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Brief report

Commercially available essential oils formulas as a repellents of common stored products pest *Alphitobius diaperinus*

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Abstract: The main scope of the presented paper is an assessment of the potential repellent effect of selected essential oils (EOs) against *Alphitobius diaperinus*, which can cause economic losses in storages and poultry industry. Due to development of pesticide resistance in *A. diaperinus* populations, as well as an attempt to limit extensive usage of potentially harmful pesticides in food-related industries, there is a strong need for development of alternative methods of management of *A. diaperinus* infestations. Because of cost-effectiveness, availability and low vertebrate toxicity EOs are promising agents in pest management. In presented paper four of-the-shelf EOs: mint, vanilla, lemon and citronella (and their mixtures) were tested as a potential repellents. Moreover, novel preference assay providing an extended analysis of preference and the locomotor response was used. The most effective EOs were, respectively: citronella and lemon. EOs mixtures were generally more repellent than single EOs, with lemon and vanilla 1:1 mixture acting as the strongest repellent. Few of tested EOs caused significant alterations in locomotor activity, although direct relation wasn't observed. In conclusion, EOs can be potentially used as a repellent agents in *A. diaperinus* management. Additionally, data on locomotor activity may lead to better design of pull-push strategies in pest management.

Keywords: lesser mealworm, essential oils, repellency, spatial preference, locomotor activity

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1. Introduction

The lesser mealworm, *Alphitobius diaperinus* (Panzer) is a cosmopolitan insect pest of stored products and a poultry industry. It infests stored grains and other amylaceous products [1]. Moreover, it is a potential vector of several pathogens and parasites [2]. Due to the lack of chitinase enzyme in some broiler strains, mealworms after being ingested may cause bowel obstruction leading to microscopic perforations in birds' intestinal wall [3]. Additionally, large-sized *A. diaperinus* population can cause structural damage in buildings, especially in thermal insulation, which leads to a drastic increase in building heating costs [4].

Insecticide resistance was reported in numerous populations of *A. Diaperinus* [5,6]. Those reports, along with growing doubts about the extensive use of synthetic pesticides in food production [7] reveal the urgency of developing new approach against lesser mealworm. Essential oils (EOs) are especially promising in fulfilling those needs: plant-derived, easy biodegradable [8], widely accessible, considered safe for vertebrates (including humans) and above all, effective [9,10]. Suitability of EO-based formulas as insecticides against *A. diaperinus* was proved recently [11]. However, the information about the repellent potential of EOs against the lesser mealworm may have crucial value in the development of the push-pull management systems [12]. Presented

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research focused on answering the question; whether (and if, how strong) the presence of EOs in the air repel lesser mealworms. In order to assess a highly developed exploratory behaviour of *A. diaperinus* the novel, non-pitfall preference (expressed as preference index – PI) test was used [13].

In the presented paper, four commercially available essential oils were tested, either single or mixed. It allows to assess potential synergistic effect which was observed in numerous experiments on microorganisms and insects [14–16]. Using the synergistic effect in creating insecticides formulas may significantly reduce the required amount of substances, thus costs.

2. Materials and Methods

2.1 Essential oils

Essential oils used in the experiment were obtained from local vendors (vanilla, lemon, mint EOs from Meister Oil and citronella EO from Vera). Tested essential oils are collected in Animal Physiology and Ecotoxicology Department, University of Silesia in Katowice, Poland.

Oils were tested separately and as a mixtures. Mixtures were formulated by adding equal parts of tested EOs together. For each oil and mixture, a series of dilutions was prepared - consequently: 0.001%, 0.01%, 0.1%, 1% 10% v/v in ultrapure deionised water. Obtained suspensions were stirred until emulsification and poured into the respective bubbler before phase separation occurred. Bubbler, providing control of airstream, was filled with an equal volume of ultrapure water. 48 individuals were used for a single concentration of essential oil or tested mixture.

