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# Phytotoxic Effect of Caraway Essential Oil and Its Main Compounds against Germination of Spring Wheat and Two Weed Species †

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**Abstract:** Caraway oil is a promising botanical herbicide. In this study the phytotoxic potential of caraway oil, carvone and d-limonene on germination and seedlings growth of spring wheat, wild oat and chamomile, was tested. As a result, an inhibiting effect of caraway oil and carvone on all the tested species was found. Contrary, d-limonene displayed a selective toxicity against chamomile and monocotyledonous species, that should be investigated further.

**Keywords:** carvone; d-limonene; wild oat; chamomile; ED50

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## 1. Introduction

Increased interest in the use of biological methods of crop protection has led to the development of research on using the allelopathic potential of various plant species to reduce agricultural pests [1,2]. Essential oils of plants' origin show phytotoxic properties that can be used to produce so-called botanical herbicides, i.e., substances of plant origin for weed control [3]. The most frequently analyzed effect of the use of essential oils against weeds is their influence on seed germination and the growth of seedlings [4,5]. One of the promising oils for that purpose is caraway oil [6]. The yield of essential oil obtained from caraway fruits is in a range of 1–6%. The oil is rich in oxygenated monoterpenes, mainly carvone and d-limonene, constituting up to 95% of all oil compounds [7].

The biological action of essential oils is attributed to synergistic effects of their compounds, as was found,i.e., for the clove oil [8,9]. Our study aimed to investigate the phytotoxic potential of water solutions of caraway oil and its main compounds: carvone and d-limonene, to the germination and initial growth of spring wheat (*Triticum aestivum* L.), wild oat (*Avena fatua* L.), and chamomile (*Matricaria chamomilla* L.).

## 2. Material and Methods

Caraway essential oil (EO) of Polish origin was purchased from the producer HerbaNordPol Sp. z o. o. (Gdansk, Poland). The fractionation of the oil and analysis of its composition were carried out at the Institute of Natural Products and Cosmetics, Lodz University of Technology. The essential oil was rectified with fractional column (Vigerous column, 3 × 20 cm), under reduced pressure. Next, the collected fractions were analyzed by the GC-MS-FID method on a Trace GC Ultra gas chromatograph connected to a DSQ II mass spectrometer (Thermo Electron). Simultaneous analysis of GC-FID and MS was performed thanks to the MS-FID splitter (SGE Analytical Science). The components of the

EO were identified by comparing the experimental retention indexes (RI) of the analyzed components with the indexes in the MassFinder 4.0 library and the NIST MS Search database (2012) and by comparing the mass spectra of the tested compounds with the patterns contained in the computer library Wiley Registry 10th Edition/NIST (2012) Mass Spectral Library.

The Petri dish (11 cm diameter) experiment was carried out in the glass dishes, in four series and three repetitions. The essential oil was applied as an oil in water (*o*/*w*) solution, with 5% acetone as a carrier. Five doses of caraway oil: 0.004; 0.007; 0.01; 0.02; 0.03 g per dish; five doses of carvone: 0.002; 0.004; 0.008; 0.012; 0.02 g per dish and five doses of d-limonene: 0.001; 0.002; 0.005; 0.007; 0.01 g per dish were used. The doses of EO components, carvone, and d-limonene reflected their percentage content in the oil, 60% and 35%, respectively. The filter paper was soaked with 7 g of an appropriate o/w solution of EO or its component. The seeds of spring wheat (*Triticum aestivum* L. cv. Tybalt) and weed seeds—wild oats (*Avena fatua* L.) and chamomile (*Matricaria chamomilla* L.) were used. Mature wild oats seeds were collected from a spring wheat field in southern Poland in 2016. Certified chamomile seeds were purchased (PlantiCo Sp. z o.o, Poland). Twenty seeds of the tested species were placed on two layers of sterile filter paper in each dish. The seeds were allowed to germinate for seven days in the dark at room temperature (±25 °C). The seedlings were counted seven days after, and the length [mm] of their roots and shoots were measured.

