Repellent effect of the caraway *Carum carvi* L. on the rice weevil *Sitophilus oryzae* L.

(Coleoptera, Curculionidae)

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Abstract

Objective
The aim of the study was to check whether Carum carvi L. essential oil and L-carvone act on Sitophilus oryzae L. as repellents and/or insecticides, in what concentrations and after what time.

Results
Caraway essential oil and L-carvone the highest repellency showed not in the highest concentrations used in the tests (1%), but in lower concentrations, respectively 0.5% and 0.1%. Caraway essential oil in all used concentrations showed repellent effects on S. oryzae. The highest repellency (60-98%) caused caraway essential oil in concentration 0.5% after 1, 2, 3, 4 and 5 h of the research. The highest repellence of L-carvone (16-100%) resulted in concentration 0.1%. The highest mortality of S. oryzae caused 0.5% caraway essential oil.

Key words: essential oil, insecticides, L-carvone, repellence, storage pests.

Introduction
It is expected that by 2050 the number of people in the world will increase to 9.1 billion and that to feed that number an additional 70% increase in food production will be needed [1, 2, 3]. The losses in world food production are enormous. At each stage of production, from producers to consumers, there are losses that constitute enormous “chain of losses”. It is estimated that 30–50% of produced food is lost each year [4]. Gitonga et al. [5] and Lesk et al. [6] report that biotic factors (insects, mites, rodents, and fungi) and abiotic factors (temperature, humidity) cause losses in total harvest reaching even up to 60%. A large number of publications indicate that the greatest losses are incurred during storage, particularly in developing countries.
In developed countries, the over-production of food is frequent, and storing the surpluses creates favourable conditions for the development of pests. Every year, only during storage, 5-10% of produced food is lost [4]. As reported by Kumar and Kalita [10], as much as 50 – 60% of cereal grains can be destroyed during storage, principally because of technical inefficiency.

It is said that the insect pest are responsible for huge losses in stored grain [11, 12, 13]. Over 20 thousand species of field and storage pests are responsible for destroying approx. one-third of the world food production [14]. One of such dangerous pest of stored cereal grain, e.g., wheat, maize, and rice, is *Sitophilus oryzae* [15, 16]. These insects bring about the reduction in germination capacity, reduction in nutritive value, and the changes in chemical composition of grains, etc.

The application of synthetic chemical insecticides rises a number of doubts associated with their adverse effects upon the environment and human health [14, 17, 18, 19]. Using natural products are effective and simultaneously friendly to the environment. Such substances include powders, oils, and extracts from plants [17, 20, 21].

The objective of the presented study was to assess the efficacy of the essential oil from common caraway and of L-carvone, which is a compound extracted from it, upon the mortality and emigration (repellence) towards *S. oryzae*. Whether the caraway essential oil and L-carvone affect *S. oryzae* as insecticides and/or repellents, the required concentrations and times of application were assessed.

**Main text**
Methods

The studies were conducted in laboratory conditions at 29°±1°C with 60±5% relative humidity (RH). Ten-day old, adult beetles of *S. oryzae* used in the tests were obtained from breeding colonies kept under the same conditions as experimental colonies. In the tests for repellence, the methodology pertaining to emigration (repellence) developed by Kłyś [21, 23]. Sets containing two plastic breeding containers were used: an inside container with 28 cm² of floor area, and an outside container with 50 cm² floor area. Forty grams of wheat grain were placed in each container. 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 grain. Four 4-cm high “screw inserts” were mounted onto the bottom of the inside container allowing the placement of the container above wheat grain in the outside container that prevented migrating beetles from returning to the inside container [21]. The insects were placed in the inside container together with a circular ring of filter paper soaked with the caraway essential oil in the subsequent series of mass concentrations of 0.1%, 0.5%, and 1%, and of L-carvone in 0.05%, 0.1%, 0.5%, and 1% concentrations. The caraway oil and L-carvone were bought from Sigma-Aldrich. The repellent effect, mortality, and the numbers of insects were recorded after 1, 2, 3, 4, 24, and 48 hours. Each variant of the experiment was conducted in nine repetitions.