2.2 Behavioral test.

Behavioral tests were conducted using the setup presented in the article [13]. In the experiments, imagoes of both sexes of *A. diaperinus* (Panzer) were used. Insects were reared in stable conditions of 50% relative humidity and the photoperiodic regime of 12/12 h LD, water *ad libitum* and standard dog food pellets (Pedigree). Each insect was placed separately in a rectangular chamber made of clear Lucite, with tubes attached to each of both ends. The tubes were providing a constant flow of humidified air from one end and humidified air with tested odour from the other [13]. Airflow was kept at 10 l/h. Inlet air was pumped through a bubbler containing mineral oil (to capture possible contaminants from the pump) and then was separated into either a water bubbler or a bubbler with an aqueous solution of odour compound. Constant homogeneous background light was provided by a red transilluminator placed underneath. Insects were able to explore chambers freely. Recordings of experimental procedure were captured by Microsoft LifeCam 500 webcam and VirtualDub 1.10.4 software as.avi files. Recordings last for 20 minutes at frame rate 15 fps and resolution of 640x860 px. The whole setup was placed in an enclosed, ventilated box, providing isolation from external visual and acoustic stimuli.

Tracking of insects' movement and analysis was performed as same as in the Baran (2018) article. For all analysed videos three parameters were calculated: preference index (PI), distance and resting time. Although, 20-minute long video was divided on half and analysed as two, 10 minutes long intervals (interval I and II). The division was caused by a difference in behavioural reaction of insects. In the first interval activity and exploration level was very high, in the second interval locomotor activity and exploration was much lower.

2.3 Statistical analysis

Statistical analyses were conducted using Statisca® software v10. Data distributions normality was verified by the Shapiro-Wilk test, which concluded that most of the variables were not normally distributed. Groups were compared using the non-parametric Kruskal-Wallis test (Kruskal-Wallis ANOVA) with the median test. For all tests the $p<0.05$ was applied. For multiple comparisons Bonferroni correction was used.

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3. Results

3.1 Behavioural effects - single oils

3.1.1 Mint EO.

No significant effects were observed, neither did PI indicated any repellence nor attractive properties (Figure 1). Locomotoric parameters did not differ from the control group (Figure 2, 3).

3.1.2 Lemon EO.

A significant repellency was observed (Figure 1) in the highest (20% v/v) concentration and was distinguishable in both of the 10 minutes intervals. Moreover, considering the locomotor activity, a twofold decrease of total travelled distance was observed (Figure 2) at 0.01% concentration in the second interval. Simultaneously, for the same concentration, the percentage of total time spent on resting (Figure 3) was significantly higher.

3.1.3 Citronella EO.

Citronella EO showed the strongest repellent effect among all tested single EOs. The repellent effect was observed at both intervals. The repellence increased with concentration (1%, 10% v/v) in the first interval. In the second interval observed repellency was also significant but the relation to the concentration differed. Only the effect of the highest concentration was statistically significant (Figure 1). The locomotor parameters did not differ significantly in comparison to the control group (Figure 2, 3).

3.1.4 Vanilla EO.

The statistically significant effect was observed only in the first interval - repellence at the 0.1% v/v concentration. It was the lowest effective concentration of the single oil in the experiment. At higher concentrations (1%, 10% v/v) the effect was not observed, thus the Pi/concentration curve for vanilla EO is U-shaped. Despite the absence of any significant effect in the second interval the same U-shaped curve is distinguishable (Figure 1). The effects on the locomotor activity level are observable only in the first interval. At the concentration of 0.1%, distance walked was much shorter and almost twofold more time was spent on resting (Figure 2, 3).