The percentage of germinated seeds was analyzed using dose-response non-linear analysis (‘drc’) [10]. Three parameters were used to fit the log-logistic curve (Y) according to [11], where the lower limit is equal to zero:

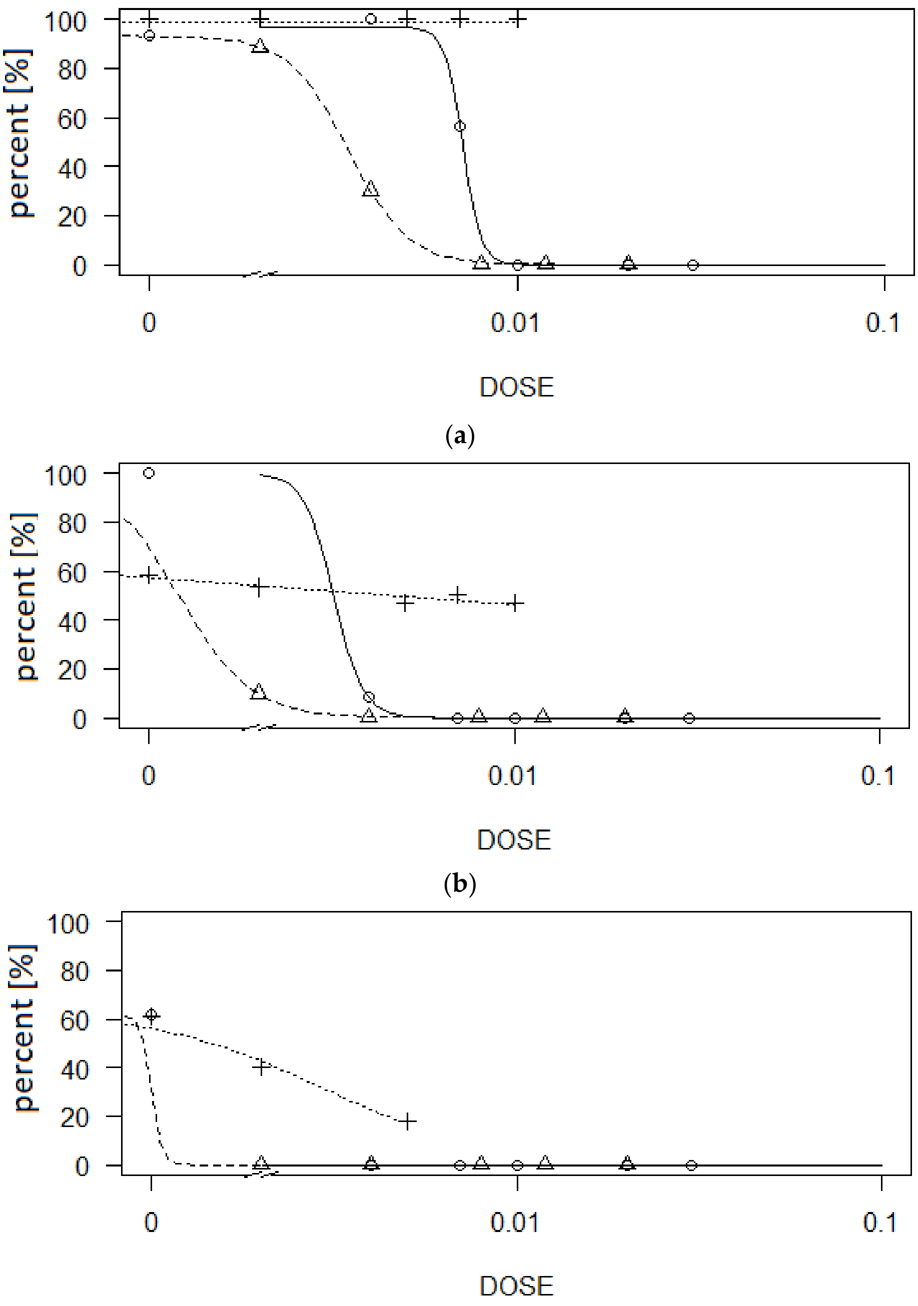
$$Y = d/(1 + \exp(b(\log x - \log e))); \tag{1}$$

where e is the ED50 value, d is the upper limit, b denotes the relative slope around e, and x is the percentage germination or root/shoot length. The ED50 value was calculated in the ‘drc’ package and further used to compare the phytotoxic effect of the EOs against the tested plants [4]. All statistical analyses were performed with the software R, ver. 4.0.1 [12].

