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# Formulation of attractive toxic sugar bait (ATSB) with safe EPA-exempt substance significantly diminishes the *Anopheles sergentii* population in a desert oasis

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ABSTRACT

Attractive toxic sugar bait (ATSB) is a highly effective method which targets mosquitoes based on their sugar foraging behavior, by presenting baits of attractive compounds in combination with sugar and oral toxin to local mosquito populations. Environmental concerns and insecticide selection-pressure have prompted investigations of novel, ecologically-harmless substances which can be used as insecticides. This study examined the efficacy of microencapsulated garlic-oil as the oral toxin component of ATSB for controlling *Anopheles sergentii* populations inhabiting desert-surrounded wetlands in Israel. ATSB solution containing 0.4% encapsulated garlic oil was applied to local vegetation around a streamlet located in the lower Jordan Valley. To determine the propensity of bait ingestion, and assess the potential ecological impact of the method, mosquito and non-target specimens were collected and tested for the presence of natural plant- or attractive sugar bait (ASB)-derived sugars. Over the experimental period, biting-pressure values in the ATSB treatment site decreased by 97.5%, while at the control site, treated with non-toxic ASB, no significant changes were observed. Approximately 70% of the mosquitoes collected before both treatments, as well as those captured following the application of ASB at the control site, were found to have ingested sugar prior to capture. Non-target insects were minimally affected by the treatment when ATSB was applied to foliage of non-flowering plants. Of the non-Diptera species, only 0.7% of the sampled non-target insects were found to have ingested ASB-solution which was applied to green vegetation, compared with 8.5% which have foraged on ASB-derived sugars applied to flowering plants. Conversely, a high proportion of the non-target species belonging to the order Diptera, especially non-biting midges, were found to have ingested foliage-applied ASB, with more than 36% of the specimens collected determined to have foraged on bait-derived sugars. These results prove that food-grade, EPA-exempt microencapsulated garlic oil is a highly effective insecticide which can be utilized for mosquito population control. The relatively short half-life of this active ingredient makes it a suitable for use in areas where repeated application is possible, limiting the accumulation of deleterious compounds and ensuring minimal environmental impact when applied in accordance with label recommendations.


## 1. Background

Mosquito-borne diseases are responsible for a significant portion of human morbidity and mortality (Tolle, 2009). Consequently, increased attention has been given for development of vector con-

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trol strategies which aim to reduce mosquito population numbers or their contact with potential human host, with some methods proving highly effective in lowering the incidence of such ailments in many affected areas (Beier et al., 2008; Enayati and Hemingway, 2010). However, the efficacy of current vector control methods is mostly limited to low-transmission environments, such as arid locations or isolated islands (Bhattarai et al., 2007; Kleinschmidt et al., 2007; Keating et al., 2011), whereas they have negligible or no impact in areas where entomological inoculation rates (EIRs) are more substantial (Beier et al., 1999; McKenzie et al., 2001; Shaukat et al., 2010). These shortcomings can be attributed to several factors. Insecticide-based methods such as long lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) have been shown to cause selection-pressure and contribute to the emergence of mosquito populations which are resistant to one or several classes of chemical compounds (Oxborough et al., 2008; Ranson et al., 2009, 2011; Beier et al., 2012). In addition, most strategies target host-seeking and blood feeding mosquitoes, relying on potential human hosts to attract the vectors to the vicinity of the insecticide, which is exclusively used within residences. Consequently, the efficacy of such treatments is behavior-dependent, with endophilic species which tend to rest and feed indoors affected more than exophilic, outdoor-feeding vectors (Fornadel et al., 2010; Russell et al., 2011; Derua et al., 2012). Accordingly, a shift in the disease transmission dynamics may perpetuate the incidence of the disease, as a readily available pool of the pathogen is maintained within the latter, impeding attempts to reduce and sustain EIRs below the desired thresholds for prolonged periods (Beier et al., 1999; McKenzie et al., 2001; Shaukat et al., 2010). Hence, while vector control remains a central aspect in the efforts to eradicate mosquito-borne diseases, such drawbacks have highlighted the need to find additional, complementing methods to be utilized in integrative vector management (IVM) for controlling vector populations.

