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Short Article

A review of *Bacillus thuringiensis* (*Bt*) production and use in Cuba

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Introduction

The insecticidal potential of bacteria belonging to the species *Bacillus thuringiensis* (*Bt*) has been known since 1915. Nowadays *Bt* is the principal biopesticide produced and used worldwide, and commercial products based on this bacterium make up over 90% of all biological products used in plant protection.

The active ingredients in these biopreparations are biotoxins and bacterial spores, which together act on susceptible insects to cause their death. Studies carried out by various researchers since the early twentieth century have allowed the broadening of the action spectrum of these microorganisms to a significant number of insect pests of agriculture and of disease vectors. Some examples are mosquito control and control of defoliating lepidopteran larvae that cause major losses in crops such as brassicas and maize. More recently, methods including genetic engineering have resulted in *Bt* strains that attack mites, nematodes and protozoa, further expanding their potential as biocontrol agents.

History of *Bt* Use in Cuba

Commercial *Bt* products from the USA, France and the former Soviet Union were first trialled in Cuba in the 1960s, for use against tobacco budworm *Heliothis virescens* (F.) and grass looper *Mocis latipes* Guenée (Lep., Noctuidae), both serious pests in the country. As a result of these studies and the high price of the products in the global market, Cuba started to develop simple and cost-effective, cottage-industry level production methods for *Bt*. The aim of in-country *Bt* production was to guarantee efficient and stable production, sufficient to meet the demand for control of these and other pests.

Bt biopesticides are now produced in Cuba, both by a cottage-industry method (using static liquid culture based on waste products from the sugar industry or other crop production) and by a fermentation process in production units designed and constructed entirely in Cuba, including the fermentation equipment. Fermentation plants produce *Bt* in liquid concentrate form, which can be stored for six months.

Production Strategies

Cottage-industry *Bt* production is carried out through a network of 220 centres known as CREES (Centres for Reproduction of Entomophages and Entomopathogens), which are distributed throughout the country and have enabled extensive use of these biopesticides in Cuban agriculture. The process involves bacterial culture in liquid media consisting of agricultural or industrial by-products or waste. The precise composition of the culture media can be adjusted according to the local availability of materials, thanks to research into suitable nutrient concentrations of different media and characteristics of bacterial growth on these media. Sterilized glass flasks with a volume ratio of 1:5 are used for the cultures and kept at temperatures of 28-30°C for 10-15 days after inoculation, according to isolate and medium. Preservative is added to the harvested product which enables it to be stored for up to three months at temperatures up to 25°C. Production costs per litre are approximately two Cuban pesos (equivalent to two US cents at the external 1:1 rate, but in fact much less).

Bt biopesticides are produced by fermentation in three Biopesticide Production Plants: two in Havana Province and a third in the central region. This industrial process results in a liquid concentrate obtained via a sedimentation stage after fermentation. Fermentation process products contain higher concentrations of infective units (spores and crystalline toxins) and stability can be maintained for up to six months at ambient temperature. Production time is much shorter and concentrate can be obtained in 72-96 hours depending on the strain and culture medium. Media are prepared based on yeasts or other sources of nitrogen and starches. *Bt* production efficiency in these plants exceeds 90% and production costs are in the range 50-60 Cuban cents per litre (equivalent to 50-60 US cents).

Bt Product Types

The need to control different pests, some of which are resistant to control by *Bt*, provided the impetus to diversify *Bt* production using isolates with more specific host ranges or greater pathogenicity to certain pests. In other cases, it is essential to alternate effective isolates against the same pest, to prevent the development of resistance. Such is the case for diamondback moth, *Plutella xylostella* (L.) (Lep., Plutellidae) which has been managed successfully for several years without any indication of *Bt* resistance, due precisely to rotation of products containing different *Bt* isolates.

Via static liquid culture, Cuba produces three *Bt* strains for Lepidoptera control and one for mites. Via submerged culture, the *Bt* industrial plants produce four different products registered under the name of THURISAV, each specific for particular pests. For example, THURISAV 24 is recommended for control of *Spodoptera frugiperda* (J. E. Smith) (Lep., Noctuidae) in maize, whilst THURISAV 26 is used preferentially against *Heliothis* spp. in tobacco.

Quality Control of *Bt* Products

Production quality is controlled in all cases by means of a State Standard for Quality Control. This standard sets out the specifications and requirements to be followed, both in the production process and for the final product. Quality control includes product purity; concentration of infective units; and biological efficacy (the last criterion assessed by laboratory bioassays). Field efficacy is also monitored by plant protection staff employed by the agricultural enterprises applying the *Bt* products. All products are registered in the Pesticide Registration Office, assigned to the national Centre for Plant Health, which is the regulatory agency for all biopesticide production throughout Cuba.

Production and Field Use

Cuban *Bt* products are used mainly in the control of the following pests: tobacco budworm (*H. virescens*), grass looper (*M. latipes*), diamondback moth (*P. xylostella*), maizeborer (*S. frugiperda*), cassava hornworm (*Erinnyis ello* L.; Lep., Sphingidae), potato leafminers (*Liriomyza* spp.), citrus leafminer (*Phyllocnistis citrella* Stainton; Lep., Gracillariidae), squash pickleworm (*Diaphania* spp.) and other lepidopteran defoliators in vegetables. The acaricide product is also used for mite control in citrus, potato and plantain. Strains of *Bt* var. *israelensis* are used for control of mosquito disease vectors.

Application rates vary in the range 3-5 litres/ha, according to product, pest and crop, with spore concentrations of 10^8-10^9/ml (8000-15,000 Infective Units). During 1997, over 1000 tonnes of *Bt* were produced in Cuba, 24% by industrial fermentation and 76% via solid substrate culture. This output enables more than 200,000 ha per annum to be treated with *Bt*, at a cost of between two and ten Cuban pesos per hectare.

One important consideration is the advanced level of farmer knowledge and acceptance of the use of *Bt* products, which ensures high field efficacy and consequently high demand. Biopesticides were first used on a massive scale in Cuba from the late 1980s, when availability of chemical pesticides was drastically reduced. However, Cuban producers have now come to appreciate the advantages of biopesticides and their superior performance compared to chemicals, particularly in the case of pests such as *P. xylostella* which had developed resistance to chemical products.

Perspectives

New *Bt* products for control of Coleoptera and nematodes are currently under development, as well as refinement of product harvesting, and formulation in powder and liquid form, in order to make Cuban *Bt* competitive on the world market.

We believe that the production and use of *Bt* in Cuba has enabled important pest problems to be overcome, including those which could no longer be controlled by any chemical insecticides, such as was the case for *P. xylostella* in cabbage and watercress. In addition, the ability to deploy different *Bt* products has encouraged application strategies which avoid rapid development of insect resistance. Further outstanding achievements are the savings for the Cuban economy in terms of no longer importing *Bt* products, and the significant reduction in imported chemical pesticides, which were traditionally used to combat pests now controlled by our own national *Bt* products. Furthermore, extension efforts have borne fruit in the current attitudes and perceptions of Cuban farmers towards the use of *Bt* products, enhanced by the successes obtained in recent years in management of pests which were conventionally hard to control even with chemical products.

Further Reading

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