The estimates of repellent effects were based on the emigration index calculated as a percentage proportion of individuals emigrating compared with the total number of individuals in the population. The calculations were made using the following formula:

\[
\frac{\bar{x}_{et} + \bar{x}_{ed}}{\bar{x}_t + \bar{x}_d} \cdot 100\%
\]
The mortality index is the percentage proportion of dead individuals compared with the total number of individuals at a given time. It was calculated from the following formula [24]:

$$\frac{\bar{x}_d}{\bar{x}_d + \bar{x}_l} \cdot 100\%$$

where:

- $\bar{x}_d$ - mean number of dead insects
- $\bar{x}_l$ - mean number of live insects

We have investigated whether there are statistically significant differences in the repellent effect of different concentrations of essential caraway oil and L-carvone on S. oryzae. The dependent variable is the insect emigration rate. Since the distribution of data in particular groups separated according to the concentration of the substances examined significantly differed from normal distribution (Shapiro-Wilk test, p<0.05), the ANOVA Kruskall-Wallis rank test was applied, followed by a multiple comparison test [25]. The test probability level "p" and the significance level "\(\alpha\)" were 0.05. The calculations were performed in the Statistica 13.3 program.
Results

The caraway essential oil applied in all concentrations used (0.1, 0.5, 1%) resulted in a major repellent effect towards *S. oryzae*. After 1, 2, 3, and 4 hours of study, the strongest repellence was confirmed by the highest values of migration index, fluctuating from 60 to 98%, was caused by the essential oil at 0.5% concentration. In the control culture, the simultaneous emigration index fluctuated between 2 to 9%. After 24 and 48 hours, the caraway essential oil in all used concentrations resulted in very high emigration (repellence) among rice weevils. At that time, in the control culture, it amounted to only 13 – 19%. It was interesting to see that the highest repellence to *S. oryzae* in the initial four hours of studies was exerted in both the highest concentration applied and in a lower one, i.e. 0.5% (Fig. 1). A similar relationship was noted during the use of L-carvone; a 0.1% concentration resulted in the highest emigration of beetles (from 16 to 100%) in each of the analysed time intervals. The subsequently lower repellence (9 – 38%) was showed by L-carvone at a 0.5% concentration, and then only after 1, 2, 3, and 4 hours, at 1% concentration (7 – 22%) (Fig. 2).

Analyzing the emigration results using the ANOVA Kruskall-Wallis test, statistically significant differences were found between the emigration of *S. oryzae* in the control culture and the emigration in the cultures with the addition of caraway essential oil at all concentrations and time intervals. However, statistically significant differences between doses are marked with asterisks in Figures 1 and 2.

At the three concentrations (0.1, 0.5, and 1%) used in the tests, the caraway essential oil caused the highest mortality among the individuals of *S. oryzae* when applied at 0.5% concentration. Statistical analysis of *S. oryzae* mortality results showed statistically significant differences (p <0.05) between mortality in the control culture and the culture using caraway essential oil at 0.5% concentration (from 2 to 24 hours). At concentrations of 0.1 and 1%, the oil
did not evoke mortality of insects throughout the initial five hours of experiments. It was only after 24 hours that the mortality of the rice weevil population treated by caraway essential oil at 0.5 and 1% concentrations amounted about 100%, and among the emigrants, it was very low, i.e. the mortality indices fluctuated from 0.6 to 1.1% (Fig. 3). Similarly as in the case of repellence, the highest mortality among *S. oryzae* was obtained after applying the caraway essential oil at the concentration of 0.5%, and not the highest used in the tests.

**Discussion**

A number of plant products and extracts were tested as repellents against *S. oryzae* with the use of various research techniques and with variable efficacy [21]. For example, the fastest repellent effect on rice weevil, after a mere 5 minutes, was exerted by the crude methanol extract of *Duabanga grandiflora* at a 0.252 mg/cm² concentration, where 63% repellence was reached. After 4 hours, the efficacy of repellence against the weevil was 100% [26].

In their studies, however, Tripathi and Upadhyay [27] obtained the efficacy of 91.1 percentage repellence (PR) against adult *S. oryzae*, after 1 hour, when they applied leaf essential oil from *Hyptis suaveolens* at a concentration of 9.2mg/cm². Kim et al. [34] assessed the insecticide activity as well as the inhibiting effect of acetylcholinesterase (AChE) by essential oils and compounds extracted from 10 species of plants of the family of Apiaceae exerted on *Sitophilus oryzae*. Among the plants included in the study, the essential oils obtained from *Anethum graveolens*, *Carum carvi*, and *Cuminum cyminum* showed strong fumigant toxicity against *S. oryzae*. The plants concerned also included *Carum carvi*. Among the compounds, (+)-carvone, (−)-carvone, cuminaldehyde, dihydrocarvone, linalool oxide, carveol, trans-anethole, and neral also displayed higher toxicities against *S. oryzae*.
as fumigants. The strongest inhibitions towards acetylcholinesterase were displayed by α-pinene, followed by β-pinene and limonene.

