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Lower dose of plant substance more effective in repelling *Rhyzopertha dominica* F. (Coleoptera, Bostrichidae) and *Sitophilus granarius* L. (Coleoptera, Dryophthoridae)

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Abstract

Introduction and Objective. The study compares the effect of two concentrations (0.1% and 1%) of plant material on repelling two dangerous stored pests – *Rhyzopertha dominica* F. and *Sitophilus granarius* L. Both species were treated with the essential oils of *Foeniculum vulgare* Mill., *Carum carvi* L., *Mentha pulegium* L., and *Cananga odorata* (Lam.) Hook.f. & Thomson, as well as the chemicals contained in these oils: anethole, L-carvone, pulegone and thymoquinone.

Materials and method. Laboratory tests were carried out at 29±1 °C with 60±5% relative humidity (RH). Repellence of insects were noted after 1, 2, 3, 4, 5, 24 and 48 h.

Results. A lower dose of essential oils and some of the tested compounds caused a stronger repellence in the specified species of beetles. In the case of *R. dominica*, the concentration of 0.1% pulegone and L-carvone had the greatest repellent effect. Differences in the repellence of *R. dominica* were found between the concentrations of 0.1%-1% among all the tested substances in each time interval. This difference ranged from 5.0%-52.92%. All substances used in the tests had a strong repellent effect on *S. granarius* (30%-100%). There were differences in the repellency of *S. granarius* between 0.1%-1% concentration in most of the tested substances in each time interval, but they were not as large as for *R. dominica*.

Conclusions. The most interesting results were obtained concerning the relationship: the lower tested substance concentration caused a much stronger repellent effect of *R. dominica* and *S. granarius*.

Key words

storage pests, plant substances, repellence

INTRODUCTION

Rhyzopertha dominica F. (Coleoptera, Bostrichidae), the lesser grain borer, and *Sitophilus granarius* L. (Coleoptera, Dryophthoridae) grain weevil, are among the most dangerous species of primary pests that destroy grains [1–4]. These pests are widely distributed worldwide. In the tropical and subtropical climate zones, they cause damage to crops as well as stored grains and their milling products. In countries with a moderate climate, among others, in Poland, their development is limited mainly to heated warehouses where they destroy the accumulated food stocks [5]. Beetles of the lesser grain borer and grain weevils are polyphages. They most often attack cereals: rice, wheat, maize, rye, triticale, barley, oats, buckwheat, sorghum, millet and grain products. *Sitophilus granarius* less frequently eat other plant materials, such as: seeds of oilseeds and legumes, acorns, chestnuts [3, 6]. On the other hand, *R dominica* also attacks the seeds of chickpeas, peas, beans and dried fruit, vegetables, herbs, as well as cork, wood and paper products [5, 7, 8]. *Rhyzopertha dominica* survives and multiplies in the seeds of some trees and shrubs and in damaged acorns [1].

Rhyzopertha dominica and *Sitophilus granarius* cause serious damage to stored food products which, losing their weight and quality, expose food producers to economic losses. Moreover, when *S. granarius* is abundant, it causes moisture and heating of the affected products. Through their physiological activity and the formation of clusters, weevil beetles locally raise the temperature and humidity in grain or other infected products, creating the so-called 'hot spots, nests' [9, 10]. The larvae and adult beetles of *R. dominica* feeding on grains produce a large amount of flour dust, leaving thin brown shells [11].

Apart from the weight loss of cereal products and the decrease in their quality, these beetles are also of medical importance related to their allergenicity. Each life stage – egg, larva, pupa and adults – can infest food and may be a potential source of allergens [12, 13]. The most important insects that can cause an occupational allergy are moths, psocids, cockroaches and beetles. There are many species of beetles known which have been proved to cause allergic occupational complaints, such as *Sitophilus granarius*, *Tenebrio molitor* and *Tribolium confusum* [12–16]. Storage insects, including *S.*

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granarius and *R. dominica*, can cause occupational allergies in humans. *Sitophilus granarius* causes allergic alveolitis AZPP (so-called pulmonary hypersensitivity), as well as itchy skin, hives, rhinitis, dyspnoea and bronchial asthma in workers exposed to these beetles [17, 18].

