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CONTROL OF INDIGENOUS AND EXOTIC MOSQUITOES IN GERMANY

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Abstract In Germany since more than three decades products based on *Bacillus thuringiensis israelensis* (Bti) and *Lysinibacillus sphaericus* have been successfully used as biological control agents against floodwater mosquitoes, mainly *Aedes vexans* and *Ochlerotatus sticticus* as well as against house mosquito *Culex pipiens*. Over 4.000 km^2 of breeding areas have been treated with various *Bti*-formulations as ice-granules by helicopters, fluid and powder formulations mixed with water by ground application, resulting in a reduction of the mosquito population year by year more than 95% and without evidence of any harmful impact on the environment. Globalization resulting in increased international trade and human mobility is responsible for the quick spread of pathogens and neozoa such as container-breeding *Aedes/Ochlerotatus* mosquitoes. Amongst them the most important ones are *Ae. albopictus* and *Oc. japonicus* in Germany. The control of container breeding mosquitoes is mainly based on environmental management and the use of Bti-tablets in the frame of community participation. Since 2013 copper as a mosquitocidal metal has been tested successfully to avoid mosquito breeding.
Key words Copper, *Bacillus thuringiensis israelensis*, container breeding mosquitoes

INTRODUCTION

The control of mosquitoes in Germany has a long history. In the 1920s and 1930s breeding sites were treated with petroleum oils. During the 1950s and 1960s adulticides were used. However, in the early 1970s, the mosquito population was extremely high because of frequent fluctuations of the water level of the Rhine. The outdoor attack rate on humans was more than 500 female mosquito bites per minute, greatly restricting the time village residents could spend outside. As a reaction to this natural disaster, towns and communities on both sides of the River Rhine merged their interests into the GMCA/KABS, a united mosquito control programme founded in 1976 (Becker, 1997). Now 102 cities and municipalities along a 310 km stretch of the Upper Rhine River, with a total population of 2.7 million people, have joined forces to control the mosquitoes, mainly *Aedes vexans* and *Ochlerotatus sticticus* over a breeding area of some 600 km^2 of the Rhine's flood plain. The budget of the programme is approximately 3.4 million Euros a year, which results in overall costs per person per year of approximately 1.2 Euros/Person/year. The overall goal of the KABS is to control mosquitoes while conserving biodiversity. This goal can be reached effectively only, when biological control methods are used. The control of *Aedes* mosquitoes by GMCA/KABS is based mainly on the use of Bti products. In the last 30 years about 340.000 hectares of floodwater breeding sites have been treated with more than 4000 tons of various Bti formulations mainly based on ice granules resulting in a reduction rate year by year by 95% of the floodwater mosquitoes. Domestic mosquitoes *Culex pipiens* are controlled mainly by the use of Culinex®/Vectobac® DT-Bti-tablets in containers and septic tanks, as well as by the application of *B. sphaericus* to eutrophic ponds and ditches.

Bti-tablets are used e.g. in Italy against *Ae. albopictus* and in Germany against all container breeding mosquitoes by millions. Also in tropical countries they can contribute to the integrated control of dengue (Kroeger et al., 1995). The fizzy tablets are sterilized by Gamma radiation before usage, that only the protein crystals produced by the bacilli as active ingredient and not spores or bacilli are applied (Becker, 2002). Another recent development has been reported with a combined formulation of *Bacillus thuringiensis israelensis* and *Lysinibacillus sphaericus*, (VectoMax®) which has a residual mosquitocidal effect against container breeders for several weeks.

Increased mobility of humans as well as the international trade, facilitate the dispersal and in some cases, the establishment of exotic mosquito species outside their original area of distribution. Within the about 30 species known to have established in new areas throughout the world, 3 species merit special recognition for their dispersal potential and also for their significance as vectors of human diseases such as *Aedes aegypti*, *Ae. albopictus* and *Ochlerotatus japonicus*. In Europe other exotic species occur such as *Oc. koreicus*, *Oc. atropalpus* and *Oc. triseriatus* (Becker et al., 2012). Once established, these species can spread by vehicles, trains and boats to neighboring countries. In Europe, *Ae. albopictus* is the most problematic species. In the early 1990s it was passively introduced into Italy, due to the international trade of used tires. Since 1999, *Ae. albopictus* has been found in various southern and central European countries, including France, Bosnia & Herzegovina, Montenegro, Belgium, Switzerland, Greece, Malta, Monaco, Croatia, San Marino, Slovenia, Spain, the Netherlands, Vatican City, Germany, Austria, Slovakia, Czech Republic, Hungary, and Romania (ECDC, 2012).

In 2000, *Oc. j. japonicus* was first recorded in Europe when larvae were found in a storage yard of imported used tires in France and Belgium. Finally in 2008 the species was detected in northern Switzerland. Since 2009 this species is recorded in several States of Germany as well as in Austria and Slovenia. Due to the threat of these exotic species in a joint cooperative project the WHO and EMCA developed 2012/13 Guidelines for the control of mosquitoes of public health importance in Europe to prevent further spread of invasive species especially *Ae. albopictus.* In Germany the rapid spread of *Ae. albopictus* and *Oc. japonicus* increased the awareness of authorities, scientists and the public and led to an intensive surveillance program for exotic mosquito species, including the development of new control tools to combat exotic mosquitoes. During the surveillance activities related to the spread of *Oc. japonicus* in Germany it has been observed that no mosquito larvae were found in copper vases in cemeteries.

In this study the efficacy of copper to combat container-breeding mosquitoes such as *Oc. japonicus*, *Cx. pipiens/torrentium* and *Ae. aegypti* has been evaluated in order to use copper coins against container-breeding mosquitoes and to limit the distribution of exotic mosquitoes such as *Oc. japonicus* and *Ae. albopictus*.

MATERIALS AND METHODS

In a series of experiments the effect of copper and other metals on the development of container breeding mosquitoes such as *Cx. pipiens* s.l., *Ae. aegypti* and *Oc. japonicus* have been investigated. Especially the effect of 1 Euro Cent (weight: 2.3 grams; surface: 2.8cm^2) and 5 Euro Cent ((weight: 3.9±0.1 grams; surface: 8.3 cm^2) applied in regular grave vases (volume: 750 ml) have been evaluated.

Series 1: Each five copper, steel, Zink and glass cups have been filled with 38 ml of tap water and 20 second/third instars of either *Cx. pipiens* s.l. or *Ae. aegypti* have been added in each 2 ml of water to the test vessels, a small amount of Tetra-Tabimin served as food resource. Mortality reading has been conducted after 1, 2, 3, 5, 7, 9, 10 and 13 days.

Series 2: 15 standard green plastic grave vases were filled each with 750 ml of tap water (pH: 7.2; conductivity: 70 uS), 20 second/third instars of *Ae. aegypti* have been added into each test vessel including some Tetra-Tabimin powder as food resource. In each of five vases one 5 cent coin or one 1 cent coin were added, respectively. 5 vases without coins served as control. The same test set-up was used for tests with *Cx. pipiens* s.l. second/third instars. The mortality reading was conducted at 1, 2, 3, 5, 7, 10, 13 and 15 days.

Series 3: In this series the effect of copper ions on larvae of *Ae. aegypti* and *Oc. japonicus* was tested. All 20 grave vases were filled with 750 ml of tap water (pH: 7.2; conductivity: 70uS). Into ten vases 20 second/third larvae of *Ae. aegypti* or *Oc. japonicus* were added in addition to small amounts of TetraTabimin. Five vases of each set were treated with one 5 cent coin and 5 vases remained untreated as control. The mortality reading was done 1, 2, 3, 5, 7, 10, 12, 13 and 14 days.

RESULTS

In the first experiment the effect of different metals on the development of *Culex pipiens* and *Ae. aegypti* larvae has been evaluated. *Cx pipiens* sl. and *Ae. aegypti* larvae in the copper vessels were all dead after 10 days, In the same time period in the Zinc vessels only 45 % and 50% of *Ae.aegypti* and *Cx, pipiens* s.l. larvae and in the steel vessels 33.3% and 41.6% died, respectively. In the glass vessels 1% of the both species died (Figure 1).

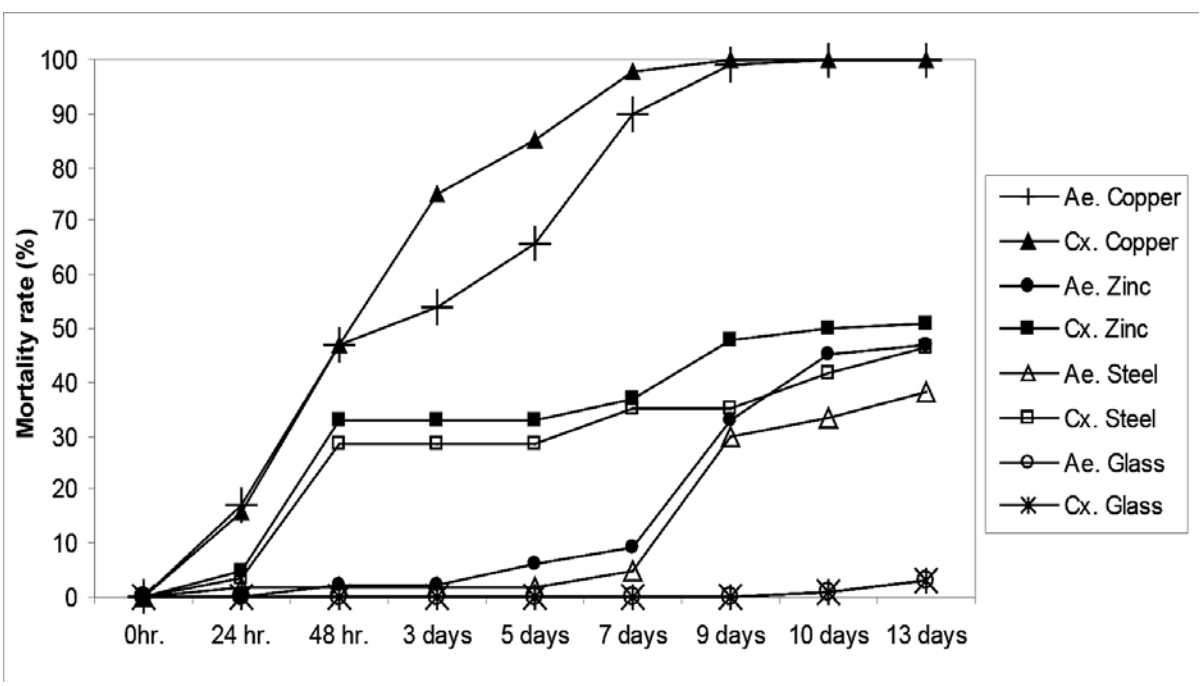

Figure 1. Effect of copper, zinc, steel and glass breeding vessels on development of *Ae. aegypti* and *Cx. pipiens* larvae.

In the second experiment the effect of one cent and 5 cent coins in standard grave vases have been tested. After 15 days all larvae were dead in the 1 cent treated as well as in the 5 cent treated vases. Not

only was there no difference recorded between the 1 or 5 cent coin treated vases, but A*edes* and *Culex* larvae were similar sensitive (Figure 2).

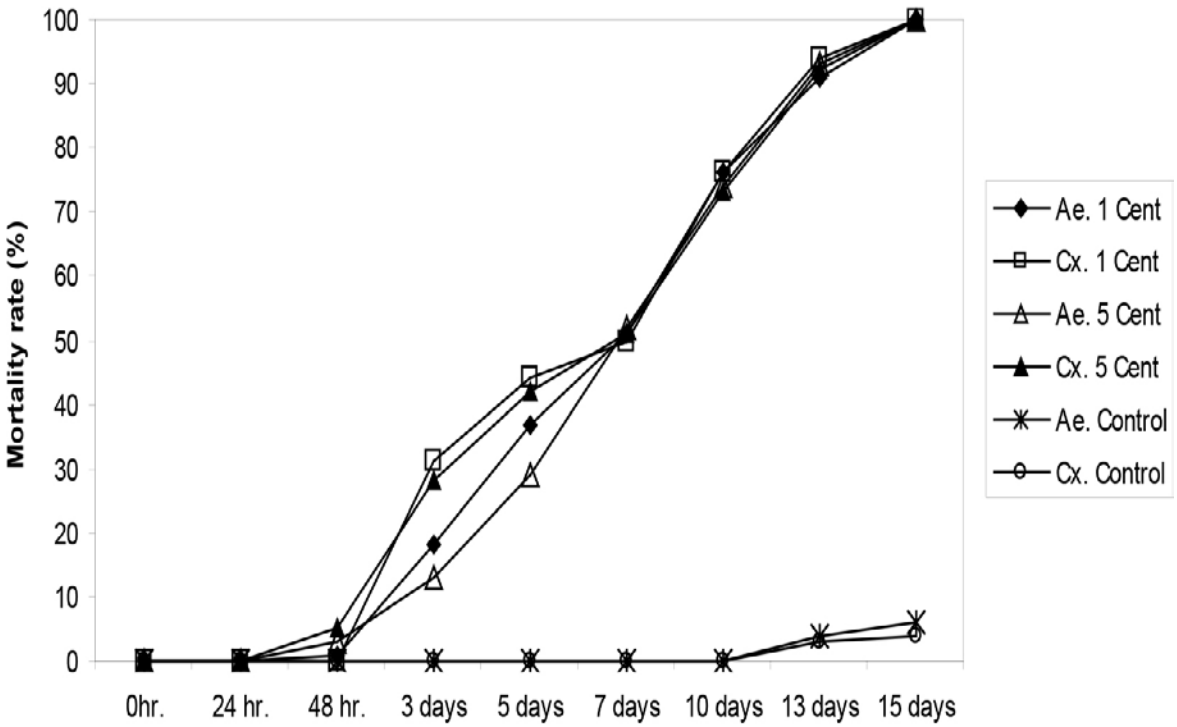

Figure 2. Effect of 5 cent and 1 cent coins on development of *Ae. aegypti* and *Cx. pipiens* larvae.

In the third experiment the effect of 5 cent coins on the development of *Ae. aegypti* and *Oc. japonicus* larvae in standard grave vases have been evaluated. After 14 days all larvae of both species were dead (Figure 3). The larvae of *Oc. japonicus* are slightly more sensitive than the larvae of *Ae. aegypti*. After 5 days 72% of the larvae of *Oc. japonicus* died whereas only 35% of the *Ae. aegypti* were dead.

DISCUSSION

In the course of the German surveillance programme of exotic mosquitoes especially cemeteries have been found infested by *Oc. japonicus*. Cemeteries seem to be an ideal breeding ground for container breeding mosquitoes because the resources are excellent for the development of container breeding mosquitoes: vases provide breeding sites, flowering plants serve for nectar feeding of the adults, humans provide blood meals and bushes are resting places for the adults. Whereas, Bti-tablets can be successfully used in wells or larger water containers, the control of mosquitoes in vases remain mainly on environmental sanitation (e.g. emptying of the vases each week) which is seldom done. In this study it was shown that container breeding mosquitoes can be easily controlled by the use of copper coins.

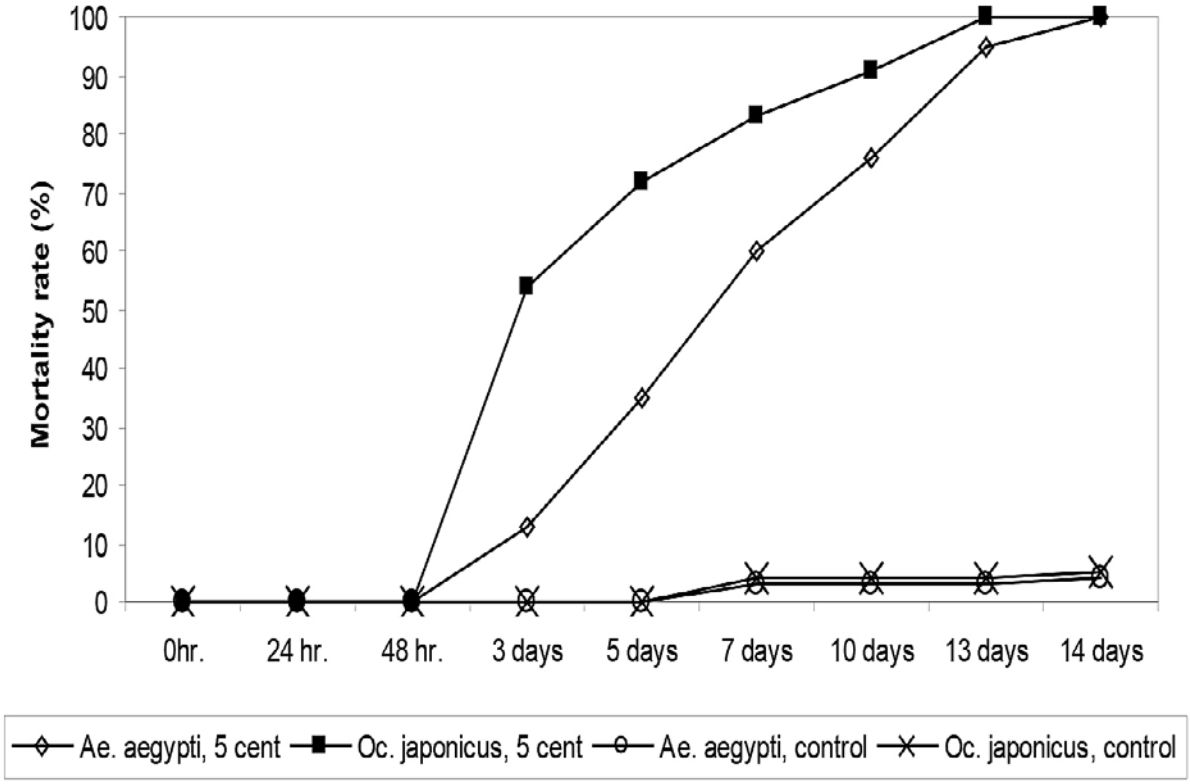

Figure 3. Effect of 5 cent coins on development of *Ae. aegypti* and *Oc. japonicus* larvae.

The results obtained here prove the results of Romi et al. (2007) who found that copper prolonged the development of mosquito larvae and even killed them at a certain copper concentration. In a research program in Florida it was shown that the toxic effect of bronze limits the development of *Ae. aegypti* and *Ae. albopictus*. Copper formulations are known as fungicides for more than 100 years. Copper is a trace element for humans and is not toxic at low dosages. WHO allows up to 2 ppm copper concentrations in drinking water. In further studies different application forms of copper will be tested to provide an easy tool for the control of container breeding mosquitoes for the general public.

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