stored for more than six months (Agriculture Canada, 1982.). Formulated products are compatible with most insecticides, acaricides, fungicides and plant growth regulators.

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