



**AQUAFIX**  
Wastewater Laboratories  
University of Wisconsin Research Park

# ***Bti*** Endotoxins

THE SCIENCE BEHIND AQUABACxt



Aquafix Laboratories

[info@teamaquafix.com](mailto:info@teamaquafix.com)

888-757-9577

## Bacillus thuringiensis

Aquafix's AQUABACxt contains *Bacillus thuringiensis* subsp. *israelensis* (*Bti*), a naturally occurring biological larvicide. This paper will discuss what *Bti* is and how it works.

*Bti* was first discovered at a mosquito breeding ground in 1976 from the soil of a stagnant pond located in the Nahal Besor Desert River Basin of Israel (Barjac & Sutherland, 1990). The following year, *Bti* was discovered to be an extremely effective larvicide to mosquitoes and black flies (Barjac & Sutherland, 1990) due to the insects' ability to activate the release of *Bti* toxins.

**Figure 1.**

Nahal Besor Desert River Basin of Israel

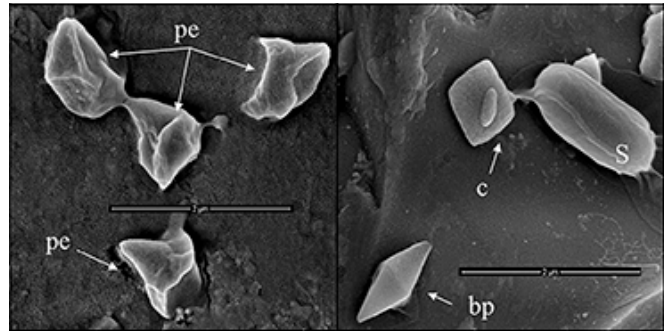


In wastewater, *Bti* was discovered to be effective against red worms/midge flies and bristle worms. The discovery of *Bti* and its capabilities is important not only due to its pest control properties, but because it poses less of a threat to non-target species, such as humans, fish, and birds in comparison to chemical larvicides, such as Methoprene.

*Bti*, when in its spore-forming stage, produces a protein crystal, or a parasporal body. This parasporal body consists of six  $\delta$ -endotoxins. These six  $\delta$ -endotoxins consist of four major polypeptides and at least two minor ones (Ben-Dov, 2014). Each of these toxins have been isolated and individually compared to the potency of the entire parasporal body; None were as toxic as the entire body (Hughes et. al., 2004).

**Figure 2.**

Scanning electron microscopy photo of *Bti* crystals (Nair et. al., 2018)



When target species consume *Bti*, the  $\delta$ -endotoxins bind to epithelial cells within their stomach lining where the internal stomach alkalinity activates the parasporal body toxins. The toxins are then released, causing the stomach to swell and burst. This leads to starvation followed by death.

Unlike target species, humans have an acidic stomach, therefore do not have the ability to activate the secretion of *Bti* toxins (Canadian Government, 2011). Due to this difference, *Bti* is not toxic to humans. However, *Bti* should still be handled with gloves and goggles due to the potential risk of eye and skin irritation. Further research also has shown *Bti* to be non-toxic to other mammals. Mammals are not the only non-target species researched. A study conducted in Florida using golf course ponds showed that *Bti* had no negative effect on microorganisms, such as daphnia and rotifers (Ali, 1981). The same cannot be said for non-target species when chemical larvicides, such as Methoprene, are used.

The most common alternative to *Bti* treatment for wastewater midge larvae is a chemical larvicide, specifically Methoprene. Methoprene utilizes a different control mechanism and can have more adverse side effects compared to *Bti*. Methoprene works by acting as an artificial hormone regulator; This hormone regulator disrupts the life cycle by preventing adulthood from being reached (National Pesticide Information Center, 2012). If insects are unable to reach adult age, they are unable to reproduce, therefore controlling the population.

Even though Methoprene works as a hormone regulator, it can be toxic to non-target species, such as some fish and crustaceans (National Pesticide Information Center, 2012). Methoprene also differs from *Bti* as it has been proven resistance to Methoprene can occur (Huffaker et. al., 1976).

