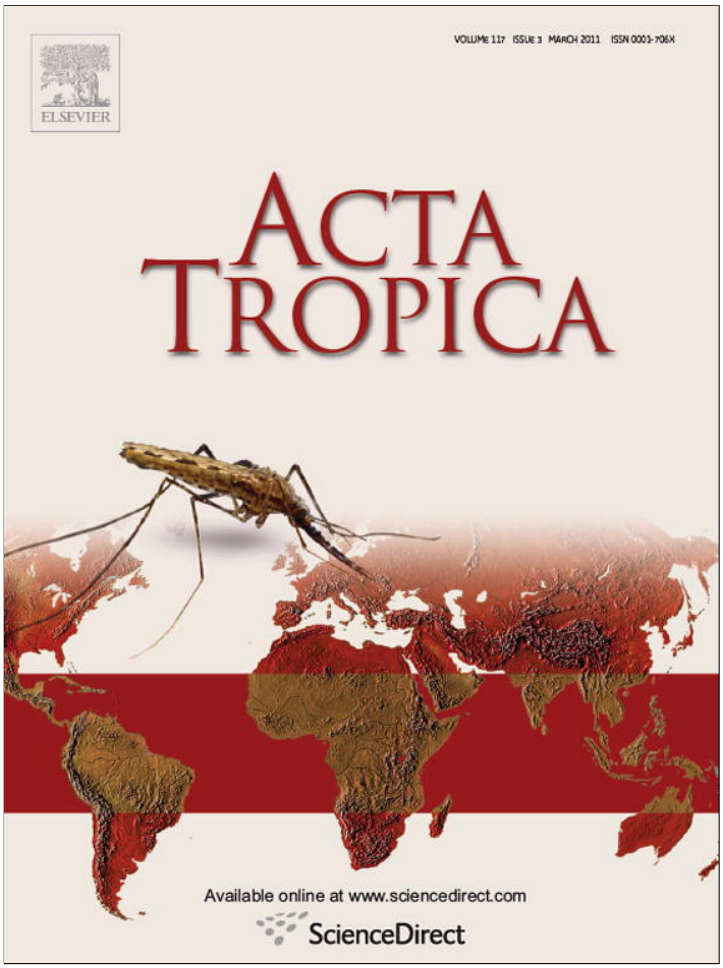

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Short communication

Modular columns to study depth-dependence behavior of mosquito larvae and toxicity of *Bacillus thuringiensis* subsp. *israelensis*

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ABSTRACT

Modular transparent column system was designed to study depth-dependence behavior of mosquito larvae. The system was used in preliminary experiments to evaluate the effect of water depth on the larvicidal activity of *Bacillus thuringiensis* subsp. *israelensis* de Barjac against bottom feeder larvae of *Aedes aegypti* (Linn.) (Diptera: Culicidae), and suggestions for increasing the efficiency of the device are discussed.

Bacillus thuringiensis subsp. *israelensis* (Goldberg and Margalit, 1977; de Barjac, 1978) is known to be one of the most promising biological alternatives in vector disease control programs around the world (Margalith and Ben-Dov, 2000). Many studies have been conducted testing the influence of biotic and abiotic factors on the larvicidal activity of *B. thuringiensis* subsp. *israelensis*, under laboratory and field conditions (Boisvert and Boisvert, 2000).

Sinking of the toxin is one reason for the difference in larval susceptibility of various mosquito species (Aly et al., 1988; Mulla, 1990). Larvae of *Anopheles* spp. filter under the surface area of the water (surface feeders), whereas larvae of *Aedes* spp. can dive (bottom feeders). This difference in feeding habits partially explains the different levels of larval susceptibility to the toxin. While the toxin is sinking below water level, surface feeder larvae are no longer exposed to it as opposed to bottom feeder species. One of the abiotic parameters influencing mosquito larvicidity of *B. thuringiensis* subsp. *israelensis* is water depth (Mulla, 1990; Aldemir, 2009), which should thus be considered in field applications (Liber et al., 1998; Russell and Kay, 2008). It is difficult to take into account water depth in natural water body experiments due to variations in nature (Liber et al., 1998). It is much easier to reach unified and controlled conditions in experiments conducted in the laboratory or at least in artificial water vessels. The efficacy of larvicides has been tested in cups (Manasherob et al., 1996; Nayar et al., 1999; Stevens et al., 2004), jars (Setha et al., 2007), tanks (Amalraj et al., 2000; Vilarinhos and Monnerat, 2004; Lima et al., 2005) and even swimming pools (Kahindi et al., 2008). A new system for this purpose was designed and is described here, that requires small amounts of water and *B. thuringiensis* subsp. *israelensis*. The advantages of this simple system are discussed, preliminary experiments were performed to demonstrate its applicability, and possible improvements are proposed.