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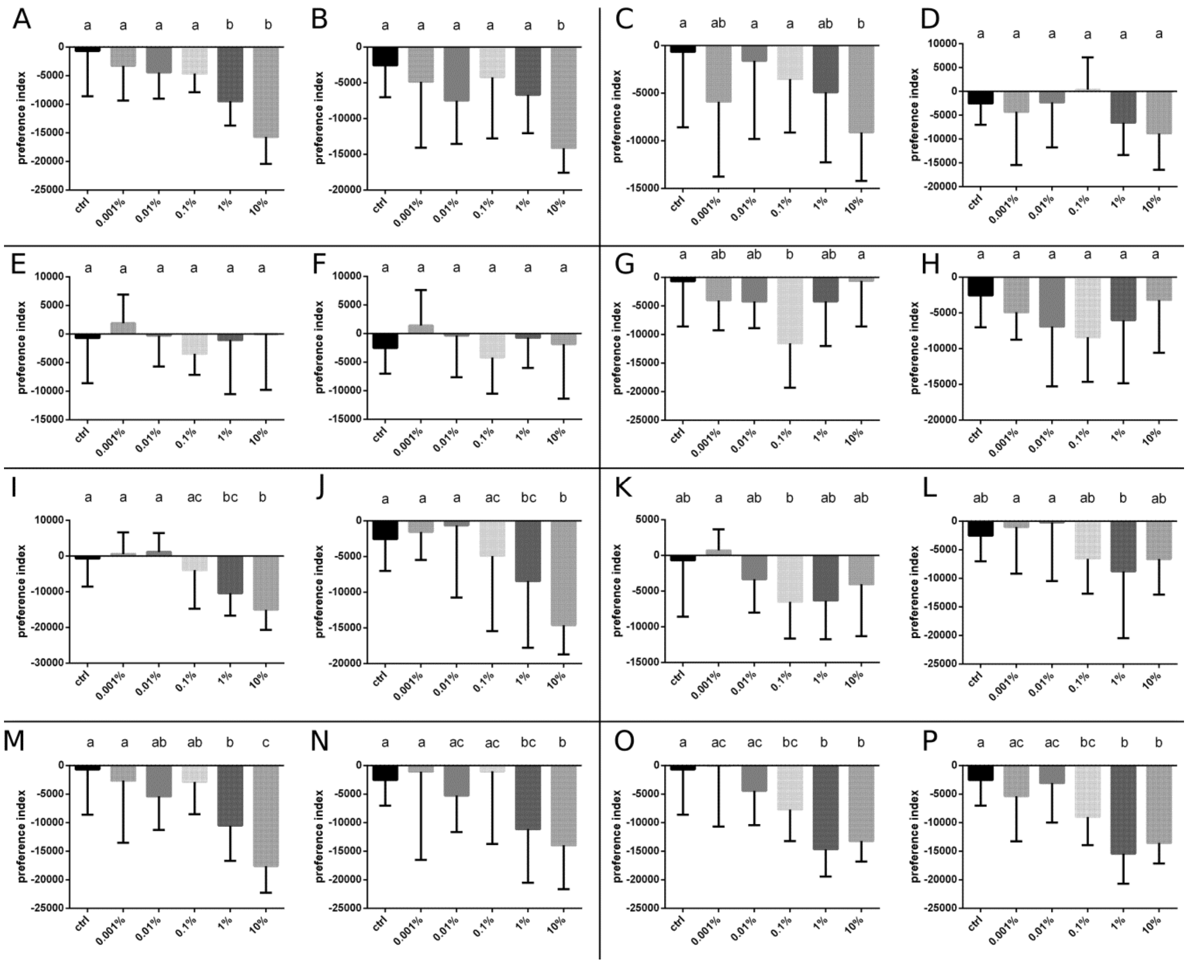

Figure 1. Preference index (PI) for insects treated with a mixture of essential oils in different concentrations and control group. Median and quartiles are presented, Kruskal-Wallis test p<0,05. Different letters indicate statistically different groups. Citronella EO A - interval 1, B - interval 2, lemon EO C - interval 1, D - interval 2, mint EO E - interval 1, F - interval 2, vanilla EO G - interval 1, H - interval 2, 1:1 mix of citronella and lemon EOs I - interval 1, J - interval 2, 1:1 mix of citronella and vanilla EOs K - interval 1, L - interval 2, 1:1 mix of vanilla and lemon EOs M - interval 1, N - interval 2, 1:1:1 mix of vanilla, citronella and lemon EOs O - interval 1, P - interval 2.