The chance of target species developing a resistance to *Bti* is very low (Land et. al. 2019). For nearly 35 years, resistance to *Bti* has been extensively researched in the field with no signs of occurring (Ben-Dov, 2014). It is theorized that due to their different modes of action and synergism with each other the four major toxins are considered to be why resistance to *Bti* does not occur (Ben-Dov, 2014). The improbability of target species gaining resistance to *Bti* is just one of the many reasons it is becoming a more widespread and favorable larvicide.

## AQUABACxt and BugJuice

Many operators prefer to use AQUABACxt with BugJuice. This is because BugJuice degrades the cocoons that protect the red worms. Cocoons are made of TSS and undigested BOD which includes F.O.G. and plant and paper fibers. BugJuice degrades these cocoons, making the red worms more susceptible to be killed by AQUABACxt. Cocoons are seen on the weirs in the clarifiers and are also present in the return lines.

At Aquafix, we pride ourselves in being environmentally friendly with all our products. This is why Aquafix chose *Bacillus thuringiensis* subsp. *israelensis* to be in our EPA-registered product, AQUABACxt.

**Figure 3.**

Red worm cocoons coating a clarifier rake



## References

- Ali, A. (1981). *Bacillus thuringiensis* serovar. *israelensis* (ABG-6108) against Chironomids and some nontarget aquatic invertebrates. *Journal of Invertebrate Pathology*, 38, 264-272.
- Barjac, H.D. & Sutherland, D. J. (Eds.) (1990). *Bacterial control of mosquitoes & black flies: Biochemistry, genetics, & applications of bacillus thuringiensis israelensis and bacillus sphaericus*. Rutgers.
- Ben-Dov, E. (2014). *Bacillus thuringiensis* subsp. *israelensis* and its Dipteran-specific toxins. *Toxins*, 6, 1222-1243.
- Canadian Government. (2011). *Bti- Bacillus thuringiensis subspecies israelensis*. [https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/cps-spc/alt\\_formats/pdf/pubs/pest/\\_fact-fiche/Bti-eng.pdf](https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/cps-spc/alt_formats/pdf/pubs/pest/_fact-fiche/Bti-eng.pdf)
- Glare, T.R. & O'Callaghan, M. (2000). *Bacillus thuringiensis: Biology, ecology, and safety*. Wiley.
- Hughes, P.A., Stevens, M.M., Park, H., Federici, B.A., Dennis, E.S., & Akhurst, R. (2005). Response of larval *Chironomus tepperi* (Diptera: Chironomidae) to individual *Bacillus thuringiensis* var. *israelensis* toxins and toxin mixtures. *Journal of Invertebrate Pathology*, 88, 34-39.
- Land, M., Bundschuh, M., Hopkins., R.J., Poulin, B., & McKie, B.G. (2019). What are the effects of control of mosquitoes and other nematoceran Diptera using the microbial agent *Bacillus thuringiensis israelensis* (*Bti*) on aquatic and terrestrial ecosystems? A systematic review protocol. *Environmental Evidence*, 8:32.
- Nair, K., Al-Thani, R., Al-Thani, D., Al-Yafei, F., Ahmend, T., & Jaoua, S. (2018). Diversity of *Bacillus thuringiensis* strains from Qatar as shown by crystal morphology,  $\delta$ - endotoxins and Cry gene content. *Frontiers in Microbiology*, 9, 708. <https://www.frontiersin.org/article/10.3389/fmicb.2018.00708>
- National Pesticide Information Center. (2015). *Bacillus thuringiensis (bt) general fact sheet*. <http://npic.orst.edu/factsheets/btgen.html>
- National Pesticide Information Center. (2012). *Methoprene general fact sheet*. <http://npic.orst.edu/factsheets/methogen.html>
- Newsom, L.D., Smith, R. F., & Whitcomb, W. H. (1976). Selective pesticides and selective use of pesticides. In C.B. Huffaker & P.S. Messenger (Eds.), *Theory and practice of biological control* (pp. 565-591).
- Pérez, C., Fernandez, L.E., Sun, J., Folch, J.L., Gill, S.S., Soberón, M., & Bravo, A., (2005). *Bacillus thuringiensis* subsp. *israelensis* Cyt1Aa synergizes Cry11Aa toxin by functioning as a membrane-bound receptor. *Proceedings of the National Academy of Sciences*, 102(51), 18303-18308.