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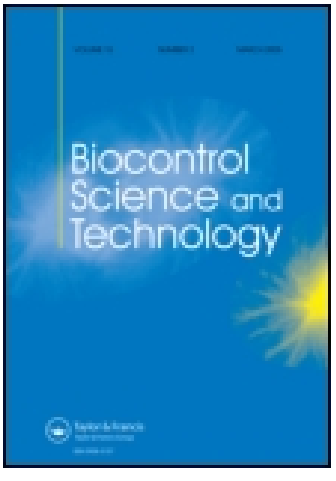

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Persistence and pathogenicity of a native isolate of Leptolegnia chapmanii against Aedes aegypti larvae in different anthropic environments

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SHORT COMMUNICATION

Persistence and pathogenicity of a native isolate of *Leptolegnia chapmanii* against *Aedes aegypti* larvae in different anthropic environments

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(*Received 15 July 2014; returned 5 August 2014; accepted 16 September 2014*)

The oomycete *Leptolegnia chapmanii* has been identified as a potential control agent of the primary vector of dengue, *Aedes aegypti*. In our assays, the persistence and pathogenicity of a native isolate of *L. chapmanii* decreased over time regardless of location. However, the mortality of *Ae. aegypti* larvae was significantly lower ($p < 0.05$) in containers located outside without sun protection (89% at first week and 9% at sixth week) compared with the containers located indoors (97% at first week and 42% at sixth week) and outside with shade (89% at first week and 29% at sixth week) possibly because of exposure to sun radiation.

Keywords: entomopathogen, biological control, oomycete, *Aedes aegypti*, *Leptolegnia chapmanii*

Aedes aegypti L. (Diptera: Culicidae) is an important vector of viral diseases such as dengue and yellow fever. Larval stages are aquatic and develop in natural and artificial containers with water located in domestic and peridomestic places. *Leptolegnia chapmanii* Seymour (Oomycetes: Saprolegniales) is an aquatic pathogen of mosquito larvae (Seymour, 1984). Its host range was previously determined (McInnis & Schimmel, 1985), as well as its life cycle, reproductive structures, the mechanisms of action on *Ae. aegypti* larvae (Zattau & McInnis, 1987) and virulence towards different larval stages of *Ae. aegypti* (McInnis & Zattau, 1982).

In the Southern hemisphere, *L. chapmanii* was reported for first time in September 1996 in La Plata, Buenos Aires Province, Argentina isolated from field-collected *Ochlerotatus albifasciatus* larvae (López Lastra, Steciow, & García, 1999). The isolate (CEP 010) was deposited in the entomopathogenic fungal culture collection of the Centro de Estudios Parasitológicos y de Vectores (CEPAVE) in La Plata and the ARSEF/USDA Collection in Ithaca, New York, USA (ARSEF 5499) and was used in the present study. Some optimal environmental variables for its infection and development on larvae of *Ae.aegypti* were previously determined (Pelizza, López Lastra, Becnel, Bisaro, & García, 2007a). It was found that water quality (physical and chemical composition) affects virulence of the zoospores.

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Under laboratory conditions, mortalities higher than 70% were reported for larvae of *Ae. aegypti* treated with *L. chapmanii* (Pelizza, López Lastra, Maciá, Bisaro, & García, 2009). Oospores developed more rapidly under temperatures between 20°C and 40°C (Pelizza, Scorsetti, López Lastra, & García, 2010a). The host range for this pathogen was found to cover different mosquito species including *Ae. aegypti*, with no impact on non-target species (López Lastra, Scorsetti, Marti, & García, 2004).

The aim of this research was to determine the mortality of *Ae. aegypti* larvae through time, with a single inoculation of *L. chapmanii* in containers located in three different anthropic areas in domestic and peridomestic environments where *Ae. aegypti* develop naturally. The study was conducted during early fall (May–June) and early spring of 2012 (September–October) and mid-spring of 2013 (October–November), in La Plata, Buenos Aires, Argentina.