1. The modular column system (Fig. 1)

The system is composed of three types of light, modular units made of transparent PVC, each 250 mm long and 50 mm internal width, with wall thickness of 2.5 mm. A column is assembled by several units flanked by connectors made of grey PVC with 10 mm wall thickness and built-in rubber band for water-proof sealing (Fig. 1A), on top of a bottom unit that is glued to a base plate of 200 mm diameter (Fig. 1B). Each column is composed of one base plate and connector-included units, the number of which varies according to the desired height (Fig. 1D). A column may or may not include one unit with a net (sieve size 0.45 mm made of 0.6 mm stainless steel wires) at its base (Fig. 1C). Several column assemblies (with heights of 50–175 cm) are depicted in Fig. 1D.

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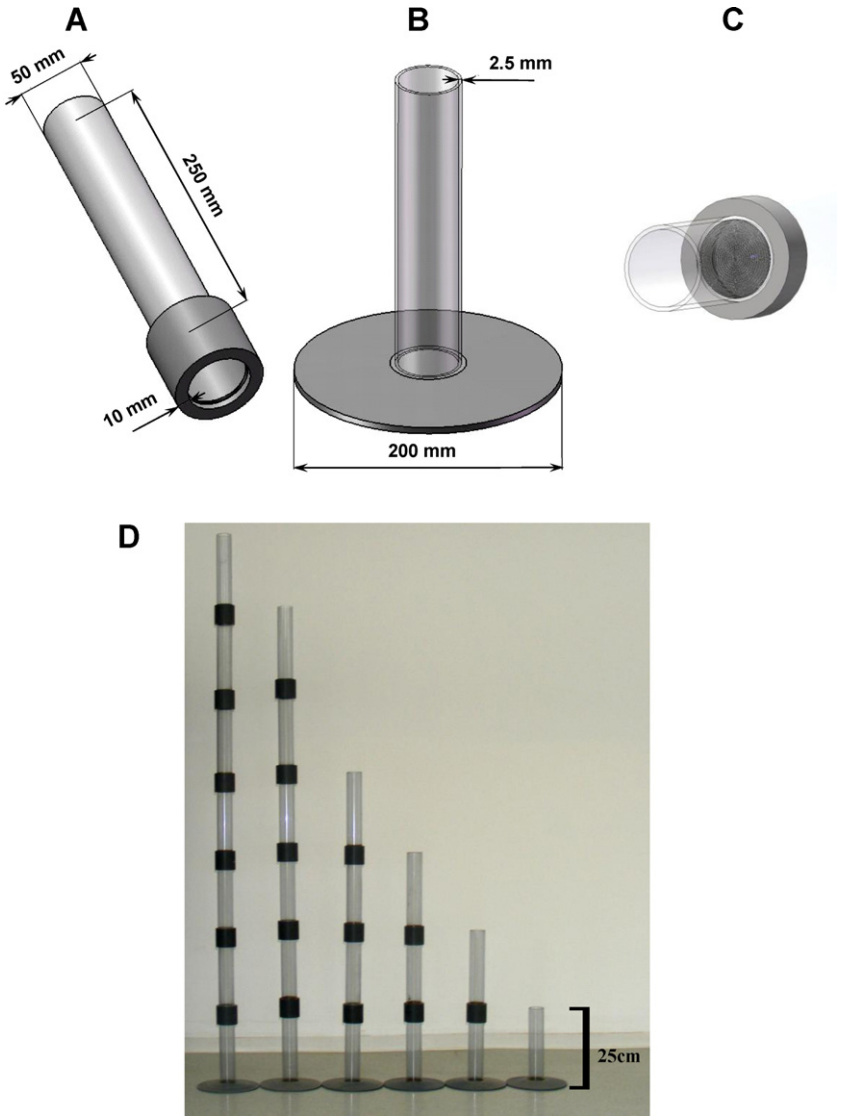

Fig. 1. Schematic representation of the three component units of the transparent water column assemblies (A–C) and a picture of several assembled columns (D).

2. Diving behavior of mosquito larvae

The transparent columns allow the observer to follow the larvae under controlled, defined conditions (e.g., the desired temperature and light intensity) in discrete depths. As an example, larval diving behavior was easily traced. As anticipated, larvae of *Aedes aegypti* reached the bottom, spending about 3-fold longer in 75 cm than in 150 cm columns (4.4 larvae ± 1.8 and 1.4 ± 1.1, respectively). No anopheline larva reached these depths during the 2 h period of examination (data not shown).

