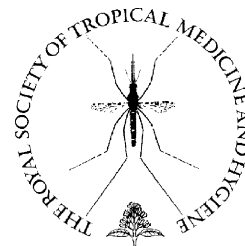




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Efficacy of toxic sugar baits against adult cistern-dwelling *Anopheles claviger*

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KEYWORDS

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Summary Sugar baits were used for mosquito control in cisterns at the building complex of Mar Saba Monastery in the Judean Hills, Israel. These cisterns provide fresh water for the monastery and are breeding and resting sites for *Anopheles claviger* (Meigen). The baits, including sucrose, fruit juice, dye marker and oral insecticide, were presented at the entrance of the cisterns and caused a drastic decline of *A. claviger* numbers in the neighbourhood. Pre-treatment catches of 16.8 ± 4.0 to 33 ± 8.1 females per trap decreased to between 2.5 ± 1.3 and 0.3 ± 0.6 females per trap six nights after treatment. Numbers of males were initially 9.2 ± 1.7 to 21.0 ± 4.8 specimens per trap and decreased to between 1.0 ± 0.4 and 0.5 ± 0.2 per trap post treatment. In the control area, where baits without toxin were presented in cisterns, there were only small fluctuations in the mosquito population. Non-poisonous bait marked >95% of the mosquitoes exiting from a cistern in the control area. Following treatment, the number of human-landing mosquitoes decreased by more than ten-fold in the experimental area, whilst in the control area there was no significant decrease.

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1. Introduction

Anopheles claviger (Meigen) is distributed from the Middle East through much of Europe. In Palestine, Syria and Lebanon it has been a primary vector of malaria (Gramiccia, 1956; Grundi, 1945; Kligler, 1930; Muir and Keilany, 1972) and in a few areas in Europe it appears to have been a secondary vector (Hargraves, 1923; Service, 1973). Today, *A. claviger* remains an important vector of malaria in Turkey where

23% of the population lives in provinces where malaria is endemic (Yurttas et al., 2005). Over most of its range, *A. claviger* is typically an exophilic and rural species. However, in countries bordering the eastern littoral of the Mediterranean, especially in limestone areas where water is stored underground in cool cisterns and wells are cut in the rock, *A. claviger* has become a highly domestic species living in close contact with man (Muir and Keilany, 1972). *Anopheles claviger* was responsible for high rates of urban malaria in Palestine during the first quarter of the 20th century and is one of the five potential malaria vectors in Israel (Barkai and Saliternik, 1968; Kitron et al., 1994).

Female and male mosquitoes obtain their essential sugar diet mostly from floral nectar but also from nectaries on

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leaves and stems, from honeydew excreted by homopterans (reviews by Foster, 1995; Yuval, 1992) and from ripe and damaged fruits (Joseph, 1970). Mosquitoes are attracted to the floral scent (Mauer and Rowley, 1999) and accordingly they concentrate on selected nectar sources (Grimstad and DeFoliart, 1974). In a previous study, we showed that some flowering trees are extreme attractants to nectar-searching mosquitoes (Müller and Schlein, 2006) and can be used for control. We presumed that mosquitoes also locate suitable ripe fruit by olfactory cues. During a previous study, we developed a sugar/marking dye/insecticide formulation to spray on attractive plants visited by mosquitoes (Müller and Schlein, 2006). Here we have added rotten/overripe fruit juice and red wine (fermented grape juice) to make the sugar bait attractive in the absence of plants. This 'attract and kill' mosquito bait worked well in an additional experiment (Müller et al., in press), so we tested the effect of this formulation on *A. claviger* populations in cisterns.

2. Materials and methods

2.1. Experimental and control study sites

The study was carried out in eastern Israel at the end of the dry season (October and November) of 2006. The experimental site was a Greek Orthodox monastery called Mar Saba that overlooks the Kidron Valley in the Judean Hills, approximately 4–5 km from the eastern outskirts of Jerusalem. Mar Saba is a large complex of buildings and caves that spreads out over an area of approximately 2 ha and is capable of accommodating hundreds of people (Patrich, 1991). All water used in the monastery, including drinking water, comes from 20 cisterns that collect surface run-off water. The cisterns have various capacities ranging from several dozen to several hundred cubic meters. They have square or rounded openings of 50–80 cm, covered by loosely fitting plates of iron, wood or stones. Some of the cisterns were dry at the time of the experiments, thus the study concentrated on nine cisterns that contained water and harboured mosquitoes.

A control site, located 5 km away from the experimental site (Mar Saba), was chosen for its similar climate and mosquito habitat. This control site comprised a group of approximately 50 small buildings in a poor neighbourhood on the eastern outskirts of Jerusalem. There was no vegetation except for a few trees and the inhabitants keep some domestic animals and fowl. Originally, the houses had wells or cisterns but most of them are now full of earth and debris. The 15 wells and cisterns that contained water and mosquitoes were used for the experiment.

2.2. Baits

The bait solution used to treat the experimental site consisted of ~85% juice of overripe to rotting nectarines (*Prunus persica* (L.) Batsch, var. nectarina: Rosacea), 5% v/v wine, 15% w/v brown sugar ('Nature Sugar' brown; Louis Dreyfus, Ashdod, Israel), 0.5% w/v red food dye (Carmoisine E122; Stern, Netanya, Israel) and 0.04% w/v oral insecticide (Spinosad, 'Tracer'®; Dow Agrosiences, Calgary,

Canada). Spinosad is a mixture of active metabolites of the soil actinomycete *Saccharopolyspora spinosa* and has a safety profile similar to benign biologicals. The solution was ripened for 48 h in covered buckets, outdoors in the sun where daily temperatures reached ~30 °C. The bait solution used to treat the control site was the same as that used to treat the experimental site, except no insecticide was added.

Clean, disposable 1.5 l plastic soft drink bottles with a hole of ~5 cm diameter at approximately two-thirds of their height were prepared. Cotton cloth wick was inserted through the holes and arranged so that both the internal and external ends reached down to the level of the bottom. The bottles were then introduced, bottom first, into large, light coloured, cotton flannel socks that had been thoroughly washed with water and dried. The socks were then wetted by dipping into the bait solution and approximately 0.5 l of the solution was poured into each bottle. Thus, the fluid evaporating from the external layer was replaced and the socks were maintained wet by passage of the internal fluid through the wick. The bottles were provided with a 60 cm diameter, umbrella-shaped plastic cover. In both the control and the experimental sites the mosquito population was initially monitored for nine nights. Then the bottles with baits, with toxin in the monastery and without toxin in the control area, were each hung at the opening of a cistern and the customary cover of the cistern was replaced.

2.3. Monitoring

The mosquito populations were monitored at each site for 36 days with six miniature CDC traps provided with black light (UV) (Model 912; John W. Hock, Gainesville, FL, USA) repeatedly hung overnight in fixed positions between houses at a height of approximately 1 m.

On Days 3 and 4 of the experiment, before treatment, two human volunteers in the experimental and control sites recorded landing and biting of *A. claviger* mosquitoes in 5 min observation periods followed by 5 min intervals. There were 12 observation periods per site before treatment. This procedure was repeated on two consecutive nights immediately after treatment.

The authors of this study served as the volunteer subjects and were therefore fully informed of the nature and purposes of the test and of any physical and mental health consequences that were reasonably foreseeable.

2.4. Marking of cistern-exiting *Anopheles claviger*

A 120-cm long cylindrical trap with an 80 cm diameter was prepared on a frame made from 6 mm metal rods. It was covered, except for one end, with mosquito mesh. A mesh funnel on a metal frame, 80 cm long, with a top opening of 80 cm diameter and a bottom opening of 10 cm diameter, was prepared and fitted, narrow end in, into the open end of the cylinder. This construction was placed on the opening of a well, with the funnel facing down, and left overnight. Caught mosquitoes were removed in the morning. This trap was used on one control cistern for ten consecutive nights after presentation of the baits.

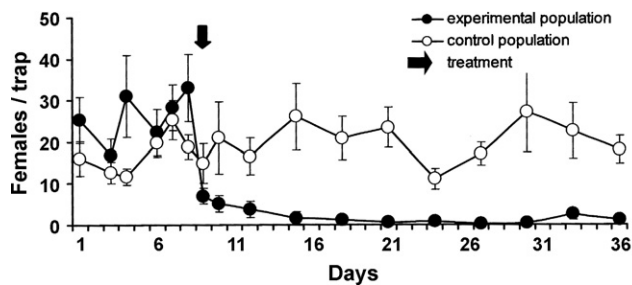


Figure 1 Catches of *Anopheles claviger* females in the experimental area, where the attractive baits at the entrance of cisterns contained oral toxin, and in the control area where similar baits were without toxin.

2.5. Statistical analysis

Catches of mosquitoes in the experimental and control sites as well as the rates of biting and landing mosquitoes were compared using the unpaired *t*-test (GraphPad Prism version 4.03 Statistical Package; GraphPad Software Inc., San Diego, CA, USA). Significance was taken at $P < 0.05$.

3. Results

3.1. Catches of *Anopheles claviger*

Initial monitoring demonstrated that there were large *A. claviger* populations in the selected sites, with that at the experimental site (the monastery) being significantly larger ($P < 0.05$, $t = 2.33$). The average catch in the monastery was 40.8 ± 3.78 mosquitoes per trap compared with 29.5 ± 3.0 mosquitoes per trap in the control area. Following the presentation of toxic baits, the number of females decreased to less than a tenth. The pre-treatment average catch during eight nights in the experimental site ranged between 16.8 ± 4.0 and 33.0 ± 8.1 *A. claviger* females per trap. After treatment on Day 9 it dropped drastically to 5.2 ± 2.0 on the 10th night, to 3.8 ± 2.0 on the 12th night and to 1.7 ± 1.4 on the 15th night. Afterwards, until the end of the experiment on Day 36, the catch ranged between 0.3 ± 0.6 and 2.5 ± 1.3 females per trap (Figure 1). The number of males was initially smaller and ranged between 9.2 ± 1.7 and 21.0 ± 4.8 males per trap during the pre-treatment period. After treatment their average number declined gradually to 3.2 ± 1.3 on the 21st night and to between 1.0 ± 0.4 and 0.5 ± 0.2 males per trap during the last week (Figure 2). The decrease in catches of females and males was highly significant ($P < 0.001$, $t = 7.32$ for females; $P < 0.001$, $t = 5.109$ for males). At the control site, the catch of *A. claviger* females and males was generally quite stable. The catch of females ranged between 11.7 ± 1.9 and 27.2 ± 9.9 per trap (Figure 1), whereas male numbers ranged between 6.2 ± 1.5 and 18.0 ± 3.9 per trap (Figure 2). The difference between mosquito catches after treatment in the experimental and control sites was highly significant ($P < 0.001$, $t = 10.75$ for females; $P < 0.001$, $t = 5.97$ for males). Most of the mosquitoes caught exiting on ten nights from a well in the control site had fed on the coloured bait and were labelled with the food dye. These included 96.2% (401/417) of female and 97.1% (297/306) of male *A. claviger*.

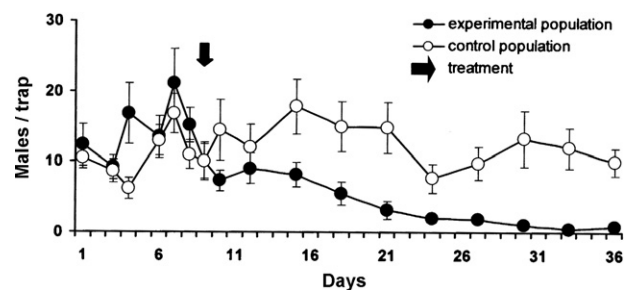


Figure 2 Catches of *Anopheles claviger* males in the experimental area, where the attractive baits at the entrance of cisterns contained oral toxin, and in the control area where similar baits were without toxin.

3.2. Mosquito biting and landing on volunteers

The landing rate of *A. claviger* females on volunteers reflects the drastic reduction of females following the use of the toxic baits. A decrease of more than ten-fold occurred in the experimental site, whilst there was no change in the control site (Table 1). Before treatment, the biting and landing rate was significantly higher at the experimental site compared with the control site ($P < 0.01$, $t = 2.826$). After treatment, the decrease in the biting and landing rate at the experimental site was highly significant compared with the control site ($P < 0.001$, $t = 11.089$).

4. Discussion

It is well documented that mosquitoes feed on nectar and other sources of plant sugars and there are also several studies on their attraction to flowers (reviews by Foster, 1995; Foster and Takken, 2004; Gary and Foster, 2004; Mauer and Rowley, 1999; Yuval, 1992). However, the potential of using plant odours for mosquito control received little attention. In a previous study (Müller and Schlein, 2006), we used the natural smell of flowering *Acacia* trees to attract *A. sergentii* Theobald to a mixture of sugar and toxin, and in the present study we used fermented fruit juice to lure and induce feeding of *A. claviger* on sugar and oral toxin.

At the beginning of the experiment, *A. claviger* catches in both study sites were high. In the experimental site (the monastery), the population was significantly higher than in the control site. The attractive, toxic sugar baits at the opening of cisterns nearly eliminated *A. claviger* from the

Table 1 Landing and biting of *Anopheles claviger* on volunteers before and after presentation of the baits

Site	Time	Volunteer	Average \pm SE
Experimental	Pre treatment	A	13.0 ± 2.02
Experimental	Pre treatment	B	18.3 ± 2.28
Experimental	Post treatment	A	0.5 ± 0.34
Experimental	Post treatment	B	1.2 ± 0.48
Control	Pre treatment	A	11.5 ± 1.43
Control	Pre treatment	B	9.0 ± 1.18
Control	Post treatment	A	10.8 ± 1.25
Control	Post treatment	B	10.0 ± 1.13

SE: standard error.

experimental area. Females almost completely disappeared 4 days after treatment; males took longer but within 2 weeks they were also very greatly reduced (Figures 1 and 2). As shown by the trapping of exiting mosquitoes from one well in the control area, virtually all the females (96.2%; 401/417) and males (97.1%, 297/306) were labelled by the food dye of the bait. In the experimental area a similar proportion of the exiting cistern-dwellers should have fed on the toxic bait and died. However, the reduction in mosquito catches was not so sudden. We therefore suggest that the samples caught in the experimental area included mostly male mosquitoes that rest in places other than the treated cisterns. Gradually, these mosquitoes also find shelter in the cisterns where they are exposed to the toxic baits. The drastic decrease (approximately 20 times) in the number of mosquitoes that came to human baits after treatment provides further evidence on the success of the toxic baits (Table 1).

In this study, the fruit attractant exerted its effect in the short range of the cistern openings. It has also been a strong attractant for *Culex pipiens* L. in release chambers (G.C. Müller and Y. Schlein, unpublished data) but its full range of effect has yet to be tested in open areas. However, even with a short range, such an attractant may be suitable for the control of subterranean mosquito populations in many places where access to the breeding sites is difficult and adult mosquitoes come out through narrow openings or pipes. For example, suitable areas may include underground storm drain systems that consist of thousands of miles of gutters and underground pipelines and hundreds of thousands of catch basins and manhole chambers. Underground storm drains collect and carry surface water as they traverse through residential and commercial areas and finally drain into the ocean. Some features such as a minimal grade, accumulation of debris and trash as well as irregular water flow result in puddles of drained water. The underground portions of the drainage systems with stable microclimatic conditions offer ideal habitats for breeding and resting sites of domestic and peridomestic mosquitoes (Chanda and Shisler, 1980; Mulligan and Schaefer, 1981; Pfunter, 1978; Smith and Shisler, 1981; Tianyun et al., 2003).

Mosquitoes that develop or find shelter in storm drain systems cause considerable public health concerns from the standpoint of arbovirus transmission (Kay et al., 2000; Marfin et al., 1993) and it is extremely difficult to control mosquitoes in these systems (Hazelrigg and Pelsue, 1980). We suggest that toxic baits on the principle described in our study might be a useful tool for this purpose.

Authors' contributions: GCM and YS were jointly responsible for the design and carrying out of the experiments. Most of the fieldwork was designed and conducted by GCM, whilst YS was responsible for the interpretation and presentation of the results. Both authors read and approved the final manuscript. YS is guarantor of the paper.

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Conflicts of interest: None declared.

Ethical approval: Not required.

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