The assays were carried out using 21 plastic containers (10 cm^2 × 13 cm high) with a final volume of 1000 ml. The containers were filled with 250 ml of dechlorinated water, and the level was kept constant by weekly addition of the required volume. Three groups (**A**, **B** and **C**) were established, each one including six treatment containers and one control. Group **A** was located inside houses and groups **B** and **C** were located in peridomestic areas, exposed to environmental conditions and climate variability. Group **B** was exposed to daily diffuse radiation and had 2 h of direct exposure to sun at noon. Incident radiation was controlled for group **C**, using 90% black polyethylene shade cloths, 20 cm far from the opening.

L. chapmanii was maintained in PYG agar media (meat peptone 1.2 g, yeast extract 1.2 g, glucose 3 g, agar 15 g, distilled water 1000 ml) at 25°C and photoperiod of 12:12 h (L:D). An initial suspension of zoospores was made submerging a 60 mm diameter disk of PYG media with *L. chapmanii* for 48 h in a beaker with 200 ml of deionized water. Mosquito larvae were infected by immersion in the zoospores suspension. According to the previous studies, a dead larva of *Ae. aegypti* produces 6.1 ± 0.2 × 10^4 zoospores during the 48 h after infection (Pelizza, López Lastra, Becnel, Bisaro, & García, 2007b). The treatment containers of each group were inoculated with five dead larvae, after 48 h of infection with *L. chapmanii*. The inoculum was equivalent to 3.05 ± 1 × 10^5 zoospores for a final concentration of 1.22 ± 0.4 × 10^3 zoospores/ml. The control containers were not inoculated.

The larvae of *Ae. aegypti* were maintained using standard methods for mosquito breeding (Gerberg, Barnard, & Ward, 1994) at the colony of the CEPAVE. They were kept in plastic pans and fed with triturated rabbit chow (Purina®). Second- (L2) and third- (L3) instar were used to test the pathogenic activity of *L. chapmanii*.

Pathogenicity of zoospore suspensions was evaluated during 6 weeks, with a weekly addition of 25 healthy larvae into each container. Dead larvae were removed each 24 h to prevent pathogen recycling, and weekly, the live larvae were eliminated. Mortality of the larvae was recorded after 48 h up to the beginning of each test. The presence of *L. chapmanii* on dead larvae was confirmed under a compound microscope with phase contrast. The test was replicated three times on different dates.

Mortality data were corrected by Abbott's formula modified by Schneider-Orelli (1947). Percentage data were transformed by arcsine square root for the analysis of variance (ANOVA). Descriptive statistics, ANOVA with one factor ($\infty = 0.05$) and the post hoc Tukey's honestly significant difference (HSD) analysis were applied to

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determine differences between groups. These analyses were performed with IBM SPPS v20 analysis software.

According to the meteorological records from the Department of Astronomy of the University of La Plata, the mean temperature in La Plata city during the period of the different assays oscillated from 11.7°C to 20.1°C. A preliminary test conducted in spring of 2011, showed that the water temperature during the day, from different containers – of the three locations – was appropriate for the development of *L. chapmanii*. The mean water temperatures of the locations **A**, **B** and **C** were of 19.3 ± 1.1, 20.1 ± 0.8 and 20.4 ± 0.8°C, respectively at 9 am. At noon, the temperatures were of 15.4 ± 2.5, 20.8 ± 3.9 and 17.5 ± 2.6°C to **A**, **B** and **C**. Finally, at 5 pm temperatures were of 14.7 ± 2.7, 17.3 ± 2.6 and 17 ± 2.6 to **A**, **B** and **C**.

The mortality of larvae had a tendency to decline with time regardless of location (Figure 1). However, there were significant differences among the groups. The mortality in the sun exposed group **B** decreased faster than the indoor group **A** and the outdoor shaded group **C**. For the group **A**, the weekly mortality of the control treatments were 0, 0.7, 3.7, 0, 2.7 and 0% (weeks 1–6, respectively). For the group **B**, they were 3.3, 4, 5.3, 0, 5.3 and 3.3%. For group **C**, the mortality of the controls were 0.7, 2.7, 0, 0 6 and 3.3%. Group **A** was exposed to conventional domestic illumination during the day and, possibly it had a more stable temperature during the day than groups **B** and **C**. Except for the incident radiation, the outdoor groups were exposed to the same environmental conditions. This suggests that the sunlight radiation could be an environmental factor affecting the viability of the zoospores.

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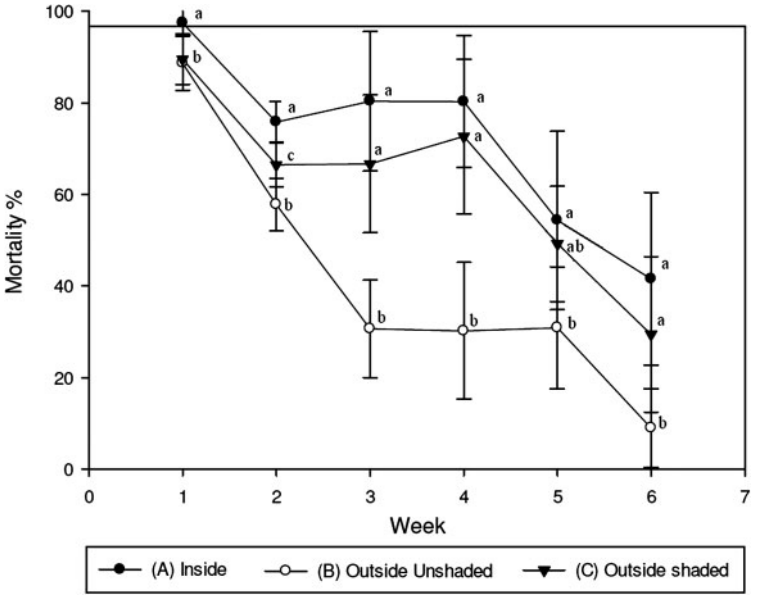

Figure 1. Mortality of *Ae. aegypti* larvae by *L. chapmanii* with time. In each week, the same letter (a, b, c) represents non-significant differences, according to Tukey's post-hoc test ($p < 0.005$).

In any case, a single application of *L. chapmanii* was able to regulate the *Ae. aegypti* larval populations over time in different places.

In previous studies, mortalities of 95 and 100% were reported for *Ae. aegypti* larvae treated with a *L. chapmanii* suspension (1.8×10^5 zoopores/ml) under semi-natural conditions, with sunlight and shade, respectively (Pelizza, Scorsetti, Bisaro, López Lastra, & García, 2010b). It has been shown that solar radiation affects the stability and persistence of different entomopathogenic fungi (Gardner, Sutlon, & Noblei, 1977; Roberts & Campbell, 1977). For conidial fungi, the germination rate is reduced after the exposure to UV-B radiation (Le Grand & Cliquet, 2013). Both solar UV-A and UV-B radiations impair conidial viability and delay germination in the entomopathogenic fungus *Metarhizium anisopliae* (Braga, Flint, Miller, Anderson, & Roberts, 2001).

In respect to other biological control agents, the most popular used against larval mosquitoes is the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti). Commercial products based on spores and toxins of this bacterium has shown residual effect for more than 7 weeks under laboratory and semi-field conditions (Fansiri, Thavara, Tawatsin, Krasaesub, & Sithiprasasna, 2006; Lee, Pe, & Cheong, 1986; Ritchie, Rapley, & Benjamin, 2010). *Lagenidium giganteum*, a similar oomycete pathogen of mosquitoes, recycles in larvae and is capable of persisting for a long time (Kerwin & Washino, 1988). Possibly, if the dead larvae infected with *L. chapmanii* are maintained inside containers, the pathogen could recycle, extending its. Equally important is that, the periodic addition of *Ae. aegypti* larvae in these assays surely reduced the zoospore concentration over time and, the densities of the mosquito larvae and the zoospores in suspension, which also affect infection rates (Pelizza et al., 2007b).

With the results of these assays, we conclude that *L. chapmanii* could potentially be used as a biological control agent for larval populations of *Ae. aegypti*, in different anthropogenic places. Under these environmental conditions, where *Ae. aegypti* develops naturally, the pathogenic action of *L. chapmanii* persists for several weeks. There are indications that the viability of the zoospores can decline with the sunlight exposure, reducing their pathogenicity against mosquito larvae with time.

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