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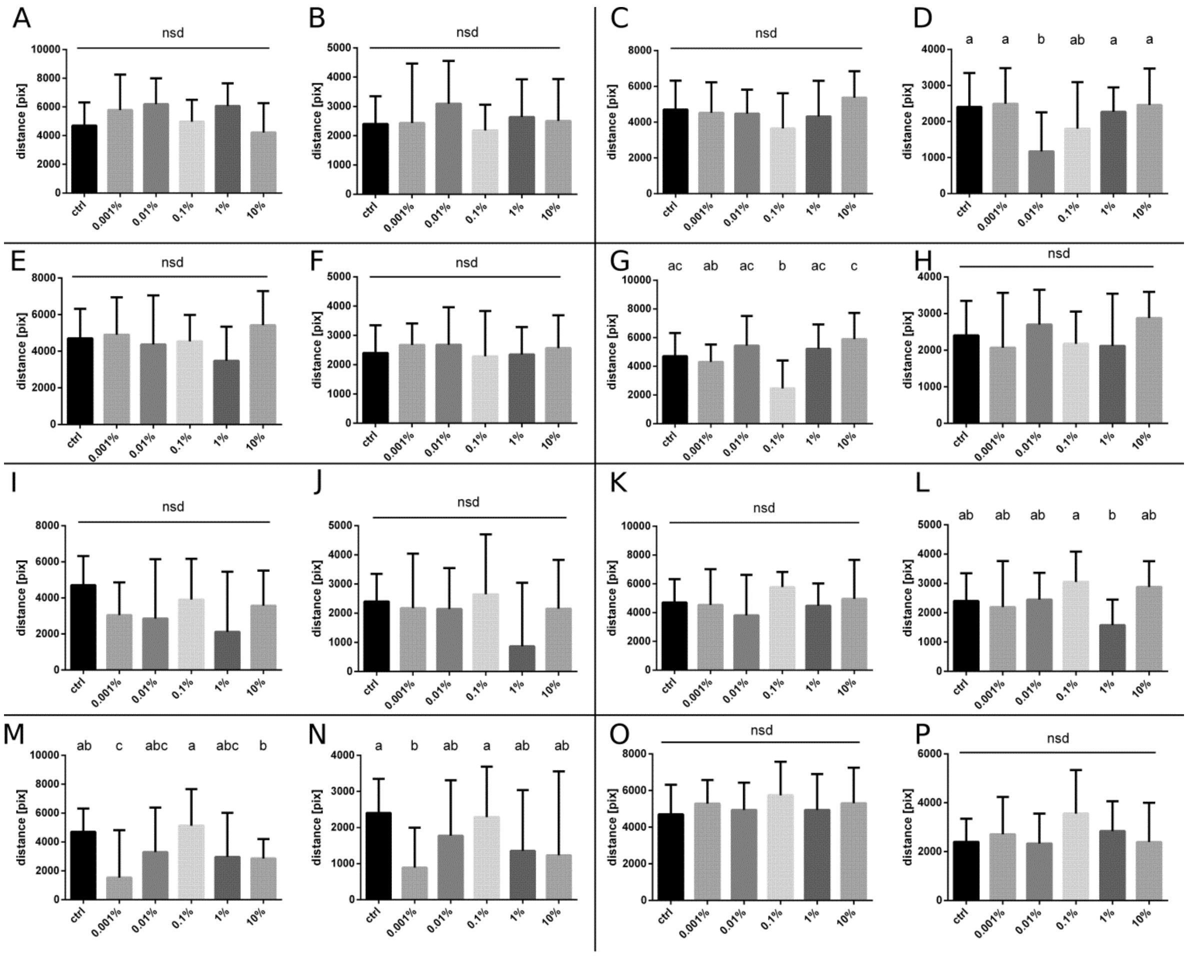