3. Water depth and mortality of *B. thuringiensis* subsp. *israelensis*-exposed mosquito larvae

To evaluate how water depth and sinking of *B. thuringiensis* subsp. *israelensis* powder (spores and toxins) affect mortality of *Ae. aegypti* larvae, units with nets (Fig. 1C) were inserted at different levels (25–150 cm) to a series of columns, all 150 cm high (Fig. 1D). Twenty or forty larvae were added to the columns containing powder at final concentrations of 15 and 25 ng ml^{-1} respectively, and the number of survivors was counted after 24 h. Larval mortality increased from about 15% at net-depths of 25 and 50 cm to an average of ca. 60% at net-depths above 75 cm (Fig. 2), consistent with diving behavior. The larger water volume above the net (about 0.5 l per column unit) and hence higher total amount of toxin that resulted in accumulation of larger amounts of the toxin that sank on the grids may be responsible for the increased mortality of the bottom feeder *Ae. aegypti* larvae.

Sinking of the toxin was tested in another set of preliminary experiments, where 40 larvae of *Ae. aegypti* were added different times (0–10 h) after *B. thuringiensis* subsp. *israelensis* powder (30 ng ml^{-1}) to water columns with nets 50 and 75 cm deep, and mortality counted after 24 h exposure. Mortality increased from about 30% at 2 h interval to values markedly higher (95%) in the deeper column than in the shallower (45%) at 10 h interval between addition of *B. thuringiensis* subsp. *israelensis* and larvae. The increased mortality with time can be explained similarly as before (Fig. 2): the bottom feeder *Ae. aegypti* larvae scraped the toxin from the net's grid (made of 0.6 mm wide wires) and hence were exposed to high concentrations of toxin, higher at 75 than at 50 cm. To isolate the sinking effect from grazing, the grid framework must be much thinner than the 0.6 mm stainless steel wires used here.

The larvae scraped also the bottom of the column when net units were omitted: value of LC_{50} (6.8 ng powder ml^{-1} – a concentration that kills 50% of exposed larvae) in columns of 75 cm – was about 3-

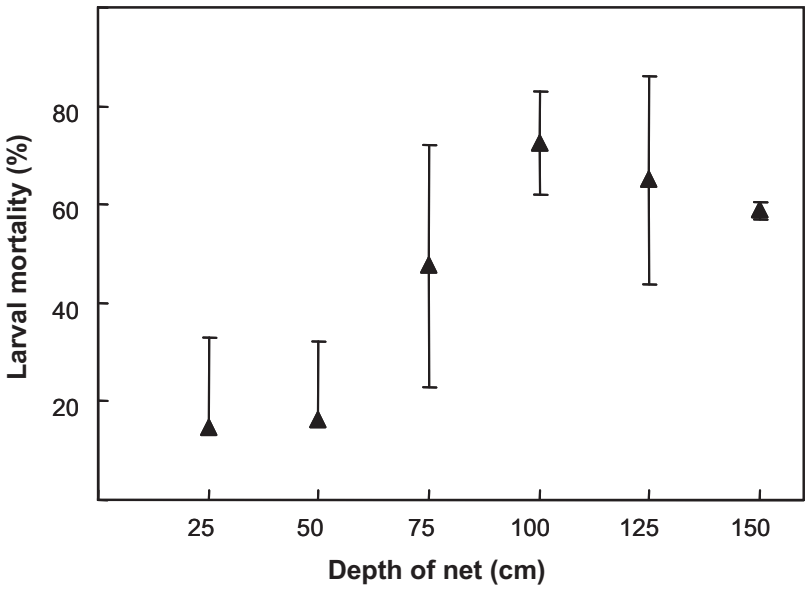

Fig. 2. The influence of water depth and sinking of free *B. thuringiensis* subsp. *israelensis* on the mortality of *Ae. aegypti* larvae. A commercial powder (Roger Bellon Laboratories, Belgium, R-153-78), which contains spores, δ-endotoxin crystals, diatom algae and debris of unknown origin, was mixed at final concentration of 15 or 25 ng ml^{-1} (▲) in 150 cm water columns with a net (impenetrable to larvae) placed at the indicated depths. Larvae were grown in 1 l of sterile tap water supplemented with 1.5 g of Pharmamedia (Traders Protein, USA) at 30 °C, and rinsed in sterile tap water upon reaching instar 3 stage before each experiment (with 20 or 40 larvae).

fold lower then in 25 cm (16.8 ng ml^{-1}) and in 150 cm (23.3 ng ml^{-1}) water columns, respectively. These results reinforced both conclusions, that (a) the larvae dived to 75 cm more frequently than to 150 cm, and (b) the toxin sank and hence its concentration at bottom rose more in 75 cm column with thrice the volume than in 25 cm column.