## 3. Results and Discussion

The first fraction, analysed in the Petri dish experiment as limonene, contained d-limonene and carvone in the proportion 91.1%:6.9%. The second fraction was composed of 53.3% of limonene and 45.4% of carvone. The residue of rectification process consisted of almost pure carvone: 99.8%, and was analysed in the Petri dish experiment as a carvone.

Germination of the tested species was affected to a different level by the tested caraway EO and its main compounds (Figure 1). Germination of spring wheat was mostly affected by caraway EO and carvone (ED50 0.006 and 0.008, respectively). Contrary, in the presence of limonene, all seeds of wheat germinated, even at the highest dose of limonene (Figure 1a). The germination of wild oat was inhibited already by the lowest doses of caraway EO (ED50 0.001) and carvone (ED50 0.003). However, in the presence of limonene, the weed germinated well (Figure 1b). Chamomile was highly susceptible to the presence of caraway EO and carvone (ED50 0.001 g for both) and less susceptible to the presence of limonene (ED50 0.003) (Figure 1c). The germination pattern of the studied species in the presence of caraway EO and its main compounds points to a strong herbicidal effect of the EO and carvone. The results are concurrent with other authors who underline inhibiting effect of caraway EO and carvone on germination of weeds and crops [4,13].



(**a**)

(**b**)

(**c**)

**Figure 1.** Dose-response curves of germination of spring wheat (**a**), wild oat (**b**) and chamomile (**c**) in the presence of different doses of caraway oil -Δ-, carvone -○- and limonene -×-.

The growth of seedlings of the tested species was highly affected by the caraway EO (Table 1), especially in weeds. Only single seedlings of wild oat grew in the lowest doses of the EO, whereas growth of chamomile seedlings was totally inhibited even at the lowest oil dose. The susceptibility of the tested species to the caraway EO is represented well also by the very low values of ED50.

**Table 1.** The response of spring wheat, wild oats, and chamomile seedlings to different doses of caraway oil (mean value ± standard error).

| Dose [g] | Shoot Length [mm] | | | Root Length [mm] | | |
|---|---|---|---|---|---|---|
| | Wheat | Wild Oat | Chamomile | Wheat | Wild Oat | Chamomile |
| 0 | 57.8 ± 0.66 | 61.7 ± 2.64 | 11.3 ± 0.7 | 103 ± 7.01 | 52.9 ± 17.2 | 3.1 ± 0.3 |
| 0.004 | 9.22 ± 0.08 | 1.31 ± 0.35 | 0 ± 0 | 12.3 ± 0.25 | 1.67 ± 0.17 | 0 ± 0 |
| 0.007 | 2.49 ± 1.99 | 0 ± 0 | 0 ± 0 | 3.83 ± 2.61 | 0 ± 0 | 0 ± 0 |
| 0.01 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 0.02 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 0.03 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **ED50** | **0.002** | **0.002** | **0.001** | **0.001** | **0.002** | **0.001** |

The seedlings' growth was inhibited by carvone even more than by the caraway EO (Table 2). Only a few seedlings of wheat grew in the presence of the lowest dose of that compound. The ED50 values of both shoot and root length were also very low.