Figure 2. Distance (px) reached by insects treated with single essential oils in different concentrations and control group. Median and quartiles are presented, Kruskal-Wallis test p<0,05. Different letters indicate statistically different groups. Citronella EO A - interval 1, B - interval 2, lemon EO C - interval 1, D - interval 2, mint EO E - interval 1, F - interval 2, vanilla EO G - interval 1, H - interval 2, 1:1 mix of citronella and lemon EOs I - interval 1, J - interval 2, 1:1 mix of citronella and vanilla EOs K - interval 1, L - interval 2, 1:1 mix of vanilla and lemon EOs M - interval 1, N - interval 2, 1:1:1 mix of vanilla, citronella and lemon EOs O - interval 1, P - interval 2.

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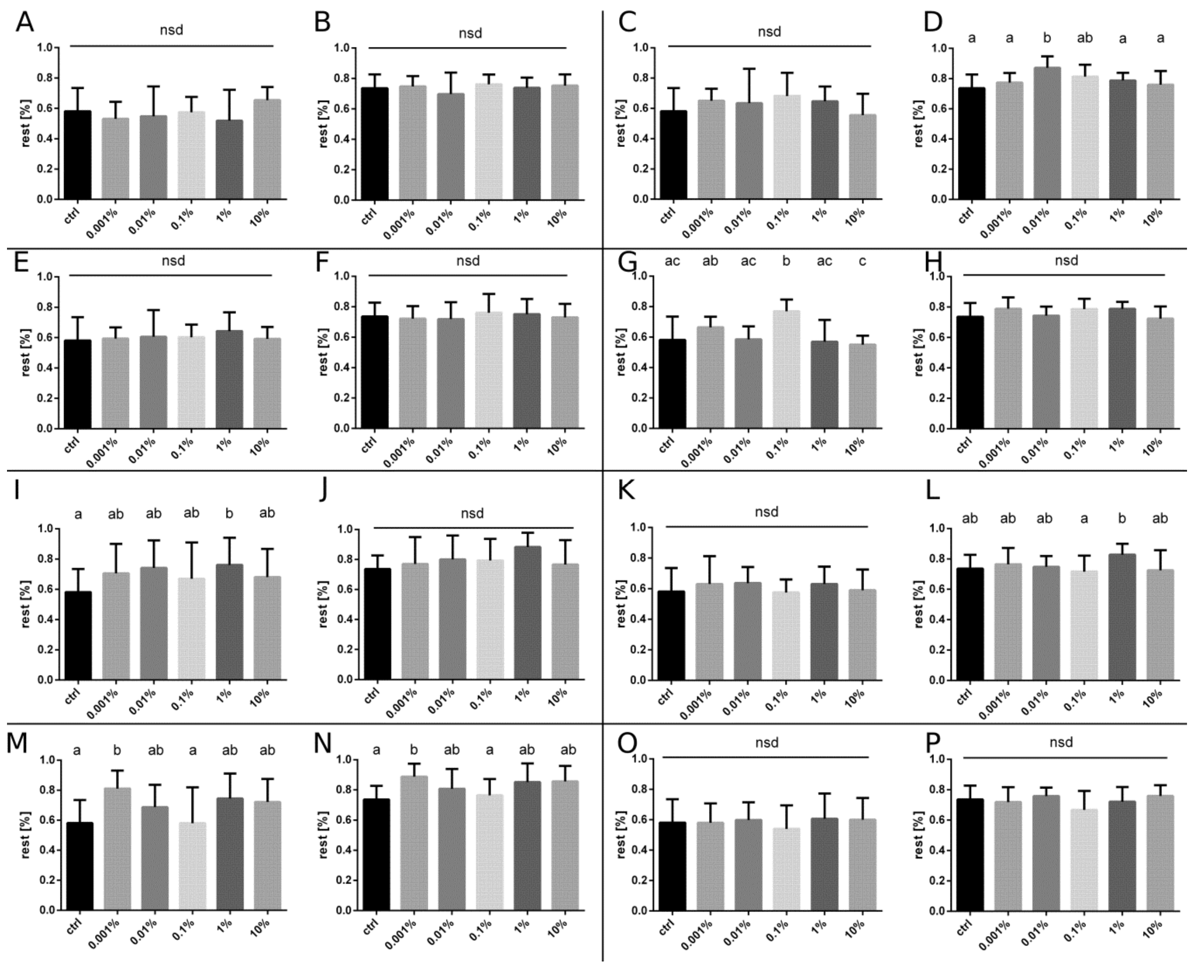