In our study, the essential oil from *Carum carvi* applied at 0.5% concentration displayed insecticidal action resulting in 100% mortality among *S. oryzae* as early as after 5 hours. López et al. [35] had also studied the effects of active substances contained in the essential oils obtained from *C. carvi* (carvon and limonene), *Coriandrum sativum* (linalool), and *Ocimum basilicum* (estragol) upon the populations of *S. oryzae*, *R. dominica*, and *Cryptolestes pusillus*. Against *S. oryzae*, the most effective monoterpenoid was carvon (1364ppm) in combination with camphor (131ppm), where, after 24 hours, 100% of the beetles were dead. Other mixtures of active substances, whose main component was caraway oil, caused high mortality in both rice weevils as in the remaining two species (approx. 90 – 100%). Against *S. oryzae*, the mortality index in the application of linalool (1723ppm) combined with camphor (185ppm) reached 63%. Against *R. dominica* and *C. pusillus*, the mortality indexes were 96% and 100%, respectively. Estragol affected the activities of rice weevil in a variable and ambiguous way.

The most interesting results obtained in our research include those which indicate the highest repellent effects of caraway essential oil and L-carvon upon *S. oryzae* in both the highest concentration applied and at the lower concentrations used, i.e. 0.5% and 0.1%, respectively. Similar results were obtained with respect to other arthropod species, e.g., mosquitoes, biting flies, fleas, and ticks applying repellents based on DEET and permethrin. DEET is a repellent with a wide spectrum of action against arthropod bites. Although the protection against arthropod bites provided by DEET is proportional to the logarithm of the dose, the higher concentrations of DEET ensure that the protection lasts longer; however, in the range up to 50%, the concentrations above 50% do not increase the efficacy of DEET [36]. Also, tests carried out on ticks showed that
permethrin reduced the reproductive rate of females not at the highest applied dose (12.5 μg), but at a lower dose of 6.25 μg [37].

In our research on the effect of *C. carvi* on *S. oryzae*, we also checked the effect of contact, oral effects, but its effectiveness was not as high as in the case of essential oil and L-carvon.

The results obtained indicate that consideration should be given to the possibility of using the essential oil from *Carum carvi* and L-carvone in the integrated protection against *S. oryzae* in stored cereal grains as well as in the control measures against that insect species.

Conclusion

In conclusion, the greatest repellent effects on *S. oryzae* were caused by lower doses of caraway essential oil and L-carvone. Which is a new discovery among plant compounds used against stored pests. This differs from the results obtained so far in relation to stored pests, in which the repellency increased with increasing concentration. Similar results to our research were obtained with respect to other arthropod species, e.g., mosquitoes, biting flies, fleas, and ticks applying repellents based on DEET and permethrin.

Limitations

Tests are limited by the number of trials because insect counting must be done quickly to maintain hourly intervals between recording data.

Declarations

Ethics approval and consent to participate

Ethics approval.
Consent for publish

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MK concept and wrote the manuscript, compile the methodology, analysis of results. AI wrote the manuscript, conducted experiments, compiled figures, collect references. NM conducted experiments, collect references. All authors read and approved the manuscript.

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**Figure legends**

**Fig.1** Repellency of *Sitophilus oryzae* caused by caraway essential oil (* 0.05 > p > 0.01; ** 0.01 > p > 0.001; *** 0.001 > p > 0.0001; NS – lack of significant differences; the figure indicates the mean of SE – standard error).

**Fig.2** Repellency of *Sitophilus oryzae* caused by L-carvone (* 0.05 > p > 0.01; ** 0.01 > p > 0.001; *** 0.001 > p > 0.0001; NS – lack of significant differences; the figure indicates the mean of SE).

**Fig.3** Mortality of *Sitophilus oryzae* caused by caraway essential oil (the figure indicates the mean of SE).