Meeting the demand for food with the ever-growing human population is a global problem. In developing countries, cereal grains are the staple food and about 70% of the human population is dependent on agriculture [19]. Unfortunately, according to Trivedi et al. [20], about 30% of global food production is deteriorated as a result of the destructive activity of crop and storage pests. By causing economic and health losses, storage pests have for many years been motivating scientists and food producers to undertake various methods of their elimination. The pest control methods currently in use are not 100% effective or are indifferent to human health and the environment. Among other things, these factors contributed to the intensification in the search for new, effective methods of pest control. Scientists are still looking for effective ways to reduce pests that safe for both humans and the environment, and are cost-effective and readily available. It seems that these conditions are met by substances of plant origin because they biodegrade more readily, pollute the environment less frequently, and are less toxic to mammals [21, 22]. Plant essential oils (EOs) have various properties, such as biodegradability and selectivity against target pests, therefore, they fit into the assumptions of the current method of safe pest reduction, which is integrated control [23].

Over the last several decades, many studies have been carried out on plants that limit the development of harmful insects, increase their mortality, and scare away pests from stored products. As a result of this research, numerous studies were undertaken which contain lists of plant species with anti-nutritional, insecticidal, repellant properties, limiting fertility and the number of offspring of storage pests [23–25]. However, despite such a large scale of research conducted in this area, currently, biopreparations are still used on a large scale to a small extent. Biopreparations are not used on a large scale due to some physical properties, including very low vapour pressure, high molecular weight and high boiling point. These features contribute to their short durability and lower toxicity than synthetic substances. Testing for their toxicity is expensive. Moreover, the implementation of botanical insecticides is associated with a long procedure, especially in the European Union, requiring approval by regulatory authorities [26–28]. Recently, the interest in natural substances of plant origin has increased. Solutions are being sought that allow the use of biopesticides on a large scale. First of all, the problems related to the short durability and toxicity of biopesticides lower than synthetics, can be eliminated thanks to constantly improved methods based on nanotechnology [26, 29].

In addition, scientists are trying to prove that the active substances contained in biopreparations do not adversely affect food products. The essential oils (EOs) and powders from *Cinnamomum cassia*, *Mentha spicata*, *Piper nigrum*, *Illicium verum*, *Cryptocarya alba* and *Myristica fragrans* were tested for repellency and/or insecticide. No negative influence of the above-mentioned plant products on the germination of wheat seeds was demonstrated [30–32].

The aim of the presented study was to compare the effect of two concentrations (0.1 and 1%) of different plant substances on repelling *R. dominica* and *S. granarius*. Both species were treated with the EOs of *Foeniculum vulgare* Mill., *Carum carvi* L., *Mentha pulegium* L., and *Cananga odorata* (Lam.) Hook.f. & Thomson, as well as the chemicals contained in these oils: anethole, L-carvone, pulegone and thymoquinone.

MATERIALS AND METHOD

The study was conducted in laboratory conditions at 29° ± 1 °C with 60 ± 5% relative humidity (RH). Adult *R. dominica* and *S. granarius* specimens were used for the tests. The methodology of emigration (repellence) developed by Kłyś [33] was used in the emigration tests. Sets containing two plastic breeding containers were used: an inside container with a 28 cm^2 of floor area, and an outside container with a 50 cm^2 floor area. Forty grams of wheat grain were placed in each container. Wheat grain was the food and place for egg laying for the beetles. The containers were tightly sealed with perforated lids. The inside container had 30 holes of 1.5 mm in diameter separated by 1.5-cm spaces in the floor and sidewalls up to the level of the grain. Four 4-cm-high 'crew inserts' were mounted onto the bottom of the inside container, allowing for the placement of the container above the wheat grain in the outside container. 'Screw inserts' prevented migrating beetles from returning to the inside container.

The insects were placed in the inside container together with a circular ring of filter paper soaked with the EOs of *F. vulgare*, *C. carvi*, *M. pulegium* L., *C. Odorata*, as well as compounds containing these oils: anethole, L-carvone, pulegone and additionally thymoquinone. EOs and substances in concentrations of 0.1% and 1% by weight were also used. All liquid substances used in the experiments were undiluted. The oils and compounds were bought from Merck KGaA. The insects were put into the inside container, numbering 40 pieces in each variant of the experiment and in each of its replications. The soaked filter paper disc was placed over the wheat, with which it had no contact. The emigration of insects from the internal to the external container was considered as the repellency. The repellent effect were recorded after 1, 2, 3, 4, 5, 24 and 48h of exposure. Each variant of the experiment was conducted in 6 repetitions.

The emigration rate was calculated according to the formula [28]:

$$\frac{\bar{X}el + \bar{X}ed}{\bar{X}l + \bar{X}d} * 100\%$$

$\bar{X}el$ – mean number of live migrants;
$\bar{X}ed$ – mean number of dead migrants;
$\bar{X}l$ – mean number of live individuals in both containers;
$\bar{X}d$ – mean number of dead individuals in both containers.