Figure 3. Resting time (%) of insects treated with single essential oil in different concentrations and control group. Different letters indicate statistically different groups. Median and quartiles are presented, Kruskal-Wallis test p<0,05. Citronella EO A - interval 1, B - interval 2, lemon EO C - interval 1, D - interval 2, mint EO E - interval 1, F - interval 2, vanilla EO G - interval 1, H - interval 2, 1:1 mix of citronella and lemon EOs I - interval 1, J - interval 2, 1:1 mix of citronella and vanilla EOs K - interval 1, L - interval 2, 1:1 mix of vanilla and lemon EOs M - interval 1, N - interval 2, 1:1:1 mix of vanilla, citronella and lemon EOs O - interval 1, P - interval 2.

3.2 Behavioral effects - oils mixtures

3.2.1 Citronella/vanilla EOs.

Significant effect on PI appeared only in the second interval, at the 1% concentration. In comparison to results obtained using single OEs, highly repellent effect of either citronella EO and vanilla EO in the first interval was suppressed in the mixture. The effect observed in the second interval could be compared to the effects of the single EOs present in the mixture, as follows: U-shaped PI curve of vanilla EO and significant repellence in the second interval same as for citronella EO (Figure 1). There wasn't any significant effect on the locomotor activity in comparison to the control group (Figure 2, 3).

3.2.2 Lemon/vanilla EOs.

In both intervals, a significant repellency of the mixture was observed in 1% and 10% concentrations (Figure 1). Even the lowest concentration (0.001%) had a significant effect on locomotor activity, decreasing the distance travelled and increasing resting time (Figure 2, 3).

3.2.3 Citronella/lemon EOs.

A significant repellent effect was observed in both intervals in case of 1% and 10% concentrations. In comparison to single EO's, a substantial amplification of effectiveness is noticeable. The repellent effect of the mixture occurred in a lower concentration than for lemon oil and lasted longer than for both oils tested separately (Figure 1).

3.2.4 Citronella/vanilla/lemon EOs.

In the first interval, repellency was observed in 1% and 10% concentrations, while the most effective were the lowest concentrations. In the second interval the repellent effect was observable from the 0.1% up to 10% concentration (Figure 1). Despite the presence of noticeable effects on spatial preference, there were no significant changes in locomotor behaviour (Figure 2, 3).

4. Discussion

For many years researchers have considered essential oils as potential pesticides against stored pests [9,17–19]. Many of them have proven insecticidal properties against common pests such as beetles. However, most studies focus on the direct effect of the oils - mortality. Results presented in this manuscript proof that EOs may also act as effective repellents, moreover it is the first report on the effectiveness of vanilla essential oil against *Alphitobius diaperinus*.

From all tasted EOs only the mint EO did not affect insect behaviour at all. While the others showed a significant repellence which occurred mainly at higher concentrations (0.1-10%) with the only exception of vanilla EO which effectively repelled *A. diaperinus* only at 0.1% vv. The strongest repellency (lowest effective concentration) was observed for citronella EO. The lowest (yet significant) repellency for single oils was observed for lemon. Similar results were reported by Wang (2014) where it was proved to have the weakest influence on the *A. diaperinus* from all tested EOs.