**Table 2.** The response of spring wheat, wild oats, and chamomile seedlings to different doses of carvone (mean value ± standard error).

| Dose [g] | Shoot Length [mm] | | | Root Length [mm] | | |
|---|---|---|---|---|---|---|
| | Wheat | Wild Oat | Chamomile | Wheat | Wild Oat | Chamomile |
| 0 | 57.8 ± 0.66 | 61.7 ± 2.64 | 11.3 ± 0.7 | 103 ± 7.01 | 52.9 ± 17.2 | 3.1 ± 0.3 |
| 0.004 | 3.54 ± 0.76 | 0 ± 0 | 0 ± 0 | 5.81 ± 1.07 | 0.5 ± 0.5 | 0 ± 0 |
| 0.007 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 0.01 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 0.02 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 0.03 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **ED50** | **0.001** | **0.001** | **0.001** | **0.001** | **0.001** | **0.001** |

Contrary to the two other compounds, the seedlings of all the tested species germinated in the presence of limonene; however, the growth of wheat and wild oat seedlings was strongly inhibited, even by the lowest dose of the EO and its main compounds (Table 3). Contrary, chamomile seedlings' growth in the presence of a majority of d-limonene doses was similar to control, except that the elongation of roots was significantly inhibited at the highest dose. The ED50 values expressed the differences in susceptibilities between grasses and chamomile to d-limonene.

**Table 3.** The response of spring wheat, wild oats, and chamomile seedlings to different doses of limonene (mean value ± standard error).

| Dose [g] | Shoot Length [mm] | | | Root Length [mm] | | |
|---|---|---|---|---|---|---|
| | Wheat | Wild Oat | Chamomile | Wheat | Wild Oat | Chamomile |
| 0 | 57.8 ± 0.66 a | 61.7 ± 2.64 a | 11.9 ± 0.45 a | 103 ± 7.01 a | 52.9 ± 17.2 a | 3.60 ± 0.30 a |
| 0.004 | 10.5 ± 0.37 b | 7.50 ± 0.35 b | 11.8 ± 0.35 a | 14.6 ± 0.34 b | 10.7 ± 0.58 b | 2.81 ± 0.21 a |
| 0.007 | 10.3 ± 0.12 b | 7.64 ± 0.29 b | 11.1 ± 0.44 a | 14.0 ± 0.28 b | 12.4 ± 0.37 b | 2.80 ± 0.28 a |
| 0.01 | 10.0 ± 0.41 b | 7.71 ± 0.33 b | 8.73 ± 0.22 a | 15.1 ± 0.09 b | 12.5 ± 0.13 b | 2.32 ± 0.23 a |
| 0.02 | 9.99 ± 0.13 b | 8.26 ± 0.20 b | 10.6 ± 0.48 a | 14.7 ± 0.68 b | 10.4 ± 0.77 b | 2.39 ± 0.21 a |
| 0.03 | 9.45 ± 0.25 b | 7.11 ± 0.36 b | 8.85 ± 1.01 a | 14.2 ± 0.18 b | 11.4 ± 0.56 b | 1.92 ± 0.43 b |
| **ED50** | **0.001** | **0.001** | **0.07** | **0.001** | **0.001** | **0.02** |

The herbicidal effect of caraway EO and carvone on the seedlings of wheat, wild oat, and chamomile was strong in laboratory conditions. Contrary, the effect of d-limonene was weaker, as seedlings were able to grow but were strongly inhibited. Interestingly, the inhibiting effect of d-limonene on shoots/roots elongation was more visible in monocotyledonous wheat and wild oat than dicotyledonous chamomile. Studies of other authors show that weed and crop species display different susceptibility to various essential oils and compounds [4,14,15], which is a good selective effect for the future development of botanical herbicides.

## 4. Conclusions

In the laboratory conditions, and with the doses applied, the caraway EO and carvone inhibit germination and seedlings growth of spring wheat, wild oat, and chamomile. Contrary, d-limonene does not affect the germination of wheat and wild oat but significantly inhibits the growth of their seedlings. D-limonene inhibits germination of chamomile in a dose-response manner, but the growth of seedlings is similar to that of control. In summary, d-limonene should be studied further for its potential selective properties.

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