The control culture was carried out in the same temperature and humidity conditions, in the same set of containers and with the same number of replications as the experimental cultures. The substrate in the control culture was 40 g of pure wheat. The filter paper disc placed in the smaller vessel was not soaked with any substance. Forty beetles were released into the containers prepared in this way.

The ANOVA Kruskall-Wallis rank test was applied, followed by a multiple comparison test. The test probability level "p" and the significance level "α" were 0.05. The calculations were performed in the Statistica 13.3 program.

RESULTS

It was found that all EOs and plant substances used in the tests at all concentrations resulted in repellence of *S. granarius* and *R. dominica* in each time interval. Only 1% of fennel oil after 1 hour of research did not cause repellence of *R. dominica*. Among EOs, the highest emigration (repellency) (40% – 57%) of *R. dominica* was caused by 0.1% of *M. pulegium* EO over 1 – 3 hours and yang oil (70% – 90%) of 4 – 48 hours, followed by 0.1% caraway oil (30% – 73%) and 0.1% fennel oil (5% – 67%). On the other hand, the lowest repellency was caused by caraway (10% – 20%) and fennel (6% – 50%) oils. On the other hand, in the control culture, the emigration index values were low throughout the entire study period, ranging from 7% – 29% (Fig. 1).

In the case of *S. granarius*, all EOs used resulted in a large emigration of beetles, confirmed by high values of the emigration index ranging from 31% – 100%. Oils in the concentration of 0.1% caused a greater repellent effect of *S. granarius* than in the concentration of 1%. The greatest repellence effect of *S. granarius* was obtained after the application of 0.1% fennel oil (83% – 100%) (Fig. 2).

It was found that only 1% of anethole from the tested chemical compounds showed no repellant properties against *R. dominica*, because the values of the emigration index were lower compared to the control culture in each time interval. The highest statistically significant repellant properties against *R. dominica* were shown by 0.1% pulegone (40% – 100%) and 0.1% L-carvone (39% – 90%) (Fig. 3).

All the chemicals used against *S. granarius* resulted in a higher repellency compared to the control culture in each time interval. The emigration rate values ranged from 48% – 98%. The greatest statistically significant repellent effect was obtained with 0.1% pulegon (84% – 96%). After 48 hours of testing, all the chemical compounds used showed high repellency (90% – 98%) (Fig. 4).

It was found that the lower 0.1% concentration of the applied EOs and chemical compounds caused a greater effect in repelling the lesser grain borer beetles in all time intervals.

In the case of *S. granarius*, all EOs at a concentration of 0.1% resulted in greater beetle repellence than the concentration of 1%. On the other hand, among the chemical compounds used, only 0.1% pulegone resulted in a greater repellent of weevil than 1% (Fig. 2, Fig. 4).