During described study both the Preference index and spatial behaviour were assessed. The analysis of various aspects of insect's response to EOs was considered to provide a more complete overview of repellent potential. The obtained results showed a large discrepancy at the level of both responses. The repellent effect isn't clearly linked with inhibition of locomotor activity. In single concentrations of vanilla (interval I 0.1%) and lemon (interval II 0.01%) EOs, reduction of traveled distance, as well as prolongation of resting time, was observed. Low concentrations of effective compounds may have triggered immobility while higher concentrations evoked the escape response [20,21]. Which manifested as increased locomotor activity and shortened immobility time. This is an important observation which would be impossible without implementing a multiparameter analysis.

The article also presents the results of the usage of a mixture of previously tested essential oils. Many available papers report increased efficiency of mixtures of substances in comparison to solutions containing single compounds [10,22]. The secondary principle of the experiment was to investigate the potential additive effect caused by the interaction of the active ingredients of previously described essential oils. The obtained mixtures potentially increased the chance of interaction between the components of each used oils. The effect on spatial behaviour highly differs between single oils and mixtures. A strong additive repellent effect was observed in the mixture containing lemon, citronella and vanilla, as well as in lemon and citronella EOs. This may serve as supporting evidence for the synergistic effect of EO's, which is a well-recognised mode of EOs action [14,23].

The most commonly used assays to measure olfactory preference are Y maze [24–26] test and the pitfall test [27–29]. Pitfall test is doubtlessly the easiest assay to assemble, and to a great extent resembles the properties of traps used in field practice [30], while the Y maze is a standard for assaying choice making in various animals. However, both assays rarely provide continuous monitoring of insects movement during the test. Moreover, after the choice is made insect is either trapped in a pitfall or removed from the setup (in Y maze assay), thus the insect cannot change the choice. Such approaches do not take into account the exploratory behaviour that may lead to random choices, therefore introducing noise to data. Our approach described in [13] and used in this study address this issue by allowing insect to freely explore the test chambers while its position is constantly tracked. This approach, based on easily accessible hardware and free software, could improve the quality of obtained data of olfactory preference and simultaneously provide additional

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information describing movement parameters (such as speed, distance travelled ect.) which may indicate a general physiological response to tested substances.

Large-scale usage of insect repellents' commonly is incorporated in integrated management strategy; consisting of planned placement of both lures and repellents, hence its named push-pull approach. The effectiveness of push-pull management of pests relies on modifying insects' spatial behaviour. We suggest that not only the preference of visiting a particular area but also general exploratory ability should be considered. The ability of a particular substance to downregulate or upregulate exploratory behaviour of insects should be considered as a key variable in designing whole pull-push setup. Terpenes contained in the oils can interact with various receptors that impact the baseline behavioural activity [31]. Moreover, alteration of exploratory behaviour may affect exposure to the EO-based insecticides, therefore considering behavioural parameters may be crucial in designing efficient insecticidal agents.

5. Conclusions

Most of the tested essential oils and their mixes may be considered as a potential way of managing *A. diaperinus* infestation. Additionally, the results of the assessments show a high potential for the use of mixtures of essential oils as a way of managing stored product pests infestations, because of observed distinct additive effect. Moreover, according to data obtained from conducted assessments, there was no direct relation between the locomotor activity and the strength of the repellent.

Author Contributions: Bartosz Baran, Jacek Francikowski conceived and designed the experiments; Bartosz Baran fabricated the experimental setup; Mikołaj Cup, Jakub Janiec performed the experiments; Bartosz Baran, Jacek Francikowski, Michał Krzyżowski analyzed the data; Bartosz Baran, Michał Krzyżowski, Jacek Francikowski wrote the paper.

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