One conclusion of the series of preliminary experiments described here is related to the efficacy of *B. thuringiensis* subsp. *israelensis* against bottom feeding mosquito larvae such as *Ae. aegypti*: at deeper water bodies, lower total concentrations may be compensated by the sinking feature of the powder. This conclusion is confirmed by decreased mortality (by 25%) of *Aedes taeniorhynchus* larvae observed when the same weight of *B. thuringiensis* subsp. *israelensis* preparation has been applied to pans of 9 cm depth than of 3 cm (Nayar et al., 1999). Thus, applications in nature must take into account depth of the water body in addition to surface area. Nayar et al. (1999) have also demonstrated "settling in water" of two different preparations (12AS and TP of VectoBac), and with different rates.

4. Potential for studying *B. thuringiensis* subsp. *israelensis* bioencapsulation in a ciliate protozoan

The ciliate protozoan *Tetrahymena pyriformis* float *B. thuringiensis* subsp. *israelensis* powder (Zaritsky et al., 1991) and efficiently deliver it in concentrated amounts (that otherwise sink and hence unavail itself) to surface feeder mosquito larvae such as *Anopheles stephensi* (Manasherob et al., 1996, 1998). This phenomenon can be studied by exploiting the modular system described here at different water column depths. To demonstrate this option, *B. thuringiensis* subsp. *israelensis* powder was loaded as before (e.g., Manasherob et al., 1996) into washed *T. pyriformis* cells by pre-incubation and diluted into two water columns, of 75 and of 150 cm. Twenty larvae of field-collected *Anopheles* sp. were added to each container. Mortality of 24 h-exposed larvae was between 80 and 90% in both, as well as in a conventional essay in water disposable cups (6 cm deep). As controls, the bioassay was conducted with the same concentrations of each of the components separately; with *B. thuringiensis* subsp. *israelensis* powder alone mortality decreased from 60% in the cups to 20% at 150 cm water column, whereas it was below 10% with *T. pyriformis* alone. Thus, even in columns of 150 cm high, the protozoan floats the spores and crystals that it bio-encapsulateed to the grazing zone of the surface feeder anopheline larvae and hence diminish the water depth effect.

5. Concluding remarks

Similar systems, albeit not modular, have previously been described (Skovmand and Guillet, 2000; Clark et al., 2006). Both were made of a single, fixed tube (of 110 and 33 cm depth), the water of which can be sampled by valves or ports respectively and used for studies of *Bacillus sphaericus* sedimentation. Skovmand and Guillet (2000) have also quantified toxicity of *B. sphaericus* products outside the tube, in either tap or sewage water, at varying intervals as a function of sedimentation. The system described here is advantageous because of its modularity and optional inclusion of units with nets (see below) thus simulating natural water ponds with varying depths. It allows simultaneous testing of toxin sedimentation and larval behavior. Addition to our modular column system of ports or valves for sampling may enhance its efficiency.

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References

Aldemir, A., 2009. Initial and residual activity of vectobac(R) 12AS, vectobac (R) WDG, and vectolex(R) WDG for control of mosquitoes in Ararat valley, Turkey. J. Am. Mosq. Control Assoc. 25, 113–116.

Aly, C., Mulla, M.S., Xu, B.Z., Schnetter, W., 1988. Rate of ingestion by mosquito larvae (Diptera: Culicidae) as a factor in the effectiveness of a bacterial stomach toxin. J. Med. Entomol. 25, 191–196.

Amalraj, D.D., Sahu, S.S., Jambulingam, P., Doss, B.P.S., Kalyanasundaram, M., Das, P.K., 2000. Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis* (Vectobac) against mosquito vectors. Acta Trop. 75, 243–246.

Boisvert, M., Boisvert, J., 2000. Effects of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field experiments. Biocontrol Sci. Technol. 10, 517–561.

Clark, J.D., Devisetty, B.N., Krause, S.C., Novak, R.J., Warrior, P., 2006. Particle distributional behavior of a spray-dried technical concentrate and a water-dispersible granule formulation of *Bacillus sphaericus* in an aqueous column. J. Am. Mosq. Control Assoc. 22, 718–724.

de Barjac, H., 1978. A new subspecies of *Bacillus thuringiensis* very toxic for mosquitoes: *Bacillus thuringiensis* var. *israelensis* serotype 14. C. R. Acad. Sci. 286, 797–800.

