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DEVELOPMENT AND EVALUATION OF TESTING METHODS FOR ANT REPELLENTS

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Abstract Repellents are biocides and a proof of efficacy is required for active substance inclusion as well as for product authorization. Here, we describe two test systems for efficacy evaluation of ant repellents with the substances DEET 50 %, Margosa extracts 100 %, baking powder: sodium hydrogen carbonate and sea sand. *Monomorium pharaonis* was tested in both systems. They are designed as choice tests and allow testing of solid and liquid substances. In the first test system, a forced-choice test, groups of 50 - 70 ant workers were set into an escape situation with a choice to escape either over a treated or an untreated surface. The second test system was a *simulated use*-test: Whole colonies, including workers, brood and queens were set into a test arena and allowed to forage. Between the nest and the food/water source, a barrier was placed which could only be passed over two small bridges, of which one was treated with the test substance and one untreated. In both test systems, ants crossing the surfaces were counted. The results of the study show that DEET repelled in both systems 100 % of the ants. Margosa repelled in the forced-choice test 96 % and 100% of the foragers were repelled in the *simulated use*-test. Baking powder had no repellent effect. 42 % of all workers in the forced-choice test and in the *simulated use*-test 51 % of the foragers crossed the substance. A comparison of the data revealed that the repellent effect of each substance was similar in both test systems.

Key words *Monomorium pharaonis*, pest control, testing method

INTRODUCTION

Most ant species in buildings in Europe are nuisances, but some have also the potential to destruct building materials or, like Pharaoh ants (*Monomorium pharaonis*), are potential mechanical vectors for pathogen bacteria (*Klebsiella* sp., *Staphylococcus* sp. or *Enterobacter* sp.; Zarzuela et. al, 2005; Moreira et. al, 2005). *M. pharaonis* is spread worldwide (Wetterer 2010). Their potential for distribution is facilitated by their small size, as they can nest in numerous small transportable objects. Pharaoh ants are polygynous and new colonies can be founded easily (Peacock et al., 1955; Petersen and Buschinger, 1971) through fission or budding (Vail and Williams, 1994). In temperate regions they occur almost entirely indoors.

Control of pharaoh ants and other tropical ant species as potential vectors is required especially in hospitals or food facilities. Ants can be controlled with baits which act slowly and are transported by foraging workers to the nest and fed to queens and brood. For ants nesting outside of buildings an alternative control method cis the use of repellents to hold back the workers from food sources. Ants living indoors may be repelled from sensitive areas in addition to control with insecticide baits. Repellents and insecticide baits are biocides according to the EU Biocides Directive (528/2012). A requirement for authorization is proof of their efficacy. However, the relevant EU biocide guidance documents (ECHA 2016) provide no tests descriptions for repellents against ants.

In this study, we compared two test designs: a short-time *forced choice*-test, where the ants are set in an escape situation, and a *simulated use*-test, conducted with whole colonies with workers, queens and brood. Repellents were tested in both systems to compare their efficacy on *Monomorium pharaonis*.

MATERIALS AND METHODS

Study Animals. Ants were taken from laboratory colonies of the German Environment Agency. The ants were provided with dead *Periplaneta americana*, sugar and a drinking trough consisting of a small petri dish with a cotton ball soaked with water. Climate conditions were 25 ± 1.5°C and 60 ± 10 % humidity.

Repellents and Substances. Substances were tested: DEET 50 % (NobiteÒ Hautspray, Tropical Concept Sarl), Margosa extract 100 % (vectrade UG), baking powder: sodium hydrogen carbonate $NaHCO_3$ (Kaiser-Natron®, Arnold Holste Wwe. GmbH & Co. KG) and sea sand (Merck KGaA).

Test Designs. The first test system (Figure 1, A) is a *forced choice*-test (modified after Dani et al., 1996). A petri dish with its walls coated with FluonÒ (Whiteford GmbH) was placed upon an inverted beaker. At two sites the walls were left blank on the position where two microscope slides served as bridges for the ants to two different beakers. One of the slides was treated with the test substance, the other slide was non-treated and served as alternative escape route for the ants. About 50 to 70 ants were put into the petri dish and had 30 minutes to escape over one of the slides, from which they subsequently fell into the beakers adjacent to them. If the ants did not move in the petri dish, they were gently disturbed with a brush. Every 10 minutes the slides and the adjacent beakers were swapped with each other to avoid possible side preferences by the ants. After 30 minutes the ants in the beakers were counted.

The second test system is a *simulated use*-test (Figure 1, B and C). Whole colonies, including workers, brood and queens were set into the test arena and allowed to forage for a week. Between the nest and the food/water source, a barrier of insect glue was applied. An untreated microscope slide served as bridge over this barrier to water and food. After a period of 7 days for acclimatisation, the untreated slide was replaced with a slide treated with the repellent substance and a second untreated slide was provided at the opposite side as alternative route to food and water. Ants passing the slides were counted over a period of 5 minutes directly after the repellent substance exposition, as well as 3 to 5 hours, 24 hours and 48 hours after exposition.