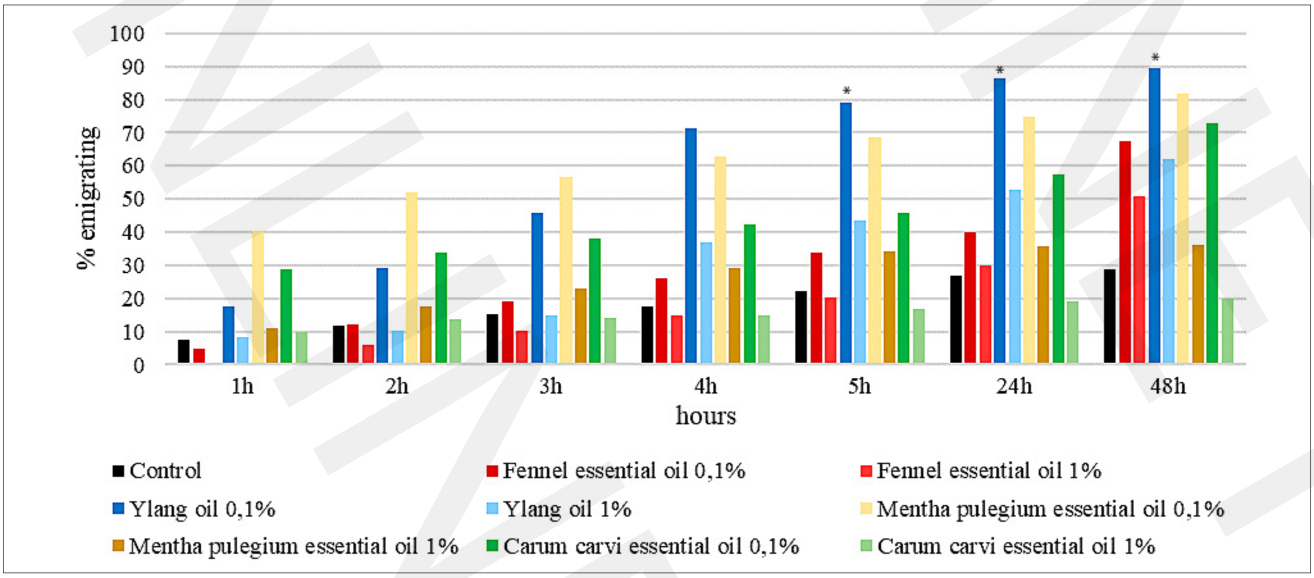

Figure 1. Repellency of *Rhyzopertha dominica* caused by essential oils. (Statistically significant differences are marked with an asterisk *)

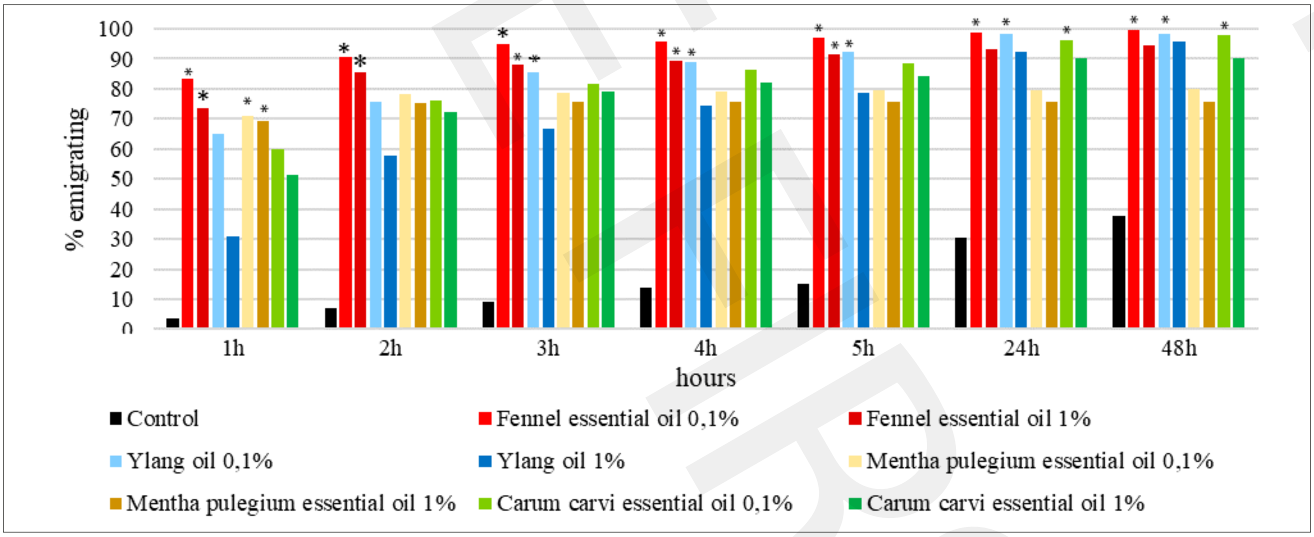

Figure 2. Repellency of *Sitophilus granarius* caused by essential oils. (Statistically significant differences are marked with an asterisk *)