Goldberg, L.J., Margalit, J., 1977. A bacterial spore demonstrating rapid larvicidial activity against *Anopheles sergentii*, *Uranotaenia uguiculata*, *Culex univitattus*, *Aedes aegypti* and *Culex pipiens*. Mosq. News 37, 355–361.

Kahindi, S.C., Midega, J.T., Mwangangi, J.M., Kibe, L.W., Nzovu, J., Luethy, P., Githure, J., Mbogo, C.M., 2008. Efficacy of vectobac DT and culinexcombi against mosquito larvae in unused swimming pools in Malindi, Kenya. J. Am. Mosq. Control Assoc. 24, 538–542.

Liber, K., Schmude, K.L., Rau, D.M., 1998. Toxicity of *Bacillus thuringiensis* var. *israelensis* to chironomids in pond mesocosms. Ecotoxicology 7, 343–354.

Lima, J.B.P., de Melo, N.V., Valle, D., 2005. Residual effect of two *Bacillus thuringiensis* var. *israelensis* products assayed against *Aedes aegypti* (Diptera: Culicidae) in laboratory and outdoors at Rio de Janeiro, Brazil. Rev. Inst. Med. Trop. São Paulo 47, 125–130.

Manasherob, R., Ben-Dov, E., Margalit, J., Zaritsky, A., Barak, Z., 1996. Raising activity of *Bacillus thuringiensis* var. *israelensis* against *Anopheles stephensi* larvae by encapsulation in *Tetrahymena pyriformis* (Hymenostomatida: Tetrahymenidae). J. Am. Mosq. Control Assoc. 12, 627–631.

Manasherob, R., Ben-Dov, E., Zaritsky, A., Barak, Z., 1998. Germination, growth, and sporulation of *Bacillus thuringiensis* subsp. *israelensis* in excreted food vacuoles of the protozoan *Tetrahymena pyriformis*. Appl. Environ. Microbiol. 64, 1750–1758.

Margalith, Y., Ben-Dov, E., 2000. Biological control by *Bacillus thuringiensis* subsp. *israelensis*. In: Rechcigl, J.E., Rechcigl, N.A. (Eds.), Insect Pest Management: Techniques for Environmental Protection. Lewis Publishers and CRC Press LLC, Boca Raton, FL, pp. 243–301.

Mulla, M.S., 1990. Activity, field efficacy and use of *Bacillus thuringiensis israelensis* against mosquitoes. In: de Barjac, H., Sutherland, D.J. (Eds.), Bacterial Control of Mosquitoes and Black Flies. Rutgers University Press, New Brunswick, NJ, pp. 134–160.

Nayar, J.K., Knight, J.W., Ali, A., Carlson, D.B., O'Bryan, P.D., 1999. Laboratory evaluation of biotic and abiotic factors that may influence larvicidal activity of *Bacillus thuringiensis* serovar. *israelensis* against two Florida mosquito species. J. Am. Mosq. Control Assoc. 15, 32–42.

Russell, T.L., Kay, B.H., 2008. Biologically based insecticides for the control of immature Australian mosquitoes: a review. Aust. J. Entomol. 47, 232–242.

Setha, T., Chantha, N., Socheat, D., 2007. Efficacy of *Bacillus thuringiensis israelensis*, VectoBac WG and DT, formulations against dengue mosquito vectors in cement potable water jars in Cambodia. Southeast Asian J. Trop. Med. Public Health 38, 261–268.

Skovmand, O., Guillet, P., 2000. Sedimentation of *Bacillus sphaericus* in tap water and sewage water. J. Invertebr. Pathol. 75, 243–250.

Stevens, M.M., Akhurst, R.J., Clifton, M.A., Hughe, P.A., 2004. Factors affecting the toxicity of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* to fourth instar larvae of *Chironomus tepperi* (Diptera: Chironomidae). J. Invertebr. Pathol. 86, 104–110.

Vilarinhos, P.T., Monnerat, R., 2004. Larvicidal persistence of formulations of *Bacillus thuringiensis* var. *israelensis* to control larval *Aedes aegypti*. J. Am. Mosq. Control Assoc. 20, 311–314.

Zaritsky, A., Zalkinder, V., Ben-Dov, E., Barak, Z., 1991. Bioencapsulation and delivery to mosquito larvae of *Bacillus thuringiensis* H14 toxicity by *Tetrahymena pyriformis*. J. Invertebr. Pathol. 58, 455–457.