In view of the above, several studies have explored the efficacy of alternative strategy which targets mosquitoes based on physiological requirements or behavioral responses other than the search for blood meals. Both male and female mosquitoes ingest sugars to meet energetic demands, (Yuval, 1992), and are highly-selective in choosing their source (Müller and Schlein, 2006; Schlein and Müller, 2008), which can include fruit, floral nectar, or honeydew. Increased knowledge of mosquito feeding-habits and preferences has enabled targeting the vectors by using attractive toxic sugar bait (ATSB) – a mixture of sugar and oral insecticide dissolved in water, which was initially sprayed on plants known to be highly-attractive to local mosquito populations (Müller and Schlein, 2006; Schlein and Müller, 2008; Müller et al., 2010; Gu et al., 2011). Following studies have improved the method by adding plant-derived attractants to the bait mixture, allowing its application on non-attracting plants (Müller and Schlein, 2008), and in portable bait-stations (Müller et al., 2008; Naranjo et al., 2013).

Most of the trials which tested the efficacy of ATSB as a vector-control strategy employed one of several chemical compounds (such as spinosad, boric acid, and dinotefuran) as oral insecticides (Müller et al., 2008; Khallaayoune et al., 2013; Naranjo et al., 2013). Possible selection-pressure and development of resistant mosquito populations, as well as ecological and environmental concerns, have prompted studies which tested alternative substances, such as eugenol and garlic oil, which were shown in several studies to possess effective insecticidal properties (Amonkar and Reeves, 1970; Amonkar and Banerji, 1971; Isman, 2000; Cetin et al., 2004; Aboelhadid et al., 2013; Zhao et al., 2013; Khater, 2014; Qualls et al., 2014). This was done in the aim to increase the range of compounds which are used as oral toxins, thereby reducing selection-pressure for specific toxins. In this study we tested the efficacy of the first commercially available ATSB formulation containing a commercial attractant and encapsulated garlic oil as the active ingredient for controlling the population size of *Anopheles sergentii*, the most common *Anopheles* species in Israel, and a main vector of malaria in the Afro–Arabian zone (Farid, 1956; Zahar, 1974).

## 2. Materials and methods

### 2.1. Study sites

The study was conducted in the Lower Jordan Valley, part of the Dead Sea Rift, which flanks the eastern part of Israel and the Palestinian territories. The climate in the region is arid, with annual precipitation ranging between 50 mm to 100 mm, and an average relative humidity of 20–30%. Local flora and fauna is typical of the Sahara–Arabian phyto-geographical zone, shifting to tropical conditions and associated biota around sporadic natural and anthropogenic water-sources.

Two sections of streamlets (ca. 1.5 km long) with similar vegetation, situated 10 km apart, were chosen as the experimental and control sites. The watercourses of both sites are encompassed by riparian vegetation, which varies between 10–40 m in width (ca. 25 m average), and mainly consists of reeds and *Tamarix* spp. thickets. A short distance from the water-flow the vegetation abruptly changes to grassland with scattered shrubs and semi-shrubs. The two sites are “island-like” isolated ecological pockets suitable for mosquito breeding, and are predominantly inhabited by the malaria vector *A. sergentii*, the principle species found in this area.

### 2.2. Preparation and field application of ATSB solutions

Commercially available industrial-grade bait concentrates were purchased from Terminix® (Memphis, TN, USA). ATSB concentrate containing 0.4% (w/w) Garlic oil encapsulated in beta-cyclodextrin was diluted in tap water (1:3 ratio) containing blue food-dye (0.4% E132, Indigotine “Food Blue No. 1”; Stern, Netanya, Israel). For preparation of ASB (attractive but non-toxic sugar bait), concentrates lacking the active ingredient were similarly diluted in tap water containing green food-dye (0.4% Tartrazine 19140 “Special green”; Stern, Netanya, Israel). ATBS and ABS solutions were applied to a double perimeter of vegetation growing near the banks of either streamlets utilizing a Back-Pack Sprayer (Killaspray, Model 4526, Hozelock, Birmingham, UK) in accordance with label recommendations.

### 2.3. Experimental design and methods of ATSB field trials

The field trials were conducted over a period of 47 days, starting on September 30th, and ending on November 16th, 2013. Adult mosquitoes were sampled every two days for the first 11 days of the experiments, and every three days following the ATSB/ASB application, which was performed 13 days into the experiment. Female mosquitoes were captured in the evening with a Power Vac Back-Pack unit (John Hock, Gainesville, FL) while attempting to land on the legs of human baits, or in the morning from the surrounding vegetation using entomological sweep-nets. Human bait samples were collected between 20:00 and 22:00, and pooled into 5 min intervals, constituting nine repetitions per day for both the treatment and the control sites. Daily catches collected on human bait and on surrounding vegetation were stored at −70 °C until further use.