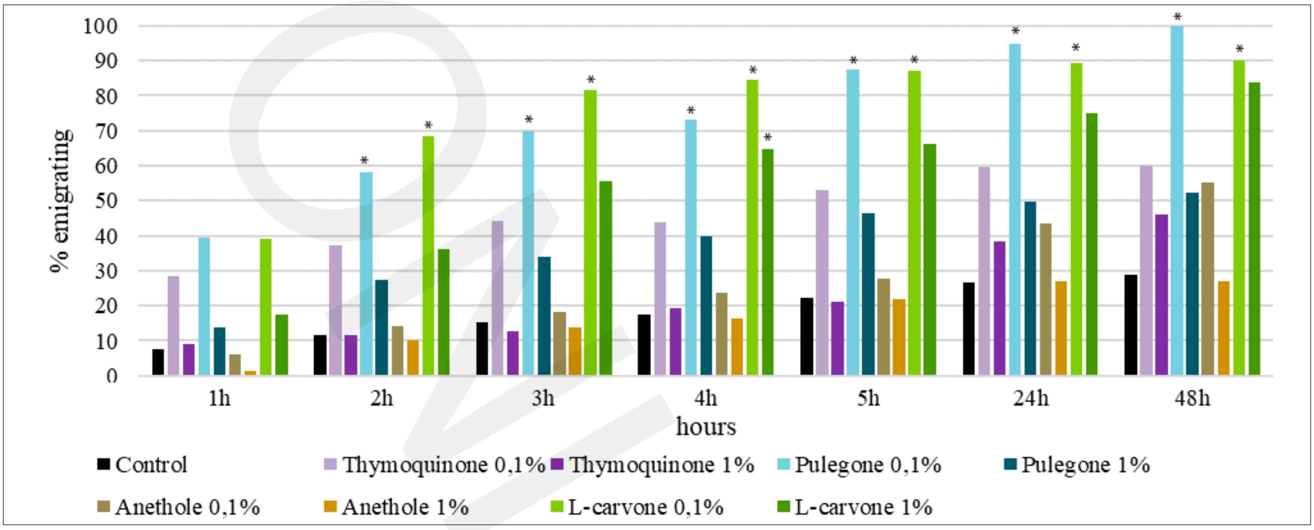

Figure 3. Repellency of *Rhyzopertha dominica* caused by substances isolated from essential oils. (Statistically significant differences are marked with an asterisk *)

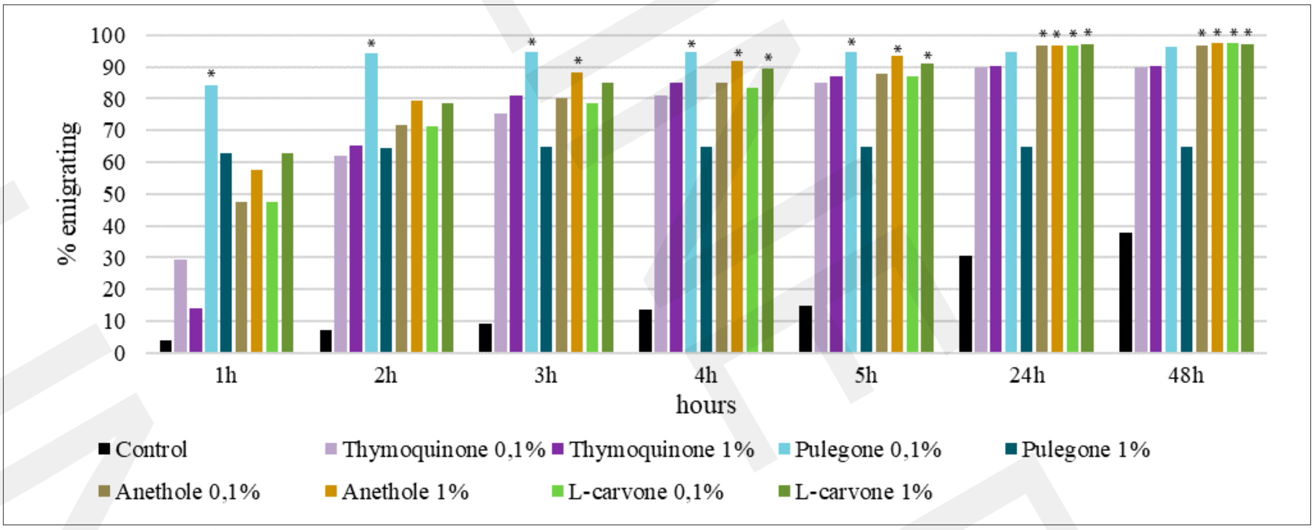

Figure 4. Repellency of *Sitophilus granarius* caused by substances isolated from essential oils. (Statistically significant differences are marked with an asterisk *)

Although not all differences are statistically significant, there is a clear tendency – lower concentration, higher repellency of both pest species tested. Much greater differences in the repelling of beetles between the concentrations of 0.1% and 1% of the plant material used were noted for the lesser grain borer beetle than in the case of the grain weevil. For the lesser grain borer, the biggest difference was recorded for caraway oil after 48 hours – 52.92%. However, for the grain weevil the differences were smaller, because in most of the results did not exceed 10%.

DISCUSSION

Substances of plant origin fulfill the assumptions of the current method of safe reduction of the number of pests, which is integrated control [23, 34, 35]. In addition, it is important that the plants used and the EOs and chemical compounds contained in them, apart from repellant and/or insecticidal effect on pests, are safe for humans and, at the same time, do not adversely affect the stored food products.

In the current study, plants were selected for research which have healing, bactericidal and antifungal properties, and are also used as food additives. These features make it possible to conclude that the substances contained therein, which exhibit repellant properties against the tested species of beetles, may find a broader protective application on the stored food products, additionally protecting them against infestation by bacteria and fungi.

In the study, the high repellent efficiency of fennel oil (73%–100%) and caraway oil (51%–98%) was demonstrated in both concentrations and throughout the entire period of the study against *S. granarius*. Carvone and thymoquinone also caused a large emigration of weevil beetles.

A high repellent effect against *S. granarius* caused all plant substances used in the tests, especially fennel oil (73%–100%) and caraway oil (51%–98%) in both concentrations and throughout the entire period of the study. The highest repellent effect of *R. dominica* (40%–90%) was caused by the lower 0.1% concentration of L-carvone.

Kłyś et al. [28] also showed a high (98%–99%) deterrent effectiveness of caraway oil in concentrations of 0.1, 0.5 and 1% after 24 hours of testing against *Sitophilus oryzae*. Similar results were obtained with L-carvone. Bedini et al. [36] found the repellant potential of substances contained in fennel. They investigated the repellent properties of two chemotypes

– estragole and anethole fennel EO and the chemicals they contain: anethole, estragole, limonene and fenchone. The subjects of their research were the caperin beetle, the greater rice weevil and the trout beetle. Both types of EOs were tested at doses ranging from 0.005–0.385 mg/cm^2, while the compounds were tested at various doses from 0.0- 0.74 mg/cm^2. The results were read after 24 hours. The RD_{50} values, i.e. the concentration that repels 50% of the tested insects, showed that estragole-type EO was a more effective repellent than anethole oil against all three species of tested insects. From among the chemical compounds, estragole turned out to be the most effective repellant in relation to the tested species of raspberries. The concentration values of this compound that scared away half of the tested beetles are as follows: 0.038; 0.126 and 0.060 mg/cm^2, respectively, for *Rhyzopertha dominica*, *Sitophilus zeamais* and *Tribolium confusum*.

When assessing the possibility of using a substance of plant origin as a repellent and/or insecticide, two parameters are important: its effectiveness and the time in which it will produce the desired effect. The effectiveness is assessed using various indicators, including the rate of emigration. The higher the value of a given index, the higher the effectiveness of the test substance. In addition, the period of time in which a given substance produces an effect is extremely important. A substance with a quick repellant and/or insecticidal effect will keep food supplies intact. No less important is the concentration of the substance used which will produce the desired effect, because a too high concentration may be unjustified for both economic and health reasons. Therefore, the most anticipated insecticide or repellent will be a substance that will produce a 100% effect in the shortest possible time, and in the lowest concentration.

Numerous publications on the repellant and/or insecticidal effect of substances of plant origin against storage pests suggest a dependence of the effect on the applied concentration. The experiments conducted by the authors of these studies show an increase in the repellent/insecticidal effect with an increase in the concentration of the tested substance [37–42].

The current study showed a stronger repellent effect on both species at a lower concentration of the tested plant substances. These results correspond with the studies by Kłyś et al. [28], who used a greater number of concentrations of L-carvone (0.05, 0.1, 0.5, 1%) and caraway oil (0.1, 0.5, 1%) against *S. oryzae*, and noted a stronger the effect of a lower concentration of L-carvone. This compound in the dose of 0.1% caused 100% beetle emigration after 24 hours. Higher concentrations of 0.5 and 1% showed a much lower repellent effect.

Benelli et al. [43] also showed that one of the tested oils from *Hyptis suaveolens* caused a higher repellency of *S. granarius* at a lower concentration of 0.1%, not 1%.

It is known that DEET is the most common active ingredient in repelling mosquitoes, ticks and other arthropods when applied to skin or clothing. The more of this compound the repellant contains, the longer it can protect against mosquito bites. Its higher concentrations provide longer protection, but the concentration of DEET above 50% does not bring much effect [44, 45].

CONCLUSION

The most interesting results obtained in the presented research concern the following relationship – lower tested substance concentration causes a much stronger repellent effect of *R. dominica* and *S. granarius*. It was proved that a further increase in the concentration did not repel the pest species further. In tests of the effects of natural plant material on storage pests, it is therefore important to find the lowest effective dose of a given substance.

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