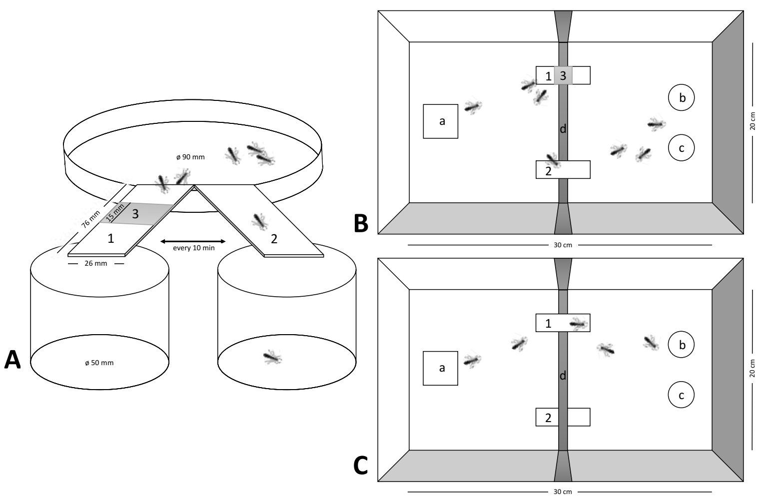

Figure 1. A – *Forced choice*-test. Petri dish and two beakers with microscope slides as bridges to escape. 1 - microscope slide/ bridge with repellent substance (3), 2 - untreated slide/bridge. B – *Simulated use*-test acclimatization period for 7 days. a – nest, b – food, c – water, d – insect glue as barrier, 1 – slide as bridge, 2 – slide with barrier. C – *Simulated use*-test after change of microscope slides. a – nest, b – food, c – water, d – insect glue as barrier, 1 – slide with repellent substance (3), 2 - slide as alternative route to food and water.

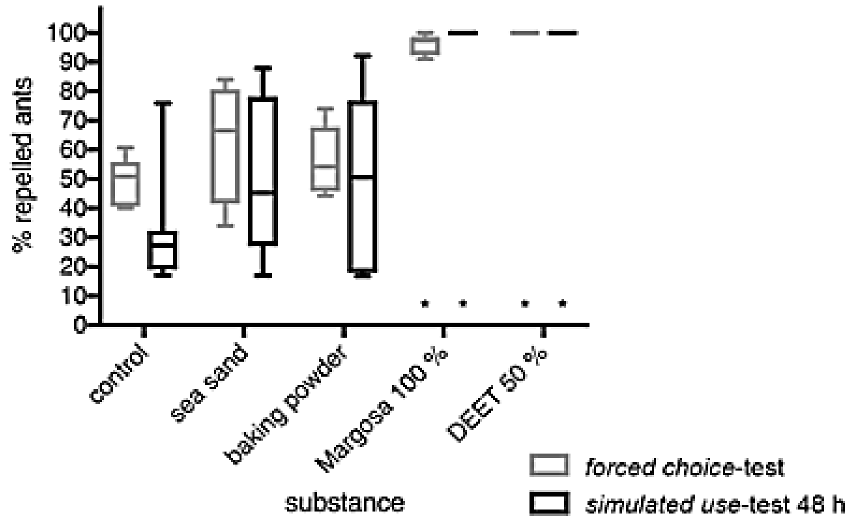

Figure 2. Repellent effect of the test substances in *forced choice*-test and in *simulated use*-test 48 h after application of the substances against *Monomorium pharaonis*. Boxplots show the percentage of workers which crossed the untreated slide.

In this study only the data of the 48 h counting periods in the *simulated use*-test were taken into account to compare the two test designs. In both systems, each substance was tested in 7 replicates. The efficacy of the substances as repellents was determined by counting the amount of workers that crossed the untreated bridge in comparison to the treated bridge.

Data analysis. Data analysis and evaluation was done with graph pad prism 7.0. A Kruskal-Wallis-test and Dunn´s post hoc test showed if the data of the substances were significantly different to the data from the control. Results for the different substances in both test systems were compared with multiple t-tests for significant differences.

RESULTS AND DISCUSSION

In both test systems, repellent effects of the test substances against pharaoh ants were observed. Substances with high repellent potential against pharaoh ants were DEET and margosa extract. Other substances like baking powder or sea sand were less efficient or showed no effect. In both tests systems, 100 % of the *M. pharaonis* foragers were repelled by DEET. Undiluted margosa extract caused strong repellent effects. In the *forced choice*-test, only 4 % *M. pharaonis* workers crossed the margosa extract and 100% of the foragers were repelled in the *simulated use*-test. Baking powder had no repellent effect to pharaoh ants. In the *forced choice*-test 42 % of all foragers, and in the *simulated use*-test 51 % of all foragers crossed the test substance. With sea sand as test substance, in the *forced choice*-test more ants 63 %) crossed the untreated slide compared to the *simulated use*-test with only 49 %. The results for efficacy of repellents were not significantly different between both test systems for all substances tested so far.

Efficacy testing of different substances, which are potentially repellent against ants, is possible in both systems. As the motivation of the ants to pass the treated or control surfaces is relatively high (escape reaction) in the *forced choice*-test, a large number of individual ants can be checked for their reaction in a relatively short period of time. Moreover, the test design is quite simple and can be realized using standard laboratory materials (microscope slides, petri dish, beakers).

In the *simulated use*-test, the motivation of ants to cross the test surfaces is closer to the practical situation in which repellents against ants are being applied. However, a disadvantage of the test system is that the number of tests is limited by their long duration, and that for each test, a complete ant colony is needed. As substances with a medium or low repellent potential show a relatively high variance in test results, the possibility to conduct more test replicates with the *forced choice*-tests in a shorter time may overweigh the less artificial test situation in the *simulated use*-tests, since both test systems deliver